# Impacts d'apports de composts de déchets urbains sur la résistance et la résilience de la microflore du sol à des évènements de type canicule/sécheresse

Meriem Ben Sassi

Université d'Avignon et des Pays du Vaucluse

**THESE**

Présentée en vue d'obtenir le grade de docteur

Spécialité : Sciences Agronomiques

par **Meriem BEN SASSI**

---

# Impacts d'apports de composts de déchets urbains sur la résistance et la résilience de la microflore du sol à des évènements de type canicule/sécheresse

---

Jury composé de :

**Mr. Thierry DUTOIT** (Examinateur)

**M$^{me}$ Claire CHENU** (Rapporteur)

**Mr. Laurent PHILIPPOT** (Rapporteur)

**Mr. Lauric CECILLON** (Examinateur)

**Mr. Antonio BISPO** (Examinateur)

**M$^{me}$ Annette BERARD** (Directrice de thèse)

**Mr. Pierre RENAULT** (Co-Directeur de thèse)

Soutenue le 16 novembre 2012

# Résumé

Face aux changements climatiques actuels et à l'augmentation des populations, la vulnérabilité du sol et des services écosystémiques qu'il rend s'accroît. En particulier dans les zones climatiques Méditerranéennes, les modèles météorologiques prévoient une augmentation des sécheresses estivales et une augmentation des températures accompagnées par l'apparition plus fréquente d'évènements extrêmes de type canicule et sécheresse. Ces événements, leur intensité, leur durée et la soudaineté avec laquelle ils arrivent, sont de nature à affecter la structure et la fonction des écosystèmes avec des conséquences principalement négatives sur leur biodiversité et leurs fonctions et services. Par ailleurs, l'apport de compost au sol pourrait constituer une solution pour prévenir et atténuer les effets des sécheresses et des canicules dans les agrosystèmes Méditerranéens. Les objectifs de ce travail étaient de caractériser les effets à court et à long-terme de perturbations de type canicule et/ou sécheresse appliquées à un sol Méditerranéen agricole (structures et fonctions des communautés microbiennes édaphiques) et d'étudier les impacts d'épandage préalable de composts sur la réponse à court et à long-terme de ces communautés microbiennes (structures et fonctions) vis-à-vis d'un événement extrême de canicule-sécheresse. Nos travaux nous ont permis d'évaluer l'influence de chacun des facteurs température élevée et sécheresse dans la perturbation canicule et sécheresse associées sur les paramètres microbiologiques et physico-chimiques du sol. Les effets de cette combinaison des deux perturbations a induit des réponses similaires à l'une ou l'autre des perturbations appliquées individuellement en bénéficiant des effets positifs et négatifs sur la communauté microbienne de chaque type de perturbation. Nous avons mis en évidence une durée seuil de la perturbation canicule-sécheresse sur la résistance de la communauté microbienne induisant un changement de structure taxonomique et fonctionnelle. Cette déstructuration de la communauté microbienne est durable et n'a pas permis de résilience. L'ajout préalable de composts de différents types au champ a amélioré la structure physico-chimique et stimulé les microorganismes indigènes du sol. Cependant, face à des perturbations de type canicule-sécheresse (telles que nous les avons testées), il semble que l'apport préalable de compost n'ait pas d'effets majeurs sur l'amélioration de la qualité du sol en terme de stabilité microbienne, mais que l'historique saisonnier influencerait cette stabilité.

# Abstract

Current climate change and increasing populations' growth enhance soil and ecosystem services vulnerability. Meteorological models predicted an increase in summer drought and higher air temperature with more frequent occurrence of extreme events like heat-waves and drought. Intensity and duration of these events may affect structure and functions of ecosystems and thereby the biodiversity and the functions of soil. The amendment of soils with composts could be an alternative to prevent and mitigate the effects of drought and heat waves in the Mediterranean agroecosystems. The objectives of this work were to characterize the effects of short and long-term high temperature and/or drought perturbation on soil Mediterranean microbial communities (structures and functions) and to study the impacts of compost amendment on short and long-term functional and taxonomic responses of microbial communities subjected to drought and high temperature. Our work allowed us to evaluate the influence of each factor (drought or high temperature) within the combined perturbation (drought and high temperature) on microbiological and physico-chemical soil properties. The effects of this combined perturbation induced similar or different responses of each of perturbations applied individually involving positive and negative effects on the microbial community. This work had shown threshold resistance duration inducing a change in taxonomic and functional microbial community structure after high temperature and drought perturbation. This abrupt shift in the community response did not allow resilience. Compost amendments improved the physico-chemical soil structure and stimulated indigenous soil microorganisms. However, it seemed that seasonal soil variations history rather than compost amendment influences soil microbial stability.

# Remerciements

Je voudrais tout d'abord remercier les membres du jury d'avoir accepté d'évaluer ce travail.

Je tiens à adresser mes plus vifs remerciements à mes directeurs de thèse Annette Bérard et Pierre Renault de m'avoir accordé leur confiance dans la réalisation de ce travail, soutenu et transmis leur passion pour la recherche tout au long de ces 3 années de thèse.

Je remercie également les membres de mon comité de pilotage Raphaël Gros, Naoise Nunan, Antonio Bispo et Fabrice Martin-Laurent de m'avoir conseillée et aiguillée.

Je remercie tous les membres de l'équipe EMMAH de l'INRA d'Avignon pour leur aide et soutien. Merci à Ghislain Sévenier, Dominique Renard, Bernard Bes et Micheline Debroux de m'avoir aidée dans les manipulations et les prélèvements de sol sur le terrain. Merci aux stagiaires Jeanne et Linda d'avoir contribué à ce travail. Un grand Merci à mes collègues de bureau Guillaume, Romain et Nicolas pour les bons moments passés ensemble. Merci Régis d'avoir résolu tous les problèmes de logiciels informatiques en un laps de temps. Merci aux secrétaires, à Bruno, Line, Claude, Dalila, Sasa, Mami et Eric pour votre soutien.

Je n'oublie pas de remercier Christian Giniès, Jérémie Béguet et David Bru pour leur collaboration dans ce travail de recherche.

Je remercie tous les membres de ma famille ainsi que mes amis pour leur soutien et leurs encouragements.

Enfin je voudrais remercier ma grand-mère, mes frères et mes parents de m'avoir encouragée et soutenue tout au long de ce cursus. Merci pour votre confiance et votre amour.

# Sommaire

# INTRODUCTION

Le sol est un système hétérogène et complexe situé à l'interface entre l'atmosphère, la lithosphère et l'hydrosphère. Il constitue une composante essentielle de l'environnement et exerce diverses fonctions dans les écosystèmes. En effet, le sol fournit les éléments nutritifs nécessaires à la production animale et végétale (Gurevich et al., 2008 ; Nannipieri et al., 2003). Il régit aussi les cycles des nutriments nécessaires à la vie sur Terre (Butler et Hooper, 2010). Le sol est aussi un élément fondamental du cycle de l'eau continentale et participe à la régulation du climat en contrôlant les émissions de gaz à effet de serre et la séquestration du carbone (Lu et Cheng, 2009). Ainsi, la fonction écologique des sols est due entre autre aux fortes interactions entre la microflore, la réactivité géochimique et les transferts d'eau, de solutés, de gaz, de matière et de chaleur. En effet, les microorganismes du sol participent à la décomposition, à la minéralisation des éléments nutritifs et à leur immobilisation (Swift et al., 1998 ; Saetre et Stark, 2005), contribuant ainsi à la nutrition des plantes en améliorant l'absorption des éléments nutritifs et leur translocation (Timonen et al., 1996). Mais de manière plus générale, ils affectent l'ensemble des propriétés du sol en modifiant les propriétés physicochimiques de sa solution, en contribuant à l'altération (et à la néoformation) de minéraux et en jouant sur la structure même du sol. En conséquence, les microorganismes édaphiques contribuent à la qualité des sols, participant ainsi à leurs services écologiques (Millennium Ecosystem Assessment, 2005).

Dans un contexte de développement durable, la qualité des sols doit être préservée. Cependant, le concept de la qualité des sols, qui a émergé au début des années 1990 (Karlen et al., 1997 ; Nortcliff, 2002), donne lieu à plus de controverses que celles de l'eau ou de l'air (Bastida et al., 2008b). Il existe différentes définitions de cette qualité et ce concept est une notion subjective et très dynamique (Gros, 2002). Toutefois, en dépit de la difficulté de fournir une définition, le maintien de la qualité des sols est essentiel pour assurer la durabilité de l'environnement et la biosphère (Bastida et al., 2008b). Cette qualité résulte d'interactions entre des composantes chimiques, physiques et biologiques. Par ailleurs, les communautés microbiennes sont primordiales pour le fonctionnement du sol et pour préserver ses services écologiques. La qualité biologique des sols fait référence à l'abondance, à la diversité et à l'activité des êtres vivants le colonisant (Chaussod, 1996). Ce concept englobe aussi les notions de résistance et de résilience (Seybold et al., 1999). La résistance est définie comme la capacité à supporter immédiatement une perturbation et la résilience comme étant la capacité à retrouver son état initial suite à une perturbation (Seybold et al., 1999; Griffiths et al., 2001;

Griffiths et Philippot, 2012; Figure 1). Ces deux composantes appliquées aux communautés microbiennes édaphiques définissent la stabilité microbiologique d'un sol (Griffiths et al., 2001). **Dans notre travail, nous nous focaliserons sur la capacité à retrouver et à maintenir la diversité fonctionnelle et structurale de la communauté microbienne édaphique après une perturbation**.

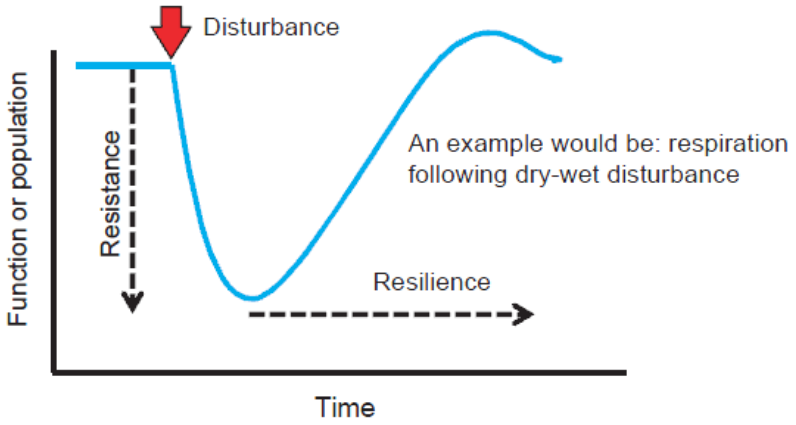


**Figure 1 :** Représentation schématique du principe de la résistance et de la résilience après une perturbation (d'après Griffiths et Philippot, 2012).

Dans une politique de réduction du rythme actuel d'appauvrissement de la diversité biologique et afin de la conserver voire de l'accroître, les apports de matières organiques exogènes tels que les composts contribuerait à la gestion durable du système écologique sol (Diacono et Montemurro, 2010). Par ailleurs, le compostage est un procédé biologique thermophile contrôlé de conversion et de valorisation des constituants organiques des déchets en un produit stabilisé, assaini et riche en composés humiques (Mustin, 1987). Il s'inscrit dans un contexte de gestion des déchets urbains et permet d'obtenir un composé, devant répondre aux critères définis dans la norme NF 44-051, valorisé essentiellement en tant qu'amendement organique en agriculture (AFNOR, 2006). Des observations expérimentales ont permis de mettre en évidence que l'incorporation de la matière organique exogène au sol améliore sa stabilité structurale et son fonctionnement biologique. En effet, des essais de longues durées au champ ont montré une augmentation des teneurs en carbone organique du sol (Albiach et al., 2001 ; Ros et al., 2006a ; Ros et al., 2006b). La matière organique exogène constitue aussi une source d'énergie pour les microbes et le réseau trophique (Chaussod, 1996) et contribue à l'augmentation de la biomasse tellurique (Garcia-Gil et al., 2000 ; Ros et

al., 2003 ; Saison et al., 2006 ; Laudicina et al., 2011), de certaines activités enzymatiques (Garcia-Gil et al., 2000 ; Bhattacharyya et al., 2001 ; Ros et al., 2003 ; Ros et al., 2006b ; Bastida et al., 2008a) ainsi qu'à l'augmentation de la synthèse des polysaccharides d'origine microbienne qui contribuent à l'augmentation de la cohésion des agrégats du sol (Albiach et al., 2001 ; Gomez et al., 2006).

Cependant, le sol est soumis à une série de processus de dégradation. Sa composition et ses fonctions microbiennes peuvent être sensibles aux perturbations environnementales et en particulier climatiques (Yuste et al., 2007 ; Wallenstein et al., 2009). En effet, le réchauffement du système climatique n'est plus mis en doute. On note déjà, à l'échelle de la planète, une hausse des températures moyennes de l'atmosphère et de l'océan, une fonte massive de la neige et de la glace et une élévation du niveau moyen de la mer (IPCC, 2007). Ce réchauffement est attribué aux activités anthropiques et aux émissions de gaz à effet de serre (IPCC, 2007). Les projections des valeurs moyennes du réchauffement en surface à l'échelle du globe selon les six différents scénarios SRES (Special Report on Emissions Scenarios) de référence analysés vont de 1,8 à 4°C d'ici 2100 (IPCC, 2007). Ces changements climatiques varient selon les régions : si les augmentations de température les plus infimes se produiront sur les océans et dans certaines régions tropicales, les modèles météorologiques prévoient des réductions significatives des précipitations, et dans les contextes climatiques de type méditerranéen une augmentation des sécheresses estivales et une augmentation des températures (Gibelin et Déqué, 2003 ; Stern, 2007 ; IPCC, 2012). Cette tendance globale, semble être accompagnée par l'apparition plus fréquente d'événements extrêmes de type pluie torrentielle, canicule et sécheresse (IPCC, 2002). Ces événements, leur intensité (durée, températures atteintes, état hydrique …) et la soudaineté avec laquelle ils arrivent sont de nature à affecter la structure et la fonction des écosystèmes avec des conséquences principalement négatives sur leur biodiversité et leurs fonctions et services (IPCC, 2007 ; Millennium Ecosystem Assessment, 2005).

Bien que la température et la teneur en eau soient les facteurs environnementaux les plus importants affectant la croissance et l'activité microbienne dans les sols (Paul and Clark 1996), peu d'études en écologie microbienne des sols de zones méditerranéennes tempérées ont été réalisées sur les effets d'épisodes climatiques extrêmes de type sécheresse et canicule

(Schimel et al., 2007) et la plupart d'entre elles traitent séparément ces effets. Certains travaux appliqués aux effets des températures sur les communautés microbiennes ont démontré que les bactéries s'adapteraient aux températures élevées alors que les champignons semblent s'adapter aux faibles températures (Pietikainen et al., 2005). D'autres études portant sur les impacts de la sécheresse ont montré que la dessiccation entraîne une augmentation relative du nombre de champignons par rapport aux bactéries (Ross and Sparling, 1993 ; Joergensen et al., 1995 ; Jensen et al., 2003). Des travaux préliminaires réalisés dans notre unité ont porté sur l'étude des effets combinés de perturbation de canicule et de sécheresse (Bérard et al., 2011). Les résultats suggèrent qu'il existe des impacts à court et à long terme et des phénomènes de seuil concernant la durée de ces perturbations sur la structure et les fonctions de la communauté microbienne édaphique, en particulier en termes de résilience. Le Groupe d'expert intergouvernemental sur l'évolution du climat suggère que "ces impacts pourraient être évités, réduits ou retardés en appliquant des mesures d'atténuation contribuant à la préservation de la qualité des sols", telles que par exemple, l'amélioration de la gestion des terres cultivées et des pâturages visant à augmenter le stockage du carbone dans les sols, l'amélioration des techniques d'épandage et le recyclage des déchets par retour de la matière organique au sol (IPCC, 2007). Plusieurs études portant sur les effets à long-terme des apports de composts sur la biomasse et l'activité microbienne des sols méditerranéens ont été réalisées (Garcia-Gil et al., 2000 ; Ros et al., 2003 ; Crecchio et al., 2004 ; Ros et al., 2006a ; Ros et al., 2006b ; Bastida et al., 2008a ; Laudicina et al., 2011). Des études ont été réalisées sur la restauration de la qualité chimique et microbiologique des sols par des apports de composts (Guerrero et al., 2001 ; Ros et al., 2003 ; Larchevêque et al., 2005 ; Guénon, 2010 ; Cordovil et al., 2011 ; Cellier et al., 2012). Cependant, aucune étude à notre connaissance n'a été réalisée sur les effets des apports de matières organiques exogènes sur les réponses à court et long terme des communautés microbiennes édaphiques aux évènements climatiques extrêmes de canicule et de sécheresse. **Ces apports de compost modifieraient-ils la réponse des communautés microbiennes du sol à une perturbation de type canicule/sécheresse, et si oui de quelle manière ?**

**Pour répondre à ces questions nos objectifs sont les suivants :**

1. En premier lieu, caractériser les effets à court et à long-terme de perturbations de type canicule et/ou sécheresse[1] appliquées à un sol Méditerranéen agricole (structures et fonctions des communautés microbiennes édaphiques).

1.1. Etudier la réponse de la communauté microbienne édaphique (structures et fonctions) à une perturbation de type canicule et/ou sécheresse en fonction de la durée de la perturbation et détecter l'existence éventuelle de seuils dans les réponses microbiennes.

1.2. Aborder la résilience (structure et fonctions) des communautés microbiennes suite à une perturbation seuil.

2. Parallèlement sur le même sol, étudier les impacts d'épandage de composts sur la réponse à court et à long-terme des communautés microbiennes édaphiques (structures et fonctions) vis-à-vis d'un événement extrême de canicule-sécheresse.

2.1. Etudier et comparer les effets d'ajout de composts de déchets urbains non issus d'unité de méthanisation et ceux issus de ces unités sur les réponses des différentes communautés microbiennes aux événements extrêmes.

2.2. Etudier les effets de la durée de la période entre l'épandage de compost et l'événement extrême appliqué et les effets de l'historique saisonnier de température et d'humidité sur les réponses à une perturbation de type canicule-sécheresse appliquée.

Pour atteindre ces objectifs des observations au champ (placettes expérimentales) ainsi que des essais au laboratoire (microcosmes) ont été réalisés.

[1] Nous utilisons le terme "canicule" pour décrire une perturbation impliquant uniquement une température élevée. Le terme "sécheresse" décrit une perturbation hydrique xérique et le terme "canicule-sécheresse" décrit une perturbation de température et une perturbation hydrique combinées.

**Ce mémoire est structuré en quatre chapitres principaux** :

Le **premier chapitre** est consacré à la présentation de deux synthèses bibliographiques portant sur les conséquences des évènements extrêmes de température et de sécheresse sur les communautés microbiennes du sol et sur les impacts d'apports de matières organiques exogènes, plus spécifiquement des composts, sur les propriétés du sol. Ce chapitre inclut une revue en préparation intitulée "Consequences of extreme events -drought and high-temperature- on soil microbial communities: A review".

Le **deuxième chapitre** est consacré à la présentation du matériel et des méthodes d'analyses utilisés au cours des différentes expériences. Nous avons développé et amélioré une méthode de microrespirométrie proposée par une équipe Ecossaise (Macaulay Institute). Ce chapitre inclut donc deux articles intitulés : "Improving the MicroRespTM substrate induced respiration method by a more complete description of $CO_2$ behaviour in closed incubation wells" soumis à *Geoderma* et "The FungiResp method : An application of the MicroResp$^{TM}$ method to assess fungi in microbial communities as soil biological indicators" publié dans *Ecological Indicators*.

Le **troisième chapitre** est consacré à l'exposé des résultats. Il traite et vérifie nos hypothèses de travail et est structuré en deux grandes parties. La première partie décrit les conséquences d'un évènement de type canicule et/ou sécheresse sur les communautés microbiennes d'un sol Méditerranéen. Elle inclut deux articles en préparation intitulés respectivement : "Heat waves and soil Mediterranean microbial communities: Differential influence of drought, high-temperature and perturbation duration" et "Functional and taxonomic stability of soil Mediterranean microbial communities subjected to drought and/or high temperature". La deuxième partie est consacrée à l'étude de l'influence de l'épandage de composts sur la réponse des communautés microbiennes à une perturbation de type canicule-sécheresse. Elle inclut un article en préparation intitulé : "Effects of compost amendment on functional and taxonomic stability of soil Mediterranean microbial communities subjected to drought and high temperature".

Le **quatrième chapitre** présente une synthèse des résultats acquis, et les rediscute au vu du contexte des travaux et de la problématique. Les limites de ce travail et les questions nées des résultats obtenus nous permettent alors de proposer quelques perspectives pour le prolonger.

**Références**


AFNOR. 2006. NF U 44-051. Amendements organiques - dénomination, spécifications et marquage. AFNOR.

Albiach, R., Canet, R., Pomares, F., Ingelmo, F., 2001. Organic matter components and aggregate stability after the application of different amendments to a horticultural soil. Bioresource Technology 76, 125-129.

Bastida, F., Kandeler, E., Moreno, J.L., Ros, M., Hernandez, T., 2008a. Application of fresh and composted organic wastes modifies structure, size and activity of soil microbial community under semiarid climate. Applied Soil Ecology 40, 318-329.

Bastida, F., Zsolnay, A., Hernández, T., García, C., 2008b. Past, present and future of soil quality indices: A biological perspective. Geoderma 147, 159–171.

Bérard, A., Bouchet, T., Sévenier, G., Pablo, A.L., Gros, R., 2011. Resilience of soil microbial communities impacted by severe drought and high temperature in the context of Mediterranean heat waves. European Journal of Soil Biology 47, 333-342.

Bhattacharyya, P., Pal, R., Chakraborty, A., Chakrabarti, K., 2001. Microbial biomass and activity in a laterite soil amended with municipal solid waste compost. Journal of Agronomy and Crop Science 187, 207-211.

Butler, J., and Hooper, P., 2010. Down to Earth: An illustration of life cycle inventory good practice with reference to the production of soil conditioning compost. Resources, Conservation and Recycling 55, 135-147.

Cellier, A., Francou, C., Houot, S., Ballini, C., Gauquelin, T., Baldy, V., 2012. Use of urban composts for the regeneration of a burnt Mediterranean soil: A laboratory approach. Journal of Environmental Management 95, S238-S244.

Chaussod, R., 1996. La qualité biologique des sols : évaluation et implication. Etude et Gestion des Sols 3, 261-278.

Cordovil, C.M.d.S., de Varennes, A., Pinto, R., Fernandes, R.C., 2011. Changes in mineral nitrogen, soil organic matter fractions and microbial community level physiological profiles after application of digested pig slurry and compost from municipal organic wastes to burned soils. Soil Biology and Biochemistry 43, 845-852.

Crecchio, C., Curci, M., Pizzigallo, M.D.R., Ricciuti, P., Ruggiero, P., 2004. Effects of municipal solid waste compost amendments on soil enzyme activities and bacterial genetic diversity. Soil Biology and Biochemistry 36, 1595–1605.

Diacono, M., and Montemurro, F., 2010. Long-term effects of organic amendments on soil fertility. Agronomy for Sustainable Development 30, 401–422.

Garcia-Gil, J.C., Plaza C., Soler-Rovira P., Polo A., 2000. Long-term effects of municipal solid waste compost application on soil enzyme activities and microbial biomass. Soil Biology and Biochemistry 32, 1173-1181.

Gibelin, A.L., and Déqué, M., 2003. Anthropogenic climate change over the Mediterranean region simulated by a global variable resolution model. Climate Dynamics 20, 327–339.

Gomez, E., Ferreras, L., Toresani, S. 2006. Soil bacterial functional diversity as influenced by organic amendment application. Bioresource Technology 97, 1484–1489.

Griffiths, B.S., and Philippot, L., 2012. Insights into the resistance and resilience of the soil microbial community. FEMS Microbiology Reviews 1–18.

Griffiths, B.S., Bonkowski, M., Roy, Ritz, J.K., 2001. Functional stability, substrate utilisation and biological indicators of soils following environmental impacts. Applied Soil Ecology 16, 49–61.

Gros, R., 2002. Fonctionnement et qualité des sols soumis à des perturbations physiques et chimiques d'origine anthropique : réponses du sol, de la flore et de la microflore bactérienne tellurique. Thèse ADEME Université de Savoie, 252p.

Guénon, R., 2010. Vulnérabilité des sols méditerranéens aux incendies récurrents et restauration de leurs qualités chimiques et microbiologiques par l'apport de composts. Thèse Université Paul Cézanne Aix-Marseille III, 248p.

Guénon, R., Vennetier, M., Dupuy, N., Ziarelli, F., Gros., R., 2011. Soil organic matter quality and microbial catabolic functions along a gradient of wildfire history in a Mediterranean ecosystem. Applied Soil Ecology 48, 81–93.

Guerrero, C., Gomez, I., Moral, R., Mataix-Solera, J., Mataix-Beneyto, J., Hernandez, T., 2001. Reclamation of a burned forest soil with municipal waste compost: macronutrient dynamic and improved vegetation cover recovery. Bioresource Technology 76, 221–227.

Gurevich, Yu.L., Manukovsky, N.S., Kovalev, V.S., Degermendzy, A.G., Hu, D., Hu, E., Liu, H., 2008. The carbon cycle in a bioregenerative life support system with a soil-like substrate. Acta Astronautica 63, 1043-1048.

IPCC, 2002. Climate Change and Biodiversity. Technical paper V: 46pp. (http://www.ipcc.ch/pub/tpbiodiv.pdf).

IPCC, 2007, Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. 21pp.

IPCC, 2012. Summary for Policymakers, in *Managing the risks of extreme events and disasters to advance climate change adaptation*, Cambridge University Press., C. B. Field et al. , ed. Cambridge, UK, and New York, NY, USA, 19 p.

Jensen, K. D., Beier, C., Michelsen, A., Emmett, B., 2003. Effects of experimental drought on microbial processes in two temperate heathlands at contrasting water conditions. Applied Soil Ecology 24, 165–176.

Joergensen, R.G., Anderson, T.H., Wolters, V., 1995. Carbon and nitrogen relationships in the microbial biomass in soils in beech (*Fagus sylvatica* L.) forests. Biology and Fertility of Soils 19, 141–147.

Karlen, D.C., Mausbach, M.J., Doran, J.W., Cline, R.G., Harris, R.F., Schuman, G.E., 1997. Soil quality: a concept, definition and framework for evaluation. Soil Science Society of American Journal 61, 4-10.

Larchevêque, M., Baldy, V., Korboulewsky, N., Ormeño, E., Fernandez, C., 2005. Compost effect on bacterial and fungal colonization of kermes oak leaf litter in a terrestrial. Mediterranean ecosystem Applied Soil Ecology 30, 79–89.

Laudicina, V.A., Badalucco, L., Palazzolo, E., 2011. Effects of compost input and tillage intensity on soil microbial biomass and activity under Mediterranean conditions. Biology and Fertility of Soils 47, 63–70.

Lu, X., and Cheng, G., 2009. Climate change effects on soil carbon dynamics and greenhouse gas emissions in *Abies fabri* forest of subalpine, southwest China. Soil Biology and Biochemistry 41, 1015-1021.

Millennium Ecosystem Assessment, 2005. Ecosystems and Human Well-being: Synthesis. Island Press, Washington, DC.

Mustin M., 1987. Le compostage, gestion de la matière organique. Ed Dubusc: 954 P.

Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. European Journal of Soil Science 54, 655–670.

Nortcliff, S., 2002. Standardisation of soil quality attributes. Agriculture, Ecosystems and Environment 88, 161–168.

Paul, E.A. and Clark, F.E., 1996. Soil Microbiology and Biochemistry. Academic Press, San Diego, pp. 245–264.

Pietikaïnen, J., Pettersson, M., Baath, E., 2005. Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. FEMS Microbiology Ecology 52, 49–58.

Ros, M., Hernandez, M.T., Garcia, C., 2003. Soil microbial activity after restoration of a semiarid soil by organic amendments. Soil Biology and Biochemistry 35, 463-469.

Ros, M., Klammer, S., Knapp, B., Aichberger, K., Insam, H., 2006a. Long-term effects of compost amendment of soil on functional and structural diversity and microbial activity. Soil Use and Management 22, 209–218.

Ros, M., Pascual, J.A., Garcia, C., Hernandeza, M.T., Insam, H. 2006b. Hydrolase activities, microbial biomass and bacterial community in a soil after long-term amendment with different composts. Soil Biology and Biochemistry 38, 3443–3452.

Ross, D.J., and Sparling, G.P., 1993. Comparisions of methods to estimate microbial C and N in litter and soil under Pinus radiate on a coastal sand. Soil Biology and Biochemistry 25, 1591–1599.

Saetre, P., and Stark, J.M., 2005. Microbial dynamics and carbon and nitrogen cycling following re-wetting of soils beneath two semi-arid plant species. Oecologia 142, 247–260.

Saison, C., Degrange, V., Olivier, R., Millard, P., Commeaux, C., Montange, D., Le Roux, X., 2006. Alteration and resilience of the soil microbial community folllowing compost amendment: effects of compost level and compost-borne microbial community. Environmental Microbiology 8, 247-257

Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its

implications for ecosystem function. Ecology 88, 1386-1394.

Seybold, C.A., Herrick, J.E., Brejda, J.J., 1999. Soil resilience: a fundamental component of soil quality. Soil Science 164, 224–234.

Stern, N., 2007. The Economics of Climate Change – The Stern Review. Cambridge: Cambridge University Press. Cambridge. ISBN: 9780521700801

Swift, M.J., Andren, O., Brussaard, L., Briones, M., Couteaux, M.,M., Ekschmitt, K., Kjoller, A., Loiseau, P., Smith, P., 1998. Global change, soil biodiversity, and nitrogen cycling in terrestrial ecosystems: three case studies. Global Change Biology 4, 729-743.

Timonen, S., Finlay, R.D., Olsson, S., Soderstrom, B., 1996. Dynamics of phosphorous translocation in intact ectomycorrhizal systems: non-destructive monitoring using a B-scanner. FEMS Microbiology Ecology 19, 171-180.

Wallenstein, D.M., McMahon, K.S., Schimel, P.J., 2009. Seasonal variation in enzyme activities and temperature sensitivities in Arctic tundra soils. Global Change Biology 15, 1631–1639.

Yuste, J.C., Baldocchi, D.D., Gershenson, A., Goldstein, A., Misson, L., Wong, S., 2007. Microbial soil respiration and its dependency on carbon inputs, soil temperature and moisture. Global Change Biology 13, 2018-2035.

# CHAPITRE I

# SYNTHESE BIBLIOGRAPHIQUE

Ce chapitre est constitué de deux synthèses bibliographiques. La première synthèse porte sur les conséquences des évènements extrêmes de température et de sécheresse sur les communautés microbiennes du sol (Article1). La deuxième synthèse porte sur les impacts d'apports de matières organiques exogènes, plus spécifiquement des composts, sur les propriétés du sol.

# Partie I.1.

# Conséquences des évènements extrêmes de température et de sécheresse sur les communautés microbiennes du sol (Article 1)

Cette partie est rédigée sous forme d'une revue en préparation intitulée : **"Consequences of extreme events -drought and high-temperature- on soil microbial communities: A review".**

# Consequences of extreme events -drought and high-temperature- on soil microbial communities: A review

Meriem Ben Sassi[a,b], Pierre Renault[a,b], Annette Bérard[a,b]*

[a] INRA, UMR1114 EMMAH, F-84914 Avignon, France

[b] UAPV, UMR1114 EMMAH, F-84914 Avignon, France

* Corresponding author: Annette Bérard *UMR 1114 INRA-UAPV Environnement Méditerranéen et Modélisation des Agro-Hydrosystèmes, Domaine Saint-Paul, Site Agroparc, 84914 Avignon Cedex 9, France.*

e-mail: annette.berard@paca.inra.fr

## Abstract

In the next decades, many soils will undergo periods of prolonged drying combined with heat wave events. These abiotic factors, varying in intensities and duration, have direct effects on soil microorganisms such as DNA–strand breaks, protein damage and change in membrane fluidity as well as indirect effects by modifying physico-chemical properties of soil and the plant rhizodeposition. Soil microorganisms can tolerate to a certain extent these conditions. They can switch to dormant forms, develop strategies to protect, repair at the cellular level and at the colony level. However strategies to cope with drying and/ or high temperatures depend on species and may lead to a change in community composition, diversity and activity. The historical and environmental contexts combined with these changes determine the stability of the soil microbial ecosystem.

**Key words:** Drought, High temperature, Microbial communities, Ecosystem stability

## 1. Introduction. Increased drought and global warming in the climate change and their effects on soil microbial communities

The Earth's climate is changing and its global warming is unequivocal (IPCC, 2007). But more and more observations suggest that mean trends in climate change would be also probably attendant with an increase in the frequency and intensity of extreme climate events such as rainfall, drought and high temperature (IPCC, 2007). This is especially true within the current Mediterranean context (Gibelin and Déqué, 2003) where changes in mean global air temperature and in spatial and temporal patterns of precipitation predicted include shifts in the frequency and intensity of drought and heat-waves events with longer drought periods and more extremely dry years (IPCC, 2007; Planton et al., 2008). These climatic changes would contribute to changes in farm and water management strategies dealing with the geographic distribution of crops (Reidsma et al., 2009) and varieties (Pray et al., 2011), and with agricultural practices like irrigation with conventional water and/or wastewaters (Rojas-Valencia et al., 2011; Zou et al., 2012), tillage (Lal, 2004), fertilization and use of organic wastes for soil amendment (Borken et al., 2002a; Hueso et al., 2011).

Climate change may affect the biodiversity and the function of terrestrial ecosystems (Millennium, 2005). The last 30 years of warmer temperatures at the end of the twentieth century have affected the phenology of organisms, the range and distribution of species, and the composition and dynamics of communities (Walther et al., 2002). Thus, Geyer et al. (2011) considered any impact of climate change on biological diversity as a stress because such effects represent (negative or positive) changes in key ecological attributes of an ecosystem or parts of it. Moreover, extreme climatic events may be more important drivers of ecosystem function than mean conditions (Heisler and Weltzin, 2006).

Soil is a complex and dynamic biological system at the interface between the atmosphere, lithosphere and hydrosphere and biosphere. It has various functions in ecosystems (Nannipieri and Eldor, 2009), and is a vital living system which governs plant and animal productivity and health (Nannipieri et al., 2003). Soil is involved in major nutrients cycles. It is often used as a bioreactor to digest and reclaim crop residues and other wastes (Rojas-Valencia et al., 2011; Abera et al., 2012), the chemical nature of its mineral fraction contributing to the stabilization of organic materials in soils (Baldock and Skjemstad, 2000). Moreover, soil is also a fundamental component of continental water cycle and participates in climate regulation by the production and/or consumption of $CO_2$, $N_2O$ and $CH_4$ greenhouse

gases, and several recent works have dealt with C sequestration (Lu and Cheng, 2009). Thus, soil behaviour results from numerous interactions between its microflora, the abiotic geochemical reactivity, and transfers of water, solutes, solids, gases, and heat.

Soil microorganisms can transform and mineralize a wide range of organic compounds and contribute in that way to organic matter and nutrient cycles, with mineralizations and immobilizations of various nutrients (Swift et al., 1998; Trevors, 1998; Saetre and Stark, 2005). Soil bacteria and fungi contribute also to plant nutrition by enhancing nutrient uptake and translocation (Timonen et al., 1996). Especially, into the rhizosphere environment, microorganisms (e.g. mycorrhizal and rhizobial communities), may produce exopolysaccharides (EPS) that contribute to significant increases in soil aggregation, which in turn affect soil moisture retention properties, reducing the impact of water deficit on plants (Hinsinger et al., 2009). Moreover rhizosphere microorganisms like Mycorrhizal fungi, can increase plant water uptake from the far surrounding soils by their mycelial network (Allen, 2007).

Soil microbial composition is generally sensitive to disturbances. Most authors have illustrated that abiotic factors such as temperature, moisture have direct effects on soil microbial community composition, diversity and activity (Ge et al., 2010; Darby et al., 2011; Prevost-Boure et al., 2011) as well as indirect effects on physical or physico-chemical properties of soil (Fierer and Schimel, 2002; Parker and Schimel, 2011). Moreover, spatio-temporal patterns of microbial diversity, as well as life history of the groups being investigated, affect the response of communities to disturbances (Bissett et al., 2010). Thus, microbial communities might be resistant and/or resilient or not. Many microorganisms have a variety of evolutionary adaptations and a high degree of physiological flexibility that allow them to survive and remain active in the face of disturbances (Schimel, et al., 2007; Allison and Martiny, 2008). Microbial communities could not change in the face of changing environmental conditions and could quickly recover to their initial composition.

At present, there is no synthesis of recent knowledge on the effects of extreme global change factors such as temperature change and/or moisture's soil change on prokaryotes and fungi, although reviews on temperature stress and or osmotic stress have been undertaken for prokaryotes (Billi and Potts, 2002) or a specified prokaryote group (Potts, 1999) and in other disciplines such as microbiological safety of food (Humpf and Voss, 2004) treated similar effects. Moreover, it is of interest to review indirect effects of these extreme events (e.g. physical and physicochemical soil parameters and interactions with plants) and to know

whether the environmental context and the life history might affect prokaryotes and fungi under severe drought and/ or heat-waves.

In this review, we will successively deal with (i) direct effects of severe drought and / or heat waves on soil microflora, (ii) indirect effects of severe drought and / or heat waves on soil microflora via the physical and physicochemical soil parameters directly impacted and interactions with plants, (iii) the strategies developed by microorganisms to withstand these events, (iv) the consequences of these stress factors on the soil microbial community, and on the soil microbial ecosystem stability.

## 2. Direct effects of drought and/or high temperature on microorganisms

### 2.1. Observations on quantities variations

Studies have shown that drying generally causes a decrease in total microbial biomass (Sparling et al., 1986; Van Gestel et al., 1993), mycelium length (West et al., 1987) and bacteria number (West et al., 1987; Rokitko et al. 2003). For example, in an out-door experimental study performed in a Denmark heathland ecosystem, Jensen et al. (2003) found that the carbon microbial biomass was reduced by 39% after almost 2 months under drought treatment. High temperatures may also lead to a rapid decrease of the soil microbial biomass (Joergensen et al., 1990). Combined desiccation and high temperature lead to the destruction of biomass and Bottner (1985) reported that air-drying of a soil at 40 °C in the laboratory destroyed up to one-third of the biomass as determined by the chloroform fumigation-incubation method. Bérard et al. (2011) reported that heating dry soil at 50 °C had a stronger effect than only drying on microbial biomass as measured by total ester-linked fatty acid methyl esters (EL-FAMEs) biomarkers.

### 2.2. Mechanisms underlying the general effects of desiccation or high-temperature

Protein damage and gene inactivation or down-regulation are part of any stress response. In that context, many proteins lose their native, functional configuration and tend to aggregate. The process may be reversible up to a degree, beyond which it becomes irreversible and generalized within the cell, which ultimately dies (Macario el al., 1999).

### 2.2.1. Mechanisms underlying the effects of desiccation

The removal of water through air drying damages membranes, proteins and nucleic acids which is lethal to the majority of organisms (Billi and Potts, 2002). The removal of the hydration shell from phospholipids of membrane bilayers increases the van der Waal's interactions between adjacent lipids, causing an increase in the phase transition temperature (Tm) of membranes and their transition to the gel phase at environmentally relevant temperatures (Billi and Potts, 2002). Membranes with a higher Tm will pass to the gel phase first and separate from those with lower Tm, which results in the aggregation of proteins (Potts, 1999; Billi and Potts, 2002). Moreover, drying cells induces conformational changes of proteins and causes enzymes efficiency restriction. It is expected that the accumulation of free radicals during drying leads to protein denaturation and lipid peroxidation and ultimately to cell lysis (Potts, 1994).

Drying induces also damage of nucleic acids (Asada et al., 1979; Dose et al., 1991). Damage to DNA may arise through chemical modification (alkylation or oxidation), cross-linking or base removal such as depurination (Potts, 1994, 1999). The increase in DNA-strand breaks during long-term exposure of microorganisms to extreme dryness may be a general phenomenon (Dose et al., 1991; Mattimore and Battista, 1996). This was especially shown for spores of *Bacillus subtilis* and for the vegetative bacterium *Deinococcus radiodurans* during long-term exposure (up to 12 weeks) to extreme dryness (Dose et al., 1991).

In consequence to changes in physiology and behavior of cells, there are changes at population and community levels. At the level of the bacterial community, air drying may lead to a change in the surface area of a community, its shrinkage, a change in texture, precipitation of salts, and color changes as pigments are oxidized (Potts, 1994).

### 2.2.2. Mechanisms underlying the effects of high-temperatures

It is well known that temperature affects the membrane composition of microorganisms. For example, the phospholipid fatty acid composition changes with temperature (Petersen and Klug, 1994; Russell et al., 1995). Thus membrane structure is modified phenotypically in response to changes in temperature.

Molecular chaperones, including the heat-shock proteins (Hsps) respond to a variety of stresses, including extremes of temperature (Parsell and Lindquist, 1993; Hecker et al., 1996; Feder and Hofmann, 1999). Hsps recognize and bind to other proteins when these are in non-

native conformations, whether due to protein-denaturing stress or because the peptides they comprise have not yet been fully synthesized, folded, assembled, or localized to an appropriate cellular compartment (Feder and Hofmann, 1999).

In consequence, the response of the soil microbial community will change if the temperature is changed. Thus, the microbial groups fungi and bacteria differ in their sensitivity to temperature (Bollen, 1969).

### 2.3. Combined effect of stresses

In reality soil systems are often subjected to combined abiotic stresses. Especially, drought and heat stress represent an excellent example of two different abiotic stress conditions that occur in the field simultaneously (Mittler, 2006). As gene expression profiles induced by different stress factors involves both overlap (the common environmental stress response), synergism (combined exposure turning on genes not induced by single exposures) and antagonism (one stress factor repressing genes induced by another factor) (Roelofs et al., 2007), the simultaneously occurrence of drought and heat stress may induce a new type of response that would not have been induced by each of stresses applied individually. In analogy with the response that would have prokaryotic cells, plant studies of Mittler (2006) suggest that the response to a combination of two different abiotic stresses is unique and cannot be directly extrapolated from the response to each of the different stresses applied individually. Interestingly, under the combination of drought and heat shock, Sharma and Kaur (2009) observed a specific induction of a protein in wheat seedlings, which is not activated when each of these stresses was applied individually. Thus, the combination of drought and heat may induce a different kind of stress to cells compared to drought alone (Roelofs et al., 2007).

There are similitudes among stressors. Thus, a broad phylogenetic spectrum of microorganisms that are adapted to desiccation can also be adapted to radiation stress, both stresses having deleterious effect on the cells, in particular they cause the same DNA lesion (Mattimore and Battista, 1996; Chanal et al., 2006). As desiccation resistance appears to require extensive DNA repair as ionizing radiation, there is a correlation between the resistance of bacterial cells to dehydration and ionizing radiations. This correlation has been demonstrated for *Deinococcus radiodurans* (Mattimore and Battista, 1996; Rokitko et al., 2003), *Methylobacterium extorquens, M. mesophilicum, Bacillus subtilis* and *B. cereus* (Rokitko et al., 2004). Desiccation helps also to maintain survival after thermal shock. Cells

of *Bacillus amyloliquefaciens* previously adapted to low water activity showed better survival to heat shock (Sartori et al., 2010). In consequence, a subsequent stress like drought may induce cell cross-protection to another stress like heat on soil microbes.

## 3. Indirect effects on microorganisms: physicochemical and biogeochemical changes

### 3.1. Wettability and accessibility to organic matter and gas diffusion

Water availability can regulate soil substrate availability, diffusion of gases, soil pH and temperature and then influence soil microbial cell physiology. As soil dries, pores within solid matrices drain and water films coating surfaces become thinner (Stark and Firestone, 1995). This reduction in conducting liquid pathways reduces liquid and nutrient transport and diffusion rates of solutes (Or et al., 2007). Thus, desiccation stress enhanced starved conditions (Lundquist et al., 1999; Bär et al., 2002). Decreasing the water content of soil alters nutrient availability (Jensen et al., 2003; Sardans and Penuelas, 2007) restricting therefore their diffusion to microorganisms and inducing a slower nutrient turn-over (Sardans and Penuelas, 2004). Increasing temperature affects also substrate availability and induced depletion of labile organic matter (Dalias et al., 2001). These changes in substrate availability could be responsible for the modification of the soil microbial activities and composition.

Soil water content affects soil gas diffusivity and soil–atmosphere gas influx and efflux. The involvement of soil moisture in controlling fluxes of important greenhouse gases has been shown for methane (Gulledge and Schimel, 1998; Schnell and King, 1996), nitrous oxide (Bollmann and Conrad, 1998), and carbon dioxide (Mielnick and Dugas, 2000; Ouyang and Zheng, 2000; Craine et al., 2001; Frank et al., 2002). However, gaseous movement is described by: (a) diffusion; (b) dissolution of gases in the soil water; (c) physical adsorption of gases by the surfaces of soil particles; (d) replacement of soil air by water when the soil is wetting and replacement of soil water by air when the soil is drying; and (e) production of gas by the respiration activities of plant roots and soil microorganisms (Ouyang and Zheng, 2000). For the last point, dry conditions impose physiological water stress to microorganisms and then limited atmospheric $CH_4$ consumption and $CO_2$ production (Schnell et al 1996; Gulledge and Schimel., 1998). However, increasing soil temperature is expected to shift gas composition towards lower concentrations of methane and oxygen, but higher concentrations of carbon dioxide (Gebert et al., 2011). Indeed, drought or high temperature had an influence

on gas movement which in turn affects the microbial aerobic respiration and then the gas composition in soil.

Low water content, particularly in combination with high temperature can increase soil water repellency (Goebel et al., 2011). This increase has been found to reduce water film thickness on particle surfaces. Thus, the water connectivity on individual particles as well as between the particles can be markedly reduced, which can change diffusional capacity and the pathways affecting gaseous and nutrient fluxes (Or et al., 2007). The increasing of soil water repellency is accompanied by increasing soil air content and water-gas interfacial area resulting in improved diffusivity and gas permeability (Or et al., 2007; Gebert et al., 2011). Therefore, soil aeration is improved during the warmer season, providing improved conditions for diffusive oxygen (Gebert et al., 2011). Even, this improved diffusion of oxygen, the soil water repellency induced reduction in water availability and may reduce microbial decomposition processes (Goebel et al., 2011).

### 3.2. Redox processes with soil drying

Makino et al. (1997) reported that changes in the chemical forms of Mn, Co and Zn subjected to air-drying differed according to element. Air-drying of soils under laboratory conditions increased exchangeable forms of Mn, Co and Zn, while it decreases Cu. A similar tendency was observed under field experiments conducted on two paddy soils collected at four different depths: After sunny days, exchangeable Mn and exchangeable Co contents in the surface soil layer markedly increased (Makino et al., 2000). Drought increased also soil Cd, As and Al concentrations (Sardans and Penuelas, 2007b). So, under drier conditions trace element accumulation can increase in soils. Particularly, the Mn is probably released by reduction through disruption and partial oxidation of soil organic matter with drying (Bartlett and James, 1980). Thus, drying and the length of drying increased Mn solubilility and exchangeability (Ross et al., 2001). As a co-factor, adequate supply of reduced Mn (II) is required for a large number of cellular functions (Tebo et al., 2005; Krishnan et al., 2007). It was also observed that added manganese resulted in significantly higher cell densities (Watanabe et al., 2012). Therefore, drying could have positive impact on microorganisms and enhanced their activities and growth. Whereas, increasing the mobilization of the trace elements (e.g. Mn, Zn, Cu) may lead also to increase their potential of ecotoxicological stress and may have a negative impact on microorganisms. The microbial growth, survival and

activity decreased with the level of metal concentration bioavailable (Kunito et al., 1999; Rajapaksha et al., 2004).

### 3.3. Interactions with plants

There is evidence for correlation between aboveground and belowground diversity (Hooper et al., 2000). Plant community composition would affect soil chemical and microbial community properties (Orwin and Wardle, 2005) which in turn affect C and N cycles (Sanaullah et al., 2011). Different plants may produce different exudates. Thus, the variation in carbon compounds exuded by the plants may modify the diversity of microorganisms (Grayston et al., 1998). As moisture deficit can stress plants, rhizodeposition (i.e. the quantity and composition of root exudates) and nutrient allocation below ground would change (Lynch and Whipps, 1990; Henry et al., 2007). It has been shown that drought stress increased the quantities of organic acid produced and that malic acid was the predominant organic acid exuded (Henry et al., 2007). The carbon compounds exuded by plant may then exert strong selective pressures on the soil microbial community (Grayston et al., 1998) and, as a result microbial communities may be affected (Lynch and Whipps, 1990). For example, Sanaullah et al. (2011) reported a significant increase of microbial biomass carbon under drought stress compared to optimum conditions, under plant mixture (two grasses: Lolium perenne and Festuca arundinacea and one legume: Medicago sativa) and indicated that plants increased rhizodeposition to facilitate soil water and nutrient transport. However, there are still controversy about these phenomena and their consequences, for example Mahieu et al. (2009) reported that water stress caused a significant reduction of N rhizodeposition by a pea (*Pisum sativum* L.).

## 4. How do microorganisms cope to stress?

To cope with stress, microorganisms involve mechanisms which span from individual level to colony level.

### 4.1. Dormancy and cellular microbial survival structure

A severe stress may cause dormancy of some microbes into (Suzina et al., 2004). Switching to dormant forms is one strategy to survive in arid conditions, as it may be illustrated by the spore-forming Bacilli (Dose et al., 2001), the akinetes developed by

cyanobacteria (Billi and Potts, 2002), the conidia developed by fungi (Dose et al., 2001) and the formation of vegetative survival states (Potts, 1994; Breeuwer et al., 2003). Saetre and Stark (2005) suggest that the increase in bacterial growth after rewetting was largely due to dormant bacteria becoming active after rewetting. Moreover upon drying, anhydrobiotes, which are organisms that tolerate complete dehydration (Mertens et al. 2008), enter metabolic dormancy and they come back to an active metabolism when water becomes available (Billi and Potts, 2002). Whereas, until now, relatively few genera of bacteria were recognized as anhydrobiotic, many of which are Cyanobacteria (Billi and Potts, 2002).

### 4.2. Strategies to protect, repair, acclimate at the cellular level

Microorganisms have a variety of physiological acclimation mechanisms that allow them to survive and remain active in the face of environmental stress (Schimel et al., 2007). The mechanisms of stress tolerance reflect both simple and complex interactions at the structural, physiological and molecular levels (Potts, 1999). For survival to stress conditions the organisms have not only to protect their hydrophobic structures and organelles but also to exhibit a pronounced capability to repair DNA lesions (Dose et al., 1991).

As mentioned above, high temperature and desiccation induced a direct phenotypically change in microbial membranes composition. In particular the composition of the fatty acid component of lipids is modified (Kieft et al., 1994; Russell et al., 1995). These changes in membrane fatty acids may be partially attributed to rapid physiological adjustments of cells (Mrozik et al., 2004). These changes in lipids are necessary to maintain physicochemical properties such as a particular state of fluidity in the membrane bilayer (Russell, 2002) and allow microbes to cope with stress.

As also mentioned above, microorganisms develop genes and proteins to respond directly to stress conditions. These genes and proteins allow coping with stress. Microorganisms synthesize a small set of proteins called the heat-shock proteins (Hsps) (Parsell and Lindquist, 1993; Hecker et al., 1996; Feder and Hofmann, 1999). And then, these heat shock regulation systems contribute to degrade or reactivate damaged proteins (Parsell and Lindquist, 1993; Hecker et al., 1996). Hsps include two important groups, namely molecular chaperones and ATP-dependent proteases. The intra-cellular chaperones prevent misfolding and unwanted protein aggregation and when stress is released, they renature the proteins and renew their biological activity (Hecker et al., 1996; Mahmood et al., 2010). Whereas, the ATP-dependent proteases are involved in the degradation of denatured proteins

(Hecker et al., 1996). Hsp expression can be correlated with resistance to stress, and species' thresholds for Hsp expression are correlated with levels of stress that they naturally undergo e.g. thermophilic species have a higher Hsp induction temperature threshold than psychrophilic ones (Feder and Hofmann, 1999). Moreover, some bacterial taxa could store high quantity of ribosomes under dried conditions allowing them to respond with rapid protein synthesis when the stress is released (Placella et al., 2012).

To reduce water cellular potential and to prevent damages to membranes and proteins during soil drying, microorganisms redirect energy and nutrients from resource acquisition and growth into survival, such as synthesizing and accumulating intracellular osmolytes (Billi and Potts, 2002; Dose et al., 2001; Schimel et al., 2007). Fungi use polyols such as glycerol, erythritol, and mannitol (Witteveen and Visser, 1995). However, bacteria protect themselves to increasing osmolarity by the rapid intracellular accumulation of ions, mainly K+, followed by the accumulation of compatible solutes such as proline, glycine betaine and trehalose (Kempf and Bremer 1998). Moreover, most gram-negative bacteria rely on pure acclimation strategies by synthesizing inducible osmolytes. However, Gram-positive bacteria and fungi could rely on a mix of inherent resistance and acclimation by producing of both inducible and constitutive osmolytes (Harris, 1981 cited by Schimel et al., 2007). Indeed, Leslie et al. (1995) showed that sugars prevent the rise of the temperature of the phospholipid phase transition of the membranes when cells are dried. Thus, the accumulation of compatible solutes not only allows the cells to withstand a given osmolality but also expands the ability of microorganisms to colonize ecological niches that are otherwise unfavourable for their proliferation (Kempf and Bremer, 1998) such as dried ones.

### 4.3. Strategies to protect, acclimate at the colony level: EPS production

In order to acclimate to a stress such as drought, microorganisms synthesize extracellular polymeric substances (EPS). Under desiccation conditions, these EPS create a favourable microenvironment (Chenu and Roberson, 1996; Or et al., 2007). Bacteria have been reported to synthesize EPS during dry periods (Roberson and Firestone, 1992). As polysaccharides are hygroscopic, bacteria may then maintain higher water content in the colony microenvironment (Potts, 1994) and therefore increase nutrient availability within the bacterial colony. For example, natural populations of $NH_4^+$ oxidising bacteria survived in air-dried soils for period greater than 3 months, which was correlated with significant amounts of

EPS, and less amounts of EPS production restricted survival of laboratory Nitrosomonas europaea strain to less than 10 weeks of dryness (Allison and Prosser, 1991).

## 5. Bulk effects on soil microbial community

### 5.1. Main factors affecting the stress expression

Intensity and duration of stress (e.g. drying, temperature) are the main factors that affect soil microbial responses. For example, Abera et al. (2012) reported that the cumulative $CO_2$–C fluxes were lower at low soil moisture content. Moreover, Sardans and Penuelas (2005) showed that an average of 21% of reduction in soil moisture had stronger effects on soil enzyme activities than 10% of reduction. Higher temperatures led also to higher rates of change in both the PLFA pattern and the bacterial activity compared with lower temperatures (Pettersson and Bååth, 2003). Concerning stress duration, 2-week droughts in birch litter reduced more strongly microbial biomass and activity than did shorter droughts (Schimel et al., 1999). Bérard et al. (2011) observed also that resilience of microbial parameters was inversely related to duration of disturbance. These researchers didn't observed microbial resilience after a 21 day heat-drought disturbance, however microbial resilience was observed in the 7 day disturbed soils. Thus, it is suggested that above a critical threshold of heat wave duration, soil microbial communities may have undergone a drastic biomass killing and restructuring associated with a shift in physiological traits.

### 5.2. Microbial activity, respiration and mineralization

Changes in soil conditions, especially humidity and temperature, are expected to affect the function of microbial communities (Van Gestel et al., 1993a; Wilkinson et al., 2002; Bell et al., 2009). For example, in an out-door experimental study performed in a Denmark heathland ecosystem, Jensen et al. (2003) found that the microbial activity was reduced by 27% almost 2 months under drought treatment. Soil moisture is also the major factor influencing enzyme activities (Brockett et al., 2012). Drought decreases soil β-glucosidase, acid phosphatase and especially urease and protease activities (enzymes involved in the nitrogen cycle) (Sardans and Peñuelas, 2005).

Rewetting of dry soils caused a short-term increase in microbial activity named the "Birch-effect" process (Birch, 1958; Bloem et al., 1992), inducing a flush of carbon and nitrogen mineralization. This respiration pulse can be explained by lysis of dead microbial

cells, release of compatible solutes and exposure of previously protected organic matter (Fierer and Schimel, 2003; Schimel et al., 2007; Borken and Matzner, 2009).

Many studies have shown that under warming scenarios the soil respiration rate increases with increasing temperature (Rustad et al., 2001). Pietikaïnen et al. (2005) conducted their experiments on two soils, an agricultural soil and a forest humus soil, and reported that the respiration rate increased over almost the whole temperature range (0-45°C), showing the highest value at around 45°C. Thus, at temperatures above 30°C there was an uncoupling between the instantaneous respiration rate and bacterial and fungal growth, since the former increased at higher temperatures, while the latter decreased (Pietikaïnen et al., 2005). Moreover, Hamdi et al. (2011) reported that at high temperatures (40 °C and 50 °C), the respiration of a Mediterranean soil decreased with increasing pre-incubation temperature and that the lower glucose-induced respiration of soils pre-incubated at 50°C may suggest a lower microbial biomass after this high temperature pre-incubation. Thus, high pre-incubation temperature may have killed part of the microbial biomass and modified microbial communities. In addition, Dalias et al. (2001) illustrated that organic material produced at higher temperatures was more recalcitrant than at lower temperatures. As the recalcitrant pool decomposes very slowly, this would disadvantage the microbial activities.

### 5.3. Variability between species and selection process

Drought treatment enhances the fungal dominance of the microbial community (Ross and Sparling, 1993; Joergensen et al., 1995) and it is often stated that fungi are more adapted to low soil moisture conditions than bacteria (Beare et al., 1992; Jensen et al. 2003). Moreover inside the bacteria group, soil drying leads to a decrease in the relative number of gram-negative bacteria ($G^-$), and to increase in the relative number of gram-positive bacteria ($G^+$) (Rokitko et al., 2003). For instance, the $G^+$ Actinomycetes group is known to be tolerant to desiccation (Leblanc et al., 2008; Uhlirova et al. 2005), but may be sensitive to osmotic up shock after rewetting (Williams 2007) and to high temperatures (Klamer and Bååth, 1998). The $G^+$ bacteria and fungi are more adapted to changes in the water potential because of their thicker and more rigid cell walls and constitutive compatible solutes that enhance osmoregulatory capabilities (Schimel et al., 1989; Kempf and Bremer, 1998; Uhlirova et al., 2005; Hamer et al., 2007). Gram-positive bacteria, as spore formers, were also reported by Potts (1994) to be more tolerant to drier conditions than $G^-$ bacteria. In sum, the general morphology and life history strategies of microbial groups suggests that soil drought should

select against $G^-$ bacteria and for $G^+$ bacteria and fungi (Nazih et al., 2001; Uhlirova et al., 2005; Schimel et al., 2007). The effects of dryness depend also on the strain. Dose et al. (1991) reported that B. subtilis-spores of strain TKJ 6312 are e.g. about 20% less resistant to long term vacuum exposure (30 to 40d) than spores of TKJ 6324, TKJ 3412 of HA 101. They also reported that D. radiodurans strains R1 and R2s are about 20% less inactivated than strain UVS78. After 6 weeks of desiccation, forty-one strains of Deinococcus radiodurans exhibited a time-dependent increase in DNA damage, measured by an increase in DNA double-strand breaks (Mattimore and Battista, 1996). These differences in resistance to dryness may in part be related to differences in the capability to repair DNA (Dose et al., 1991).The drying of soil samples reduced also the abundance of predominant species and the diversity of bacteria isolated from these samples, making easier the isolation of rare bacterial species. Thus, some bacterial species that were minor before soil drying became dominant in dried soil samples (Rokikto et al., 2003). For example, some species decreased of only about one order of magnitude (e.g. the sporeformer *Bacillus cereus*) and were thereafter relatively more abundant, whereas other ones greatly decreased and were not more detected in dried soil (e.g. G- bacteria *Pseudomonas sp.* and *Enterobacter sp*.) (Rokitko et al., 2003). Drought stress thus induces a reduction of bacterial diversity Rokitko et al. (2003).

Many organisms responsible for litter and organic matter breakdown are found in the drought tolerant $G^+$ bacteria and fungi. Whereas many of the organisms carrying out "specialized" or "narrow" functions such as nitrifiers, sulphur oxidisers and $CH_4$ oxidisers are $G^-$ bacteria and may be more drought sensitive (Schimel, 1995 cited by Schimel et al., 2007). For example, methanogens (e.g. *Methanosarcina barkeri)* are obviously able to survive short $O_2$ exposure periods (<30 h). By contrast, rapid desiccation (30 minutes) in anoxic or oxic conditions greatly impact their viability (Fetzer et al., 1993). This taxonomic restructuration by drying stress in depends of specialized groups, could then induce shifts in functional diversity (see part 6, Chowdhury et al., 2011a). Thus, environmental stress tolerance varies widely depending on the species (genome) and on cell type and differentiation state (proteome) (Kültz, 2005).

It seems also that drying alter the relative abundance of bacterial taxa. Indeed, *Acidobacteria* tend to be favoured in oligotrophic soils with lower carbon availability (Fierer et al., 2007) and then were most predominant within the dry treatment soils (Castro et al., 2010). However, Gram-negative bacteria like *Proteobacteria*, which are r-strategists (or “copiotrophic, Fierer et al., 2007) have the ability to grow fast. This biological trait may

favour them in the context of rewetting after a severe drought. For example, Thompson et al. (2010) observed high $CO_2$-C efflux correlated with the development of *Proteobacteria* in soils previously dried and rewetted. However, the speed of response after wet-up of *Proteobacteria* is class level dependent (Placella et al., 2012). Indeed, Placella et al. (2012) showed that there are three-step resuscitation strategies of the indigenous microbial community after wet-up (linked to the relative ribosomal abundance under dried conditions) and that most bacteria could be classified as rapid responders (within 1 h), intermediate responders (between 3 and 24 h), or delayed responders (24–72 h). Therefore, within the *Proteobacteria* they classified *Deltaproteobacteria* and *Epsilonproteobacteria* as rapid responders and *Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria* as delayed responders. However other bacteria phyla, *Actinobacteria* and *Verrucomicrobia* which had relatively high ribosomes per cell at pre-remoistening stage were generally rapid responders and *Firmicutes* were classified as intermediate responders. All these biological traits (e.g. resuscitation strategies, "trophic category") could have consequences on the "recovery trajectories" of microbial communities after an extreme event (see part 6).

At higher temperatures, the decrease of growth rates is more drastic for fungi than for bacteria resulting in an increase in the ratio of bacterial to fungal growth rate (Pietikaïnen et al., 2005). Moreover, with increasing temperatures from 60 °C to 80 °C the survival of fungi decreases much faster than of bacteria (Bollen, 1969). Even, fungi proved to be the most sensitive group to heat treatment, the heat tolerance of the species inside this group showed that many species of the saprophytic fungal flora were more heat-tolerant than those of pathogenic fungi and that *Gilmaniella humicola* was the most heat tolerant fungus found, and some Ascomycetes *(Byssochlamys nivea, Carpenteles brefeldianum* and *Sartorya fumigata)* and few Hyphomycetes *(Gilmaniella humicola* and *Trichocladium piriformis)* were only found after treatment of the soil at 55 °C or more (Bollen, 1969). Thus the heat tolerance is probably species-specific. Recently, the FungiResp method was developed and applied to heated soils to give further data on the catabolic structure of microbial and fungal communities. This study showed that the catabolic structure of fungi in the microbial community seems to be heavily impacted by the heat disturbances (Ben Sassi et al., 2012). An original in situ study that examined soil microbial community structure soon after a sudden temperature shift resulting from changes in the underlying geothermal activity (Yellowstone National Park), reported that genetic fingerprint measurements from heated soils appeared less complex than those from the unaffected soils and that thermopiles or thermotolerant

species were probably distributed throughout the soil temperature gradient (Norris et al., 2002). The authors suggest that these thermotolerant species are subsisting at low levels or perhaps as spores in low-temperature environments, “awaiting” favourable conditions (high temperature) for growth and that the localized thermal activity selected for them. As for drought stress, heat is a component of natural selection in soil ecosystems.

## 6. What are the consequences of drought and/or high temperature disturbance on the soil microbial ecosystem stability?

In order to describe the impact of stress and disturbance events on ecosystems stability, Pimm (1984) developed the concepts of resistance and resilience. Resistance is defined as the magnitude of change after a system is disturbed. Resilience definition could be related to engineering or ecological resilience (Griffiths and Philippot, 2012). Engineering resilience indicates the capacity of a system to recover after disturbance (Pimm, 1984; Seybold et al., 1999). While ecological resilience considers how much disturbance is required to move the system from one stable state to another alternate stable state, using the “ball and cup” model (Griffiths and Philippot, 2012). Resistance and resilience of a variety of soil properties and processes are emerging as key components of soil quality (Griffiths et al, 2005). Soils with the highest biodiversity seem to be more resistant and resilient to experimental perturbations (Griffiths et al., 2000). Indeed, different microbial parameters may display various degrees of resistance and resilience (Seybold et al., 1999). Pesaro et al. (2004) found that while respiration rates recover quickly following drought, biomass and certain specific groups remain depressed for at least one month. Thus, even if the biodiversity of a soil microbial community was reduced the functions were generally maintained implying that there was a high degree of functional redundancy in the original soil community (Griffiths et al., 2000; Griffiths et al., 2001). However, this functional stability was related to specific components of the microbial community rather than biodiversity per se (Griffiths et al., 2004) and as we said taxonomic restructuration by physical stress in depends of groups of organisms carrying out "specialized" functions or “specialized” niches, may induce shifts in functional diversity (Chowdhury et al., 2011a). For example, in finer-textured soils, the fraction of microbes located in small pores may be exposed to less severe water stress than those in larger pores and can, therefore, recover more rapidly after rewetting (Chowdhury et al., 2011b).

Resistance and resilience could depend on the historic of habitats (Zelles et al., 1991; Feder and Hofmann, 1999). The moisture regime to which a soil is naturally exposed may be responsible for the resistance of microbial communities to drying and rewetting stress (Zelles et al., 1991). Moreover, stress-hardening (increased tolerance of a stress after preconditioning at low doses of that stress) and cross-tolerance (increased tolerance of one stress after preconditioning by another) may happen (Kültz, 2005). Indeed at the community level, the resistance and resilience to additional stressors may depend on species tolerances being positively correlated, as named by Vinebrooke et al. (2004) the "positive species cotolerance". Initial exposure to a stressor combined with positive species co-tolerance should reduce the impacts of other stressors, which Vinebrooke et al. (2004) term "stress-induced community tolerance". This concept was largely developed in the case of chemical stressor (Pollution-Induced community tolerance) in water (e.g. Seguin et al., 2002; Tlili et al., 2011) and in soil (e.g. Bérard et al., 2004; Brandt et al., 2009). It has been studied in a lesser extend with physical stressors like pH (Pettersson and Bååth, 2004) and temperature (Ranneklev and Bååth, 2001). In the context of soil microbial communities regularly submitted to drought, precedent studies have shown a lower impact of additional drought stress and suggested that it was because of a pre-selection by drying–rewetting stresses (Fierer et al., 2003; Wiliams, 2007). Bérard et al. (2012) suggested also that the physiological stress imposed by a severe drought of 75 days may have induced a community tolerance to the subsequent heat–drought disturbance of 21 days.

Recovery depends not only on the nature of the perturbation, but also on the soil characteristics and the nutrient availability. Indeed in another stress context (chemical), samples from soil amended experimentally with heavy metal contaminated sewage sludge showed that the physical and biological resilience to subsequent perturbation were affected by the previous land use management and potentially coupled through the microbial community (Griffiths et al., 2005). Thus, land use management affects the soil characteristics. Moreover, recovery depends on soil organic matter content. For example, the total amount of microbial biomass recovered completely to the level before air-drying in a soil with low organic matter content while it recovered partly in soil with high organic matter content (Hamer et al., 2007). By contrast, De Nobili et al. (2006) found that recovery after rewetting of dry soil was more rapid in soil with higher soil organic matter. Thus, recovery is context dependent and it can not be ruled out that recovery is correlated with organic matter and resource availability.

Recovery can be partial or complete and the length of time required for recovery varies (Bérard et al., 2011; Chowdhury et al., 2011b). Indeed, the initial resistance of function to stress was not predictive of recovery of function over time (Kuan et al., 2006). Chowdhury et al. (2011b) reported that even after 54 days at optimal water content recovery of microbial respiration after drying of soil for 14 days, may be not complete. They suggested that drying of soil can have a significant and long-lasting impact on microbial functioning. Bérard et al. (2012) also observed that microbial catabolic activities did not recover to control values 63 days after the end of a severe drought of 75 days followed by a heat–drought disturbance of 21 days. However, following rewetting of dried intact soil monoliths over a 2-month period, Griffiths et al. (2003) observed through culture-based analyses and Biolog measurements a physiological recovery of the microbial population.

Recovery of the biotic capacity of the soil after disturbance can be reached by microorganisms' re-colonization. Bacterial colonization of a spherical remoulded sterilized of 27 mm diameter soil put in contact with the same non-sterile soil (2-4 mm soil aggregates) was monitored from 0 to 14 days in three concentric portions of the sterilized clods (outer, intermediate and inner) and in the surrounding non-sterile soil, by measuring cell numbers and substrate-induced respiration and showed that the sterilized soil clod was colonized rapidly (within 2 days) during incubation time from its outer to its inner portion. Indeed, the modes of soil re-colonization could involve resistant or protected bacteria of the upper soil layers (that could be called ''inner way'' colonization) and/or bacteria of the unperturbed lower soil layers (that could be called ''outer way'' colonization) (Wertz et al., 2007), as previously mentioned the microbes located in small pores less dried impacted compared to larger ones (Chowdhury et al., 2011b). These mechanisms could explain the observations of Norris et al. (2002), concerning the soil microbial community structure following a geothermal heating event in the Yellostone National Park: Growth of thermophiles and thermotolerant species following a geothermal heating of soils in situ may include transport of these species from thermal locations.

The microbial colonization of soil can occur through the transport of bacteria by water flow (passive cell movements), bacterial motility (active cell movements) and bacterial growth (Wertz et al., 2007). The advantages of the motility of the strain in the re-colonization mechanism are more pronounced on a larger scale or when a longer timescale is considered (Turnbull et al., 2001). Indeed, the motile strain of *Pseudomonas fluorescens* SBW25 survived in significantly greater numbers than the non-motile strain after 21 days in soil

(Turnbull et al., 2001). It was shown that *B. cereus* and various soil-isolated *Bacillus* species are able to translocate from their point of inoculation in soil to a distal one by sliding (Vilain et al., 2006). Thus motility and sliding allow bacteria to migrate from unfavorable environment to favorable ones. The capacity of microbes to produce EPS could be in that context an advantage for colonization by creating a favourable microenvironment under desiccation conditions (Or et al., 2007). Moreover copiotrophic microorganisms (as Gram-negative bacteria Proteobacteria) would be advantaged for colonization of soil environments previously disturbed (Fierer et al., 2007).

Interestingly, the re-colonization depends on the physical and chemical characteristics of the soil, for example its pH. Indeed, the short-term re-colonization of soil by fungi and bacteria studied after heat treatments of a humus soil with high carbon content and low pH, and a calcareous soil with lower carbon content and high pH followed by addition of fresh soil as inoculum, showed that the re-colonization by fungi after this heating event was favoured in low pH soils, while the re-colonization at high pH was dominated by bacteria (Barcenas Moreno et al., 2011). Thus, physical and chemical characteristics of the soil after a disturbance selected microbial species or groups which can drive the re-colonization.

## 7. Conclusion. Considering historical and environmental context for understanding drought and heat-wave impacts on soil microbial communities

Many studies illustrated that the composition and the function of the microbial community vary according to land-use type plant species, pollutants, soil structure, nutrients and oxygen availability, temperature, humidity and other environmental variables linked to anthropological, geographical and meteorological contexts (Smolders et al., 2004; Juottonen et al., 2005; Grüter et al., 2006). As we have illustrated above, other studies showed that resistance and resilience to drought and high temperature stresses depend on the community composition, the characteristics of the soil and on the historic of habitats. In consequence, the response of microbial communities to climatic warming and climatic extreme events may differ between soils from different latitudes and from different land soil managements. For example, in the context of the inter-European research projects “Climate-driven changes in the functioning of heath and moorland ecosystems” (CLIMOOR) and “Vulnerability assessment of shrubland ecosystems in Europe under climatic changes” (VULCAN), Jensen et al. (2003) showed that soil microbial carbon biomass was reduced by 39% after almost 2

months of drought treatment at the relatively dry heath at Mols site (Denmark), while this parameter was unaffected at the wetter site heath at Clocaenog site (Wales). In the context of agricultural practices, Makino et al. (1997) reported that the effects of air-drying differed according to soil type and that these effects were more strongly exhibited in paddy soil than in upland soil.

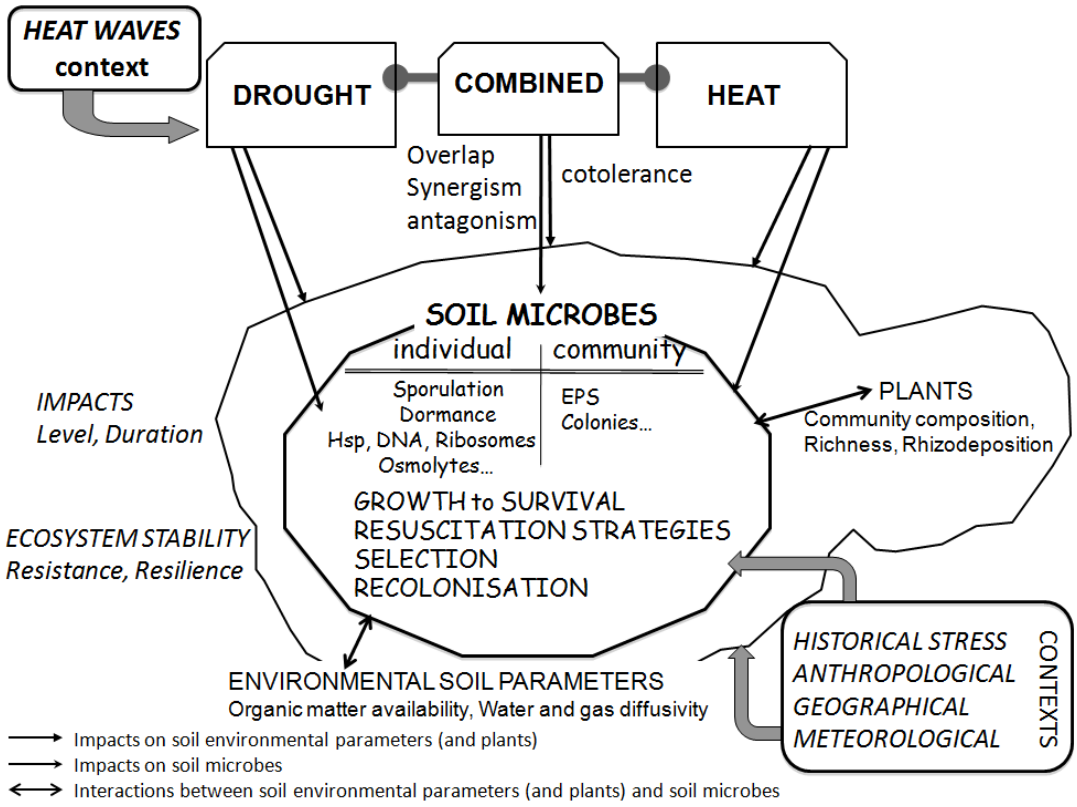


**Fig.1.** Impacts of heat-waves on soil microbial communities in their environmental contexts.

Moreover as we mentioned above, the size, the structure and functioning of the microbial community was strongly affected by its stress history (Schimel et al., 1999). Indeed, the moisture regimen to which a soil is naturally exposed may be responsible for the resistance of microbial communities to drying and rewetting stress (Zelles et al., 1991). In addition, microbial communities in soil with stable environmental conditions may be more sensitive to environmental parameters shifts like temperature than those from more variable soil environment (Waldrop and Firestone, 2006). Moreover, the resistance to stress could be correlated to the level of stress that the soil community naturally undergo. For example, Brockett et al. (2012) showed that potential activities of extra-cellular enzymes (indicating the

functional potential of the soil microbial community) vary with the moisture to which a soil is exposed. Drought decreases soil enzyme activity in a Mediterranean Quercus ilex L. forest depending on season and soil depth (Sardans and Penuelas, 2005).

To conclude, in this review we highlighted that heat waves induce combined abiotic stresses as drought and high temperature occurring in the field simultaneously and involving responses such as overlap, synergism, antagonism and cotolerance for soil microorganisms. Moreover, these stressful factors have direct effects on soil microorganisms such as DNA–strand breaks, protein damage and change in membrane fluidity, but also indirect effects via the environmental soil parameters such as substrate availability, water and gaseous diffusion and biological interactions with plants. In turn, these microbiological effects modified environmental soil parameters and interactions with plants. The level and the duration of these stressful environments mainly affect these impacts. To withstand with these conditions microorganisms involve mechanisms, which span from individual level to colony and community level and induce consequences in ecosystem stability in terms of resistance and resilience. It is also clear that the resistance and resilience of the microbial community to these extreme events like heat waves are strongly affected by the types and levels of stress that they naturally undergo. In addition to stress history, the survival and the selection of soil microbial communities and the soil microbial recolonization depend on the soil characteristics and the nutrient availability strictly connected with anthropological, geographical and meteorological contexts (Fig. 1). Whether we reviewed the impacts of heat waves furthermore particular interest would be taken to develop mathematical models describing the potential impact of heat waves factors on the biomass, the diversity and the functions of the soil microbial community. The incorporation of these microbial parameters into the models used to study future climate changes impacts would allow to adopt policies to avoid the loss of biodiversity and improve the soil quality and thus to achieve sustainable agriculture tools.

## References

Abera, G., Wolde-meskel, E., Bakken, L.R., 2012. Carbon and nitrogen mineralization dynamics in different soils of the tropics amended with legume residues and contrasting soil moisture contents. Biology and Fertility of Soils 48, 51–66.

Allen, M.F., 2007. Mycorrhizal Fungi: Highways for Water and Nutrients in Arid Soils. Vadose zone journal 6, 291-297.

Allison, S.D., and Martiny, J.B.H., 2008. Resistance, resilience, and redundancy in microbial communities. Proceedings of the National Academy of Sciences 105, 11512-11519.

Allison, S.M., and Prosser, J.I., 1991. Survival of ammonia oxidising bacteria in air-dried soil. FEMS Microbiology Letters 79, 65-68.

Asada, S., Takano, M., Shibasaki, I., 1979. Deoxyribonucleic Acid Strand Breaks during drying of Escherichia coli on a hydrophobic filter membrane. Applied and Environmental Microbiology 37, 266-273.

Baldock, J. A., Skjemstad, J. O., 2000. Role of the soil matrix and minerals in protecting natural organic materials against biological attack. Organic Geochemistry 31, 697-710.

Bär, M., von Hardenberg, J., Meron, E., Provenzale, A., 2002. Modelling the survival of bacteria in drylands: the advantage of being dormant. Proceedings of the Royal Society of London 269, 937–942.

Bárcenas-Moreno, G., Rousk, J., Bååth, E., 2011. Fungal and bacterial recolonisation of acid and alkaline forest soils following artificial heat treatments. Soil Biology and Biochemistry 43, 1023-1033.

Beare, M.H., Parmalee, R.W., Hendrix, P.F., Cheng, W., Coleman, D.C., Crossley, D.A., 1992. Microbial and faunal interactions and effects on litter nitrogen and decomposition in agroecosystems. Ecological Monographs 62, 569–591.

Bell, C.W., Acosta-Martinez, V., McIntyre, N.E., Cox, S., Tissue, D.T., Zak, J.C., 2009. Linking Microbial Community Structure and Function to Seasonal Differences in Soil Moisture and Temperature in a Chihuahuan Desert Grassland. Microbial Ecology 58, 827–842.

Ben Sassi, M., Dollinger, J., Renault, P., Tlili, A., Bérard, A., 2012. The FungiResp method: An application of the MicroResp$^{TM}$ method to assess fungi in microbial communities as soil biological indicators. Ecological Indicators 23, 482-490.

Bérard, A., Ben Sassi, M., Renault, P., Gros, R., 2012. Severe drought-induced community tolerance to heat wave. An experimental study on soil microbial processes. Journal of Soils Sediments 12, 513-518.

Bérard, A., Bouchet, T., Sévenier, G., Pablo, A.L., Gros, R., 2011. Resilience of soil microbial communities impacted by severe drought and high temperature in the context of Mediterranean heat waves. European Journal of Soil Biology 47, 333-342.

Bérard, A., Rimet, F., Capowiez, Y., Leboulanger, C., 2004. Procedures for determining the pesticide sensitivity of indigenous soil algae: a possible bioindicator of soil contamination? Archives of Environmental Contamination and Toxicology 46, 24–31.

Billi, D., and Potts, M., 2002. Life and death of dried prokaryotes. Research in Microbiology 153, 7–12.

Birch, H.F., 1958. The effect of soil drying on humus decomposition and nitrogen availability. Plant and Soil 10, 9-31.

Bissett, A, Richardson, A.E., Baker, G., Wakelin, S., Thrall, P.H., 2010. Life history determines biogeographical patterns of soil bacterial communities over multiple spatial scales. Molecular Ecology 19, 4315-4327.

Bloem, J., De Ruiter, P.C., Koopman, G.J., Lebbink, G., Brussaard, L., 1992. Microbial numbers and activity in dried and rewetted arable soil under integrated and conventional management. Soil Biology and Biochemistry 24, 655-665.

Bollen, G.J., 1969. The selective effect of heat treatment on the microflora of a greenhouse soil. Netherlands Journal of Plant Pathology 75, 157-163.

Bollmann, A., and Conrad, R., 1998. Influence of $O_2$ availability on NO and $N_2O$ releae by nitrification and denitrification in soils. Global Change Biology 4, 387-396.

Borken, W., and Matzner, E., 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. Global Change Biology 15, 808–824.

Borken, W., Muhs, A., Beese, F., 2002 a. Application of compost in spruce forests: effects on soil respiration, basal respiration and microbial biomass. Forest Ecology and Management 159, 49-58.

Borken, W., Muhs, A., Beese, F., 2002 b. Changes in microbial and soil properties following compost treatment of degraded temperate forest soils. Soil Biology and Biochemistry 34, 403-412.

Bottner, P., 1985. Response of microbial biomass to alternate moist and dry conditions in a soil incubated with 14C- and 15N-labelled plant material. Soil Biology and Biochemistry 17, 329–337.

Brandt, K.K., Sioholm, O.R., Krogh, K.A., Halling-Sorensen, B., Nybroe, O., 2009. Increased pollution-induced bacterial community tolerance to sulfadiazine in soil hotspots amended with artificial root exudates. Environmental Science and Technology 43, 2963–2968.

Breeuwer, P., Lardeau, A., Peterz, M., and Joosten, H.M., 2003. Desiccation and heat tolerance of *Enterobacter sakazakii*. Journal of Applied Microbiology 95, 967–973.

Brockett, B.F.T., Prescott, C.E., Grayston, S.J., 2012. Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. Soil Biology and Biochemistry 44, 9-20.

Castro, H.F., Classen, A.T., Austin, E.E., Norby, R.J., Schadt, C.W., 2010. Soil microbial community responses to multiple experimental climate change drivers. Applied and Environmental Microbiology 76, 999-1007.

Chanal, A., Chapon, V., Benzerara, K., Barakat, M., Christen, R., Achouak, W., Barras F., Heulin, T., 2006. The desert of Tataouine: an extreme environment that hosts a wide diversity of microorganisms and radiotolerant bacteria. Environmental Microbiology 8, 514–525.

Chenu, C., and Roberson, E.B., 1996. Diffusion of glucose in microbial extracellular polysaccharide as affected by water potential. Soil Biology and Biochemistry 28, 877-884.

Chowdhury, N., Burns, R.G., Marschner, P. 2011b. Recovery of soil respiration after drying. Plant and Soil 348, 269–279.

Chowdhury, N., Marschner, P., Burns, R.G., 2011a. Response of microbial activity and community structure to decreasing soil osmotic and matric potential. Plant and Soil 344, 241–254.

Craine, J. M., Wedin, D. A., and Reich., P. B., 2001. The response of soil CO2 flux to changes in atmospheric CO2, nitrogen supply and plant diversity. Global Change Biology 7, 947–953.

Dalias, P., Anderson, J.M., Bottner, P., Coûteaux, M., 2001. Temperature responses of carbon mineralization in conifer forest soils from different regional climates incubated under standard laboratory conditions. Global Change Biology 6, 181-192.

Darby, B.J., Neher, D.A, Housman, D.C., Belnap, J., 2011. Few apparent short-term effects of elevated soil temperature and increased frequency of summer precipitation on the abundance and taxonomic diversity of desert soil micro- and meso-fauna. Soil Biology and Biochemistry 43, 1474-1481.

De Nobili, M., Contin, M., Brookesn, P.C., 2006. Microbial biomass dynamics in recently air-dried and rewetted soils compared to others stored air-dry for up to 103 years. Soil Biology and Biochemistry 38, 2871–2881.

Dose, K., Bieger-Dose, A., Ernst, B., Feister, U., Gomez-Silva, B., Klein, A., Risi, S., Stridde, C., 2001. Survival of microorganisms under the extreme conditions of the Atacama Desert. Origins of Life and Evolution of the Biosphere 31, 287-303.

Dose, K., Biegerdose, A., Kerz, O., Gill, M., 1991. DNA-Strand breaks limit survival in extreme dryness. Origins of Life and Evolution of the Biosphere 21, 177-187.

Feder, M.E., and Hofmann, G.E., 1999. Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and Ecological Physiology. Annual Review of Physiology 61, 243–82.

Fetzer, S., Bak, F., Conrad, R., 1993. Sensitivity of methanogenic bacteria from paddy soil to oxygen and desiccation. FEMS Microbiology Ecology 12, 107-115.

Fierer, N. and Schimel, J.P., 2002. Effects of drying-rewetting frequency on soil carbon and nitrogen transformations. Soil Biology and Biochemistry 34, 777-787.

Fierer, N. and Schimel, J.P., 2003. A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. Soil Science Society of America Journal 67, 798-805.

Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology 88, 1354-1364.

Fierer, N., Schimel, J. P. and Holden., P. A., 2003. Influence of drying-rewetting frequency on soil bacterial community structure. Microbial Ecology 45, 63–71.

Firestone, M.K., Davidson, E.A., 1989. Microbiological basis of NO and $N_2O$ production and consumption. In: Andreae MO, Schimel DS (eds) Exchange of trace gases between terrestrial ecosystems and the atmosphere. Wiley, New York.

Franco-Correa, M., Quintana, A., Duque, C., Suarez, C., Rodrigueza, M.X., Barea, J.M., 2010. Evaluation of actinomycete strains for key traits related with plant growth promotion and mycorrhiza helping activities. Applied Soil Ecology 45, 209–217.

Frank, A. B., Liebig, M. A., and Hanson., J. D., 2002. Soil carbon dioxide fluxes in northern semiarid grasslands. Soil Biology and Biochemistry 34, 1235–1241.

Gadd, G.M., 2004. Microbial influence on metal mobility and application for bioremediation. Geoderma 122,109-119.

Ge, G.F., Li, Z.J., Fan, F.L., Chu, G.X., Hou, Z.A., Liang, Y.C., 2010. Soil biological activity and their seasonal variations in response to long-term application of organic and inorganic fertilizers. Plant and Soil 326, 31–44.

Gebert, J., Rachor, I., Gröngröft, A., Pfeiffer, E.M., 2011. Temporal variability of soil gas composition in landfill covers. Waste Management 31, 935–945.

Geyer, J., Kiefer, I., Kreft, S., Chavez, V., Salafsky, N., Jeltsch, F., Ibisch, P.L., 2011. Classification of climate-change-induced stresses on biological diversity. Conservation Biology 25, 708–715.

Gibelin, A.L., and Déqué, M., 2003. Anthropogenic climate change over the Mediterranean region simulated by a global variable resolution model. Climate Dynamics 20, 327–339.

Goebel, M.O., Bachmann, J., Reichstein, M., Janssens, I.A., Guggenberger, G., 2011. Soil water repellency and its implications for organic matter decomposition – is there a link to extreme climatic events? Global Change Biology 17, 2640–2656

Grayston, S.J., Wang, S., Campbell, C.D., Edwards, A.C., 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biology and Biochemistry 30, 369-378.

Griffiths, B.S., Hallet, P.D., Kuan, H.L., Aitken, M.N., 2005. Biological and physical resilience of soil amended with heavymetal-contaminated sewage sludge European. Journal of Soil Science 56, 197–205.

Griffiths, B.S., Kuan, H.L., Ritz, K., Glover, L.A., McCaig, A.E., Fenwick, C., 2004. The relationship between microbial community structure and functional stability, tested experimentally in an upland pasture soil. Microbial Ecology 47, 104–113.

Griffiths, B.S., Ritz, K., Bardgett, R.D., Cook, R., Christensen, S., Ekelund, F., Sorensen, S.J., Bååth, E, Bloem, J., de Ruiter, P.C., Dolfing, J., Nicolardot, B., 2000. Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: An examination of the biodiversity– ecosystem function relationship. Oikos 90, 279–294.

Griffiths, B.S., Ritz, K., Wheatley, R., Kuan, H.L., Boag, B., Christensen, S. et al. 2001. An examination of the biodiversity– ecosystem function relationship in arable soil microbial communities. Soil Biology and Biochemistry 33, 1713–1722.

Griffiths, R.I., Whiteley, A.S., O'Donnell, A.G., Bailey, M.J., 2003. Physiological and community responses of established grassland bacterial populations to water stress. Applied and Environmental Microbiology 69, 6961–6968.

Grüter, D., Schmid, B., Brandl, H., 2006. Influence of plant diversity and elevated atmospheric carbon dioxide levels on belowground bacterial diversity. BMC Microbiology 6:68 doi: 10. 1186/1471-2180-6-68.

Gulledge, J., and Schimel., J. P., 1998. Moisture control over atmospheric CH4 consumption and CO2 production in diverse Alaskan soils. Soil Biology and Biochemistry 30, 1127–1132.

Hamer, U., Unger, M., Makeschin, F., 2007. Impact of air-drying and rewetting on PLFA profiles of soil microbial communities. Journal of Plant Nutrition and Soil Science 170, 259–264.

Hecker, M, Schumann, W., Volker, U., 1996. Heat-shock and general stress response in Bacillus subtilis. Molecular Microbiology 19, 417-428.

Heisler, J.L., Weltzin, J.L., 2006. Variability matters: towards a perspective on the influence of precipitation on terrestrial ecosystems. New Phytologist 172, 189-192.

Henry A., Doucette W., Norton J., Bugbee B., 2007. Changes in Crested Wheatgrass Root Exudation Caused by Flood, Drought, and Nutrient Stress. Journal of Environmental Quality 36, 904-912.

Hisinger, P., Bengough, A.G., Vetterlein, D., Young, I.M., 2009. Rhizosphere: biophysics, biogeochemistry and ecological relevance. Plant and Soil 321, 117–152.

Hooper, D., Bignell, D., Brown, V., Brussaard, L., Dangerfield, J., Wall, D., Wardle, D., Coleman, D., Giller, K., Lavelle, P., Van der Putten, W., De Ruiter, P., Rusek, J., Silver, W., Tiedje, J., Wolters, V., 2000. Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: patterns, mechanisms, and feedbacks. BioScience 50, 1049–1061.

Hueso, S., Hernández, T., García, C., 2011. Resistance and resilience of the soil microbial biomass to severe drought in semiarid soils: The importance of organic amendments. Applied Soil Ecology 50, 27–36.

Humpf, H.U., and Voss, K.A., 2004. Effects of thermal food processing on the chemical structure and toxicity of fumonisin mycotoxins. Molecular Nutrition and Food Research 48, 255 – 269.

Illeris, L., Michelsen, A., Jonasson, S., 2003. Soil plus root respiration and microbial biomass following water, nitrogen and phosphorus application at a high arctic semi desert. Biogeochemistry 65, 15-29.

IPCC, 2007, Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. 21pp.

Jensen, K. D., Beier, C., Michelsen, A., Emmett, B., 2003. Effects of experimental drought on microbial processes in two temperate heathlands at contrasting water conditions. Applied Soil Ecology. 24, 165–176.

Joergensen, R.G., Anderson, T.H., Wolters, V., 1995. Carbon and nitrogen relationships in the microbial biomass in soils in beech (*Fagus sylvatica* L.) forests. Biology and Fertility of Soils 19, 141–147.

Joergensen, R.G., Brookes, P.C., Jenkinson, D.S., 1990. Survival of the soil microbial biomass at elevated temperatures Soil Biology and Biochemistry 22, 1129-1136

Juottonen, H., Galand, P.E., Tuittila, E.S., Laine, J., Fritze, H., Yrjälä, K., 2005. Methanogen communities and *Bacteria* along an ecohydrological gradient in a northern raised bog complex. Environmental Microbiology 7, 1547–1557.

Kempf, B., and Bremer, E., 1998. Uptake and synthesis of compatible solutes as microbial stress responses to high osmolality environments. Archives of Microbiology 170, 319–330.

Kieft, L.T., Soroker, E., Firestone, M.K., 1987. Microbial biomass response to a rapid increase in water potential when dry soil is wetted. Soil Biology and Biochemistry 19, 119-126.

Kieft, T.L., Ringelberg, D.B., White, D.C., 1994. Changes in ester-linked phospholipid fatty acid profiles of subsurface bacteria during starvation and desiccation in a porous medium. Applied and Environmental Microbiology 60, 3292–3299.

Klamer, M., and Bååth, E., 1998. Microbial community dynamics during composting of straw material studied using phospholipid fatty acid analysis. FEMS Microbiology Ecology 27, 9-20.

Kuan, H.L., Fenwick, C., Glover, L.A., Griffiths, B.S., Ritz, K., 2006. Functional resilience of microbial communities from perturbed upland grassland soils to further persistent or transient stresses. Soil Biology and Biochemistry 38, 2300–2306.

Kültz, D., 2005. Molecular and evolutionary basis of the cellular stress response. Annual Review of Physiology 67, 225–257.

Kunito, T., Saeki, K., Oyaizu, H., Matsumoto, S., 1999. Influences of Copper Forms on the Toxicity to Microorganisms in Soils. Ecotoxicology and Environmental Safety 44, 174-181.

Lal, R., 2004. Soil carbon sequestration to mitigate climate change. Geoderma 123, 1 –22

Leblanc, J. C., Gonçalves, E. R., Mohn, W. W., 2008. Global response to desiccation stress in the soil actinomycete Rhodococcus Jostii RHA1. Applied and Environmental Microbiology 74, 2627–2636.

Leslie, S.B., Israeli, E., Lighthart, B., Crowe, J.H., Crowe, L.M., 1995. Trehalose and Sucrose Protect Both Membranes and Proteins in Intact Bacteria during Drying. Applied and Environmental Microbiology 61, 3592–3597.

Lu, X., and Cheng, G., 2009. Climate change effects on soil carbon dynamics and greenhouse gas emissions in *Abies fabri* forest of subalpine, southwest China. Soil Biology and Biochemistry 41, 1015-1021.

Lundquist, E.J., Scow, K.M., Jackson, L.E., Uesugi, S.L., Johnson, C.R., 1999. Rapid response of soil microbial communities from conventional, low input, and organic farming systems to a wet/ dry cycle. Soil Biology and Biochemistry 31, 1661–1675.

Lynch, J. M., and Whipps., J. M., 1990. Substrate flow in the rhizosphere. Plant and Soil 129, 1–10.

Macario, A.J.L., Lange, M., Ahring, B.K., De Macario, E.C., 1999. Stress genes and proteins in the archaea. Microbiology and Molecular Biology Reviews 63, 923–967.

Mahieu, S., Germon, F., Aveline, A., Hauggaard-Nielsen, H., Ambus, P., Jensen, E.S., 2009. The influence of water stress on biomass and N accumulation, N partitioning between above and below ground parts and on N rhizodeposition during reproductive growth of pea (Pisum sativum L). Soil Biology and Biochemistry 41, 380–387.

Mahmood, T., Safdar, W., Abbasi, B.H., Naqvi, S.M.S., 2010. An overview on the small heat shock proteins. African Journal of Biotechnology 9, 927-949.

Makino, T., Hasegawa, S., Sakurai, Y., Ohno, S., Utagawa, H., Maejima, Y., Momohara, K., 2000. Influence of Soil-Drying under Field Conditions on Exchangeable Manganese, Cobalt, and Copper Contents. Soil Science and Plant Nutrition 46, 581-590.

Makino, T., Takahashi, Y., Sakurai, Y., 1997. The influence of air-drying treatment on chemical forms of Mn, Co, Zn and Cu in soils. Japanese Journal of Soil Science and Plant Nutrition 68, 409-416.

Mattimore, V., Battista, J.R., 1996. Radioresistance of Deinococcus radiodurans: Functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. Journal of Bacteriology 178, 633–637.

Mertens, J., Beladjal, L., Alcantara, A., Fougnies, L., Van Der Straeten, D., Clegg, J.S., 2008. Survival of dried eukaryotes (anhydrobiotes) after exposure to very high temperatures. Biological Journal of the Linnean Society 93, 15–22.

Mielnick, P. C., and Dugas, W. A., 2000. Soil CO2 flux in a tallgrass prairie. Soil Biology and Biochemistry 32, 221–228.

Millennium Ecosystem Assessment, 2005. Ecosystems and Human Well-being: Synthesis. Island Press, Washington, DC.

Mittler, R., 2006. Abiotic stress, the field environment and stress combination. Trends in Plant Science 11, 1360-1385.

Mrozik, A., Piotrowska-Seget, Z., Łabużek, S., 2004. Cytoplasmatic bacterial membrane responses to environmental perturbations. Polish Journal of Environmental Studies 13, 487-494.

Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. European Journal of Soil Science 54, 655–670.

Nannipieri, P., Eldor. P., 2009. The chemical and functional characterization of soil N and its biotic components. Soil Biology and Biochemistry 41, 2357-2369.

Nazih, N., Finlay-Moore, O., Hartel, P.G., Fuhrmann, J. J., 2001. Whole soil fatty acid methyl ester (FAME) profiles of early soybean rhizosphere as affected by temperature and matric water potential. Soil Biology and Biochemistry 33, 693–696.

Norris, T.B., Wraith, J.M., Castenholz, R.W., McDermott, T.R., 2002. Soil Microbial Community Structure across a Thermal Gradient following a Geothermal Heating Event. Applied and Environmental Microbiology 68, 6300–6309.

Or, D., Smets, B.F., Wraith, J.M., Dechesne, A., Friedman, S.P., 2007. Physical constraints affecting bacterial habitats and activity in unsaturated porous media – a review. Advances in Water Resources 30, 1505–1527.

Orwin, K., and Wardle, D., 2005. Plant species composition effects on belowground properties and the resistance an resilience of the soil microflora to a drying disturbance. Plant and Soil 278, 205-221.

Ouyang, Y., and Zheng, C. 2000. Surficial processes and CO2 flux in soil ecosystem. Journal of Hydrology 234, 54–70.

Parker, S.S., and Schimel, J.P., 2011. Soil nitrogen availability and transformations differ between the summer and the growing season in a California grassland. Applied Soil Ecology 48, 185–192.

Parsell, D.A., and Lindquist, S., 1993. The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. Annual Review of Genetics 27, 437-496.

Pesaro, M., Nicollier, G., Zeyer, J., Widmer, F., 2004. Impact of soil drying-rewetting stress microbial communities and activities and on degradation of two crop protection products. Applied and Environmental Microbiology 70, 2577–2587.

Petersen, S.O., and Klug, M.J., 1994. Effects of Sieving, Storage, and Incubation Temperature on the Phospholipid Fatty Acid Profile of a Soil Microbial Community. Applied and Environmental Microbiology 60, 2421-2430.

Pettersson, M., and Bååth, E., 2003. Temperature-dependent changes in the soil bacterial community in limed and unlimed soil. FEMS Microbiology Ecology 45, 13-21.

Pietikaïnen, J., Pettersson, M., Bååth, E., 2005. Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. FEMS Microbiology Ecology 52, 49–58.

Pimm, S. L., 1984. The complexity and stability of ecosystems. Nature 307, 321–326.

Planton, S., Déqué, M., Chauvin, F., Terray, L., 2008. Expected impacts of climate change on extreme climate events. Comptes Rendus Geoscience 340, 564-574.

Potts, M., 1994. Desiccation tolerance of prokaryotes. Microbiological Reviews 58, 755–805.

Potts, M., 1999. Mechanisms of desiccation tolerance in cyanobacteria, European Journal of Phycology 34, 319-328.

Pray, C., Nagarajan, L., Li, L., Huang, J., Hu, R., Selvaraj, K.N., Napasintuwong O., Chandra Babu R., 2011. Potential impact of biotechnology on adaption of agriculture to Climate Change: The case of drought tolerant Rice Breeding in Asia. Sustainability 31, 723-1741.

Prevost-Boure, N.C., Maron, P.A., Ranjard, L., Nowak, V., Dufrene, E., Damesin, C., Soudani, K., Lata, J.C., 2011. Seasonal dynamics of the bacterial community in forest soils under different quantities of leaf litter. Applied Soil Ecology 4, 14–23.

Rajapaksha, R. M. C. P., Tobor-Kapłon, M. A., Bååth, E., 2004. Metal toxicity affects fungal and bacterial activities in soil differently. Applied and Environmental Microbiology 70, 2966–2973.

Ranneklev, S.B., and Bååth, E., 2001. Temperature-Driven Adaptation of the Bacterial Community in Peat Measured by Using Thymidine and Leucine Incorporation. Applied Environmental Microbiology 67, 1116–1122.

Reidsma P., Lansink A.O., Ewert F., 2009. Economic impacts of climatic variability and subsidies on European agriculture and observed adaptation strategies. Mitigation and Adaptation Strategies for Global change 14, 35–59.

Roberson, E.B., Firestone, M.K., 1992. Relationship between desiccation and exopolysaccharide production in soil Pseudomonas spp. Applied and Environmental Microbiology 58, 1284–1291.

Roelofs, D., Aarts, M.G.M., Schat, H., van Straalen, N. M., 2008. Functional ecological genomics to demonstrate general and specific responses to abiotic stress. Functional Ecology 22, 8–18.

Rojas-Valencia, M.N., Orta de Velásquez, M.T., Franco, V., 2011. Urban agriculture, using sustainable practices that involve the reuse of wastewater and solid waste Agricultural Water Management 98, 1388– 1394.

Rokitko, P.V., Romanovs'ka, V.O., Malashenko, Iu. R., Chorna, N.A., 2004. Effect of UV-radiation and drying on bacterium diversity in soil. Mikrobiolohichnyi zhurnal 66, 68-77.

Rokitko, P.V., Romanovskaya, V.A., Malashenko, Y.R., Chernaya, N.A., Gushcha, N.I., Mikheev, A.N., 2003. Soil drying as a model for the action of stress factors on natural bacterial populations. Microbiology 72, 756-761.

Ross, D.J. and Sparling, G.P., 1993. Comparisions of methods to estimate microbial C and N in litter and soil under Pinus radiate on a coastal sand. Soil Biology and Biochemistry 25, 1591–1599.

Ross, D.S., Hales, H.C., Shea-McCarthy, G.C., Lanzirotti, A., 2001. Sensitivity of Soil Manganese Oxides: Drying and Storage Cause Reduction. Soil Science Society of America Journal 65, 736–743.

Russell, N.J., 2002. Bacterial membranes: the effects of chill storage and food processing. An overview. International Journal of Food Microbiology 79, 27– 34.

Russell, N.J., Evans, R.I., terSteeg, P.F., Hellemons, J., Verheul, A., Abee, T., 1995. Membranes as a target for stress adaptation. International Journal of Food Microbiology 28, 255-261.

Rustad, L.E., Campbell, J.L., Marion, G.M., Norby, R.J., Mitchell., M.J., Hartley, A.E., Cornelissen, J.H.C., Gurevitch, J., 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia 126, 543–562.

Saetre, P., and Stark, J.M., 2005. Microbial dynamics and carbon and nitrogen cycling following re-wetting of soils beneath two semi-arid plant species. Oecologia 142, 247–260.

Sanaullah, M., Blagodatskaya, E., Chabbi, A., Rumpel, C., Kuzyakov, Y., 2011. Drought effects on microbial biomass and enzyme activities in the rhizosphere of grasses depend on plant community composition. Applied Soil Ecology 48, 38–44.

Sardans, J., and Peñuelas, J., 2004. Increasing drought decreases phosphorus availability in an evergreen Mediterranean forest. Plant and Soil 267, 367–377.

Sardans, J., and Peñuelas, J., 2005. Drought decreases soil enzyme activity in a Mediterranean Quercus ilex L. forest. Soil Biology and Biochemistry 37, 455–461.

Sardans, J., and Peñuelas, J., 2007b. Drought changes the dynamics of trace element accumulation in a Mediterranean Quercus ilex forest. Environmal Pollution 147, 567-583.

Sardans, J., and, Peñuelas, J., 2007. Drought changes phosphorus and potassium accumulation patterns in an evergreen Mediterranean forest. Functional Ecology 21, 191–201.

Sardans, J., Penuelas, J., Estiarte, M., 2008. Changes in soil enzymes related to C and N cycle and in soil C and N content under prolonged warming and drought in a Mediterranean shrubland. Applied soil ecology 39, 223 – 235.

Sartori, M., Nesci, A., Etcheverry, M., 2010. Impact of osmotic/matric stress and heat shock on environmental tolerance induction of bacterial biocontrol agents against Fusarium verticillioides. Research in Microbiology 161, 681-686.

Schimel ,J.P., Gulledge, J.M., Clein-Curley, J.S., Lindstrom, J.E., Braddock, J..F., 1999. Moisture effects on microbial activity and community structure in decomposing birch litter in the Alaskan taiga. Soil Biology & Biochemistry 31, 831–838.

Schimel J., Balser T.C., Wallenstein M., 2007. Microbial stress response physiology and its implications for ecosystem function. Ecology 88, 1386-1394.

Schimel, J., 1995. Ecosystem consequences of microbial diversity and community structure. In F. Chapin and C. Korner, editors. Arctic and alpine biodiversity: patterns, causes, and ecosystem consequences. Springer-Verlag, Berlin, Germany, 239–254.

Schimel, J., Scott, W., Killham, K., 1989. Changes in cytoplasmic carbon and nitrogen pools in a soil bacterium and a fungus in response to salt stress. Applied and Environmental Microbiology 55, 1635–1637.

Schimel, J.P., and Gulledge, J., 1998. Microbial community structure and global trace gases. Glob. Change Biology 4, 745–758.

Schnell, S., and King, G. M., 1996. Responses of methanotrophic activity in soils and cultures to water stress. Applied and Environmental Microbiology 62, 3203–3209.

Seguin, B., 2007. Les impacts du changement climatique sur l'agriculture, en particulier en Europe. Sciences 1, 17-23.

Seybold, C. A., Herrick, J. E., Brejda, J. J., 1999. Soil resilience: a fundamental component of soil quality. Soil Science 164, 224–234.

Sharma, D., and Kaur, P., 2009. Combined effect of drought stress and heat shock on Cyclophilin protein expression in *Triticum aestivum.* General and Applied Plant Physiology 35, 88–92.

Smolders, E., Buekers, J., Oliver, I., Mc Laughlin, M.J., 2004. Soil properties affecting toxicity of Zinc to soil microbial properties in laboratory-spiked and field-contaminated soils. Environmental Toxicology and Chemistry 23, 2633–2640.

Sparling, G. P., Speir, T. W., and Whale, K. N., 1986. Changes in microbial biomass C, ATP content, soil phospho-monoesterase and phospho-diesterase activity following air drvine. of soils. Soil Biology and Biochemistry 18, 363-370.

Stark, J., and Firestone., M., 1995. Mechanisms for soil moisture effects on activity of nitrifying bacteria. Applied and Environmental Microbiology 61, 218–221.

Suzina, N. E., Mulyukin, A. L., Kozlova, A. N., Shorokhova, A. P., Dmitriev, V. V., Barinova, E. S., Mokhova, O. N., El'-Registan, G. I., Duda., V. I., 2004. Ultrastructure of resting cells of some non-spore-forming bacteria. Microbiology 73, 435–447.

Swift, M.J., Andren, O., Brussaard, L., Briones, M., Couteaux, M.,M., Ekschmitt, K., Kjoller, A., Loiseau, P., Smith, P., 1998. Global change, soil biodiversity, and nitrogen cycling in terrestrial ecosystems: three case studies. Global Change Biology 4, 729-743.

Thompson, B.C., Ostle, N.J., McNamara, N.P., Whiteley, A.S., Griffiths, R.I., 2010. Effects of sieving, drying and rewetting upon soil bacterial community structure and respiration rates. Journal of Microbiological Methods 83, 69–73.

Timonen, S., Finlay, R.D., Olsson, S., Soderstrom, B., 1996. Dynamics of phosphorous translocation in intact ectomycorrhizal systems: non-destructive monitoring using a B-scanner. FEMS Microbiology Ecology 19, 171-180.

Tlili, A., Marechal, M., Montuelle, B., Volat, B., Dorigo, U., Bérard, A., 2011. Use of the Microresp™ method to assess Pollution-Induced Community Tolerance for lotic biofilms. Environmental Policy 159, 18–24.

Trevors, J.T., 1998. Bacterial biodiversity in soil with an emphasis on chemically contaminated soils. Water Air and Soil Pollution 101, 45– 67.

Turnbull, G.A., Morgan, J.A.L., Whipps, J.M., Saunders, J.R., 2001. The role of bacterial motility in the survival and spread of *Pseudomonas fluorescens* in soil and in the attachment and colonisation of wheat roots. FEMS Microbiology Ecology 36, 21-31.

Uhlirova, E., Elhottova, D., Triska, J., Santruckova, H., 2005. Physiology and microbial community structure in soil at extreme water content. Folia Microbiologica 50, 161–166.

Van Gestel, M., Merckx, R., Vlassak, K., 1993. Microbial biomass and activity in soils with fluctuating water contents. Geoderma 56, 617-626.

Van Gestel, M., Merckx, R., Vlassak, K., 1993a. Microbial biomass response to soil drying and rewetting: the fate of fast- and slow-growing microorganisms in soils of different climates. Soil Biology and Biochemistry 25, 109–123.

Vilain, S., Luo, Y., Hildreth, M.B., Brozel, V.S., 2006. Analysis of the Life Cycle of the Soil Saprophyte *Bacillus cereus* in Liquid Soil Extract and in Soil. Applied and Environmental Microbiology 72, 4970–4977.

Vinebrooke, R.D., Cottingham, K.L., Norberg, J., Scheffer, M., Dodson, S.I., Maberly, S.C., Sommer, U., 2004. Impacts of multiple stressors on biodiversity and ecosystem functioning: the role of species co- tolerance. Oikos 104, 451–457.

Waldrop, M.P., Balser, T.C., Firestone M.K., 2000. Linking microbial community composition to function in a tropical soil. Soil Biology and Biochemistry 3, 1837–1846.

Waldrop, M.P., Firestone, M.K., 2006. Response of microbial community composition and function to soil climate change. Microbial Ecology 52, 716–724.

Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesank, C., Beebee, T.J.C., Fromentin, J.M., Hoegh-Guldberg, O., Bairlein, F., 2002. Ecological responses to recent climate change. Nature 416, 389-395.

Wertz, S., Czarnes, S., Bartoli, F., Renault, P., Commeaux, C., Guillaumaud, N., Clays-Josserand, A., 2007. Early-stage bacterial colonization between a sterilized remoulded soil clod and natural soil aggregates of the same soil. Soil Biology and Biochemistry 39, 3127–3137.

West, A. W., Sparling, G.P., Grant, W.D., 1987. Relationships between mycelial and bacterial populations in stored, air-dried and glucose-amended arable and grassland soils. Soil Biology and Biochemistry 19, 599–605.

West, A.W., Sparling, G.P., Feltham, C.W., Reynolds, J., 1992. Microbial activity and survival in soils dried at different rates. Australian Journal of Soil Research 30, 209-222.

Wilkinson, S., Anderson, J., Scardelis, S., Tisiafouli, M., Taylor, A., Wolters, V., 2002. PLFA profiles of microbial communities in decomposing conifer litters subject to moisture stress. Soil Biology and Biochemistry 34, 189–200.

Williams, M.A., 2007. Response of microbial communities to water stress in irrigated and drought-prone tallgrass prairie soils, Soil Biology and Biochemistry 39, 2750-2757.

Witteveen, C.F.B., and Visser, J., 1995. Polyol Pools in Aspergillus Niger. FEMS Microbiology Letters 134, 57–62.

Zelles, L., Adrian, P., Bai, Q. Y., Stepper, K., Adrian, M. V., Fischer, K., Maier, A., Ziegler, A., 1991. Microbial activity measured in soils stored under different temperature and humidity conditions. Soil Biology and Biochemistry 23, 955–962.

Zou, X.X., Li, Y.E., Gao, Q.Z., Wan, Y.F., 2012. How water saving irrigation contributes to climate change resilience-a case study of practices in China. Mitigation and Adaptation Strategies for Global change 17, 111–132.

# Partie I.2.

# Impacts d’apports de matières organiques exogènes sur les propriétés du sol

## Apports de Matières Organiques (MO) au sol: le compost

Les orientations réglementaires tant européennes que françaises visent à réduire la production de déchets ultimes et à développer leur recyclage autrement que par incinération, pour limiter les mises en décharge définitives (ADEME 2011). C'est notamment le cas pour les déchets organiques. La France s'est ainsi fixée différents objectifs dans le cadre du Grenelle de l'Environnement (Septembre-Octobre 2007) :

- passer de 24% de recyclage matière ou organique des déchets ménagers et assimilés (DMA, déchets collectés par le service public de collecte) en 2004, à 35% en 2012 puis à 45% en 2015 ;
- diminuer les quantités totales de déchets stockés ou incinérés ;
- poursuivre le développement d'une filière de production de compost de qualité.

La valorisation des déchets par compostage joue donc un rôle important dans les stratégies Européennes et Françaises de gestion des déchets. En France, les déchets organiques (hors papiers cartons non souillés) de toutes origines (ménages, entreprises, collectivités …) représentent environ 33 Mt dont environ 10 épandues directement et 6 Mt générant 2,3 Mt de compost (ADEME, 2011). Cette valorisation organique apparaît comme un mode de gestion des déchets respectueux de l'environnement, car mettant en œuvre des phénomènes biologiques « naturels » et permettant, sous réserve des principes d'innocuité et d'efficacité agronomique, le « retour au sol » de la matière organique (ADEME, 2005). Le compostage considéré ainsi comme une écotechnologie puisqu'elle permet la réinsertion de la matière organique dans les cycles écologiques (Mustin, 1987) et la valorisation agronomique des déchets biodégradables constitue une voie d'action prioritaire de la politique de gestion des déchets, aussi bien au niveau Européen qu'au niveau national (ADEME, 2005). Le compostage et la valorisation agronomique du compost constitueraient aussi une solution adéquate et d'un intérêt majeur pour les pays en développement. En particulier en Tunisie, l'Agence De l'Environnement et de la Maîtrise de l'Energie (ADEME) a soutenu techniquement et financièrement des programmes de recherche pour la mise au point de méthodes de caractérisation des déchets, le développement des procédés de compostage et la mise en place d'essais agronomiques (Ben Ammar, 2006).

## 1. Définition et principales étapes du compostage

Le compostage est une méthode ancestrale de fabrication d'amendement organique. Il peut être défini comme un procédé biologique thermophile contrôlé de conversion et de valorisation des constituants organiques des déchets en un produit stabilisé, assaini et riche en composés humiques ; le compost (Mustin, 1987).

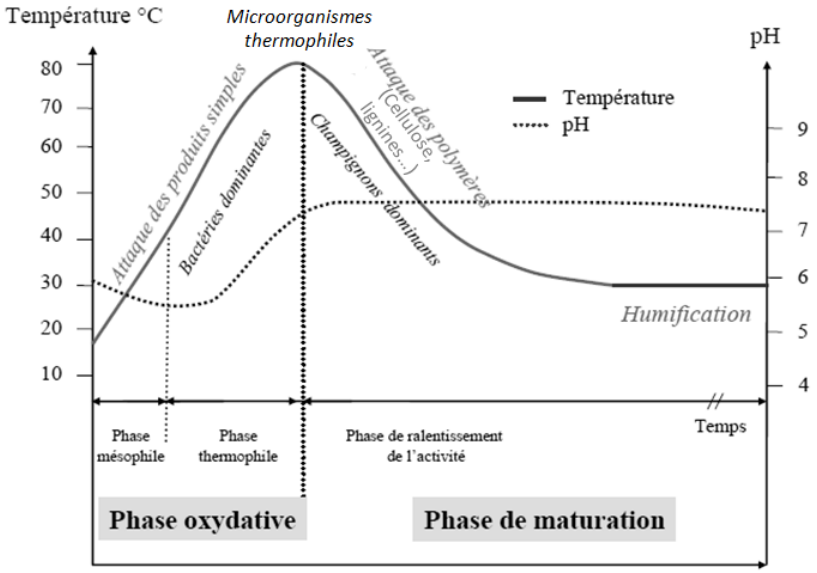


**Figure 1 :** Courbe théorique d'évolution de la température et du pH au cours du compostage d'après Mustin (1987).

L'évolution de la température au cours du compostage permet de distinguer différentes phases (Figure 1) :

- la phase mésophile : les micro-organismes dont la température de croissance optimale est comprise entre 20 et 45°C se multiplient et décomposent la matière organique facilement biodégradable. Leur activité engendre une forte production de chaleur et une montée rapide de la température au cœur du compost. Cette phase peut durer de quelques heures à quelques jours ;

- la phase thermophile au cours de laquelle la température augmente jusqu'à 60°C voire 70°C. La matière organique est alors fortement dégradée et un assèchement du compost lié à l'évaporation de l'eau est souvent observé. Cette phase peut durer de quelques jours à quelques semaines ;

Ces deux premières phases correspondent à des phases de dégradation aérobie intense de la matière organique. Certains auteurs parlent de fermentation aérobie (Harada et al., 1981).

- la phase de refroidissement se produit lorsque la quantité de matières facilement dégradables diminuent, provoquant un ralentissement de l'activité microbienne ;

- la phase de maturation dure jusqu'à l'utilisation du compost. Les processus d'humification prédominent durant cette phase et les composés résistants sont lentement dégradés. Cette phase peut durer de quelques semaines à quelques mois.

### 2. Les microorganismes au cours du compostage

Le processus de compostage résulte de l'activité microbiologique complexe d'une multitude de populations microbiennes évoluant en milieu aérobie (Sharma et al., 1997). L'inventaire des microorganismes présents lors d'un compostage a révélé la présence de 155 espèces de procaryotes, dont 33 Actinomycètes, et de 408 espèces fongiques (Ryckeboer et al., 2003a). Ces populations microbiennes se succèdent au cours des différentes phases du compostage (Mustin, 1987 ; Hassen et al., 2001). Afin de suivre ces successions microbiennes, différentes méthodes sont utilisées allant de la culture microbienne aux analyses moléculaires pour caractériser une biomasse, une structure et/ou un profil d'activités. Ces analyses montrent que les bactéries sont toujours présentes et largement dominantes (Figure 2.A-B), en particulier pendant la phase thermophile du compostage (Ryckeboer et al., 2003b). Elle concerne principalement les bactéries gram-positives, dont l'abondance augmente rapidement avec l'augmentation de la température. A l'inverse au cours de cette phase, l'abondance des bactéries gram-négatives et des champignons diminue (Klamer et Bååth, 1998). La phase thermophile permet une hygiénisation du compost. En effet, Hassen et al. (2001) ont observé que les températures élevées (50-60°C) induisent une diminution d'*Escherichia coli* et des *Streptocoques* fécaux de $2\times10^7$ à $3.1\times10^3$ $10^7$ à $1.5\times10^3$ cellules/g de matière sèche respectivement et une élimination des spores de *Salmonelles*.

Les Actinomycètes ont une grande importance. Ils sont caractérisés par une croissance lente et par conséquent n'entrent pas en compétition avec les organismes stratèges-r caractérisés par une croissance rapide et qui apparaissent préférentiellement en présence de matières organiques fraiches (Ryckeboer et al., 2003a ; Fierer et al., 2007). Ainsi, leur abondance est faible pendant la phase mésophile mais augmente durant les phases de refroidissement et de maturation (Klamer et Bååth, 1998). Cependant, contrairement à Xiao et al. (2011) qui ont rapporté un développement des *Actinomycètes* lorsque la température excède 50°C, Klamer et Bååth (1998) ont observé une inhibition des *Actinomycètes* au cours de la phase thermophile.

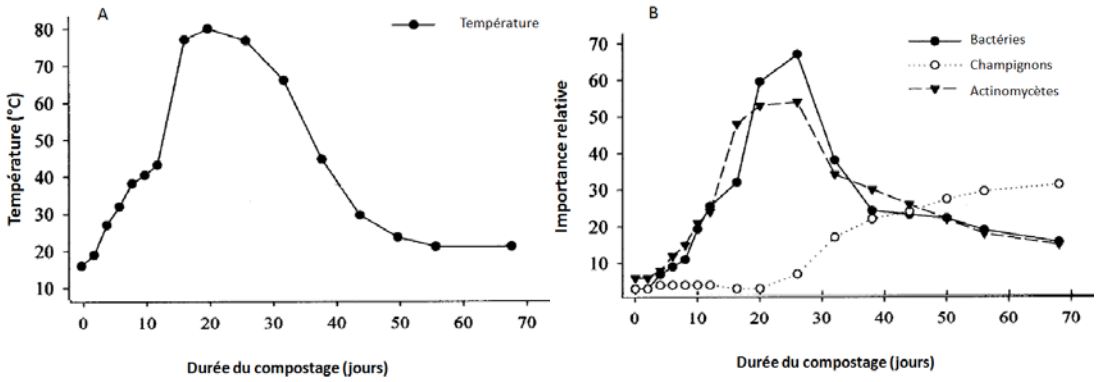


**Figure 2 :** Evolution de la température (A) et de l'importance relative des bactéries, des Actinomycètes et des champignons au cours du compostage d'après Ryckeboer et al. (2003a).

La baisse de la température au cours des phases de refroidissement et de maturation entraîne donc une reprise de la croissance des bactéries et des champignons, avec un changement de la composition microbienne. En effet, les *Staphylocoques* dominent durant la phase mésophile et le début de la phase thermophile alors que les *Bacilles* prédominent durant les phases de refroidissement et de maturation (Hassen et al., 2001). L'abondance des champignons augmente aussi au cours des deux dernières phases du compostage (Figure 2.A-B; Ryckeboer et al., 2003a). De même, comme nous l'avons déjà mentionné, l'abondance des *Actinomycètes* augmente en comparaison avec la première phase mésophile (Klamer et Bååth, 1998 ; Ryckeboer et al., 2003a). Les champignons sont essentiellement actifs pendant la phase de maturation et sont responsables de la dégradation de la cellulose et de la lignine tandis que

les *Actinomycètes* dégradent les substances non dégradées par les bactéries et les champignons (e.g. la chitine ; Mustin, 1987). Malgré l'accroissement de l'activité des champignons et des *Actinomycètes*, l'activité microbienne globale décroît au cours de la phase de maturation alors que la diversité microbienne augmente (Ryckeboer et al., 2003b).

### 3. Intérêt du compostage et Législation

Le procédé de compostage présente des intérêts écologiques et agronomiques :

- il permet de diminuer le volume et la masse des déchets d'environ 50%. Ces réductions sont dues à la minéralisation des composés organiques, à la perte d'eau ;
- les températures atteintes au cours du compostage permettent d'hygiéniser le produit final par une destruction des micro-organismes pathogènes ;
- le compost produit, assez riche en substances humiques, constitue un amendement organique permettant d'améliorer la stabilité structurale et la fertilité des sols.

Il existe une grande diversité de composts liée à la nature des déchets compostés et à la diversité des procédés de compostage. Ces amendements organiques peuvent être commercialisés et épandus s'ils sont conformes à la norme NF U 44-051 qui fixe leurs dénominations et spécifications. Les produits à base de boues d'épuration font l'objet d'une norme différente : la norme NF U 44-095.

La norme NF U 44-051 à la quelle doivent répondre les composts que nous utilisons dans le cadre de cette thèse, s'applique aux produits destinés à améliorer les sols en place et/ou les supports de culture. A l'exception des amendements organiques avec engrais, cette norme s'applique aux composts dont la somme des formes nitrique, ammoniacale et uréique ne doit pas dépasser 33 % de l'azote total, et le rapport C/N doit être supérieur à 8. La norme impose que les amendements organiques doivent respecter un taux de matière sèche (MS) ≥ 30 % de la matière brute (MB) et des spécifications dépendant de la nature des amendements. Les teneurs en éléments traces métalliques (ETM) inclus dans les apports d'amendements organiques doivent être inférieures à des valeurs limites et ne pas aboutir à dépasser des flux d'importation maximaux annuels.

## 4. Impacts sur les propriétés du sol

Plusieurs études ont montré l'importance de l'apport des amendements organiques dans l'amélioration des propriétés physiques, chimiques et biologiques du sol. Ces matières organiques sont variées : boue, fumier, compost, etc. Cependant, tous les types de matières organiques ne permettent pas d'atteindre les mêmes améliorations. En comparaison avec les boues et le fumier, le compost est riche en matières organiques stables qui améliorent les propriétés du sol et par conséquent sa fertilité physique et sa productivité.

### 4.1. Impacts sur les propriétés chimiques

Les composts constituent une source de substances organiques et minérales contribuant à l'amélioration de la fertilisation des sols. L'incorporation de compost au sol augmente le stock de carbone organique (Albiach et al., 2001 ; Ros et al., 2006a ; Ros et al., 2006b ; Bastida et al., 2008 ; Arthur et al., 2011). A même dose, le compost enrichit plus le sol en carbone organique total que le fumier (Tabuchi et al., 2008 ; Jemai et al., 2011). Ainsi la valorisation agronomique du compost permet d'entretenir, voire d'augmenter la séquestration du carbone dans les sols contribuant à la réduction des émissions de gaz à effet de serre. Une augmentation de ces stocks de 0,2% par an (6 Mt) permettrait de compenser 4% des émissions brutes annuelles des gaz à effet de serre (GES), ou un quart environ des missions des secteurs agricole et forestier (Arrouays et al., 2002). Les composts constituent aussi une source d'azote (Tabuchi et al., 2008 ; Arthur et al., 2011), de phosphore (Caravaca et al., 2002 ; Bastida et al., 2008 ; Alburquerque et al., 2011), et de potassium (Alburquerque et al., 2011). Cependant la valeur fertilisante azotée et phosphatée des composts diminue en cas de grande stabilisation de la matière organique des composts alors que la valeur amendante, associée à la capacité à augmenter le stock de matières organiques du sol, augmente avec la stabilité de la matière organique (Houot et al., 2003).

L'ajout de compost contribue aussi à une augmentation de la capacité d'échange cationique (CEC) (Ouédraogo et al., 2001 ; Arthur et al., 2011) augmentant ainsi le pool de nutriments potentiellement disponibles pour les végétaux sous forme cationique (Larchevêque, 2004). Les composts pourraient par ailleurs servir d'amendements calciques et réduire l'acidité des sols (Bougnom et al., 2010). Cette augmentation de pH provoquerait une immobilisation des ETM. En effet, l'abaissement du pH favorise la mobilité des ETM, notamment par mise en solution de sels métalliques ou la destruction de la phase de rétention.

Inversement, l'augmentation du pH provoque l'immobilisation par formation de composés insolubles ou l'accroissement de la capacité d'échange cationique engendrant la rétention des cations métalliques. La fixation des métaux et des polluants organiques modifie ainsi leurs propriétés de transfert dans le sol et réduit leurs toxicités **(**Alburquerque et al., 2011). Par conséquent, l'ajout de compost s'insère dans les techniques de remédiation des sols contaminés par les métaux lourds **(**Alburquerque et al., 2011).

### 4.2. Impacts sur les propriétés physiques

Le mélange du compost avec le sol améliore ses propriétés physiques. En effet, l'ajout de compost, matériau riche en matières organiques, fournit davantage d'acides fulviques et humiques et des cations polyvalents au sol (Jemai et al., 2011). Il se forme ainsi de nouveaux agrégats stables par adhésion des particules du sol aux molécules organiques (Chenu et al., 2000 ; Tejada et Gonzales, 2003). En effet, les cations polyvalents lorsqu'ils sont apportés en quantité suffisante au sol agissent en présence des substances humiques comme agents de liaison et forment ainsi des complexes argile-cations polyvalents-matière organique (Jemai et al., 2011). Ces associations organo-minérales diminuent la mouillabilité des agrégats (Chenu et al., 2000 ; Jemai et al., 2011) et contribuent à l'amélioration de la stabilité de la structure du sol (Chenu et al., 2000 ; Tejada et Gonzales, 2003). L'amélioration de la stabilité structurale peut être aussi en partie expliquée par l'action indirecte des microorganismes du sol. En effet, l'ajout de compost stimule l'activité biologique bactérienne et fongique laquelle produit des agents agrégeant tels que les filaments mycéliens et les polysaccharides (Albiach et al., 2001 ; Gomez et al., 2006).

L'augmentation de la concentration des sels solubles de sodium, de magnésium et de calcium dans les sols conduit à une augmentation de leur conductivité électrique et une atténuation de leur stabilité structurale (Tejada and Gonzales, 2005). L'utilisation des amendements organiques permet de réduire les effets négatifs des sols salins ou irrigués avec des eaux salines (Lakhdar et al., 2008) et pourrait par conséquent être exploitée comme stratégie favorisant la remédiation de ces sols (Tejada et al., 2006).

L'amélioration de la stabilité des agrégats, fortement corrélée à l'accroissement de la teneur en matières organiques du sol permet d'augmenter sa porosité et diminue sa densité apparente (Caravaca et al., 2002 ; Arthur et al., 2011). Ainsi, l'érosion par ruissellement est ralentie par une meilleure infiltration de l'eau dans le sol (Bresson et al., 2001). L'amélioration de la rétention en eau pourrait atténuer les phénomènes de dessiccation liés

aux épisodes de sécheresse (Culot, 2005) ; ceci présente un intérêt fondamental en région méditerranéenne où le stress hydrique est un facteur primordial limitant la croissance et l'activité microbienne dans les sols (Paul and Clark 1996).

### 4.3. Impacts sur les propriétés biologiques

L'incorporation de compost a un effet positif sur l'abondance des organismes vivants dans le sol. Les matières organiques apportées par le compost servent de source d'énergie permettant aux organismes de croître (Drenovsky et al., 2004). Globalement, la biomasse microbienne varie dans le même sens que la teneur en carbone organique (ADEME, 2005). Le relargage du carbone organique dissous (COD) du compost et l'amélioration de la biodisponibilité des nutriments pourraient favoriser la croissance de la communauté microbienne autochtone du sol (Borken et al., 2002a). Ainsi, les composts contribuent à l'augmentation de la biomasse microbienne tellurique (Garcia-Gil et al., 2000 ; Borken et al., 2002a,b ; Caravaca et al., 2002 ; Ros et al., 2003 ; Saison et al., 2006 ; Bastida et al., 2008 ; Laudicina et al., 2011).

Le compost stimule aussi l'activité des organismes du sol. Il constitue un apport de substrats mais aussi de cofacteurs contribuant à l'augmentation de l'activité microbienne (Albiach et al., 2000). Borken et al. (2002b) ont montré que les composts de déchets ménagers constituent une source importante de phosphore et de cations basiques contribuant à l'amélioration de l'activité microbienne des sols forestiers. Dans leur étude, l'amendement de compost a permis une augmentation de la respiration des sols forestiers allant jusqu'à 65% après une année d'épandage. Plusieurs études ont montré une augmentation de la respiration des sols après un apport de compost (Tejada et Gonzales, 2003 ; Saison et al., 2006 ; Bastida et al., 2008). Cependant, cette respiration peut être attribuée à une minéralisation du carbone labile apporté par le compost et/ ou à une minéralisation de la matière organique stable du sol (i.e. "priming effect", Leifeld et al., 2002). Au-delà de la respiration des microorganismes, l'activité microbienne peut être aussi évaluée à travers l'étude de leurs activités enzymatiques. Certaines de ces activités peuvent être stimulées après un apport de compost. Gárcia-Gil et al., (2000) ont montré une augmentation des activités enzymatiques oxydo-réductrices (déhydrogénases et catalases) et des activités hydrolases (β-glucosidases et protéases) à la suite de leurs amendements en compost. Les activités uréases liées au cycle de l'azote et phosphatases liées au cycle du phosphore peuvent aussi augmenter après un apport de compost (Crecchio et al., 2004 ; Bastida et al., 2008). L'augmentation de ces activités

enzymatiques peut provenir d'une part d'une contribution directe des enzymes provenant des composts et d'autre part d'une stimulation de la production d'enzymes par les micro-organismes du sol (Guénon, 2010).

Cet accroissement des activités microbiennes pourrait aussi être attribué à une modification de la structure de la communauté microbienne du sol. L'apport de compost constitue un apport d'éléments nutritifs mais aussi une source de micro-organismes. Au cours des différentes étapes du compostage il se produit une succession de microorganismes mésophiles et thermophiles qui sont intégrés directement dans le sol au moment de l'épandage. Ainsi l'augmentation de la biodisponibilité des substrats associée à l'apport d'un inoculum de microorganismes pourrait se traduire par un remaniement de la communauté microbienne indigène du sol. Il en résulterait une augmentation de la biodiversité se traduisant par un changement de la structure de la communauté microbienne comme l'ont montré Bossio et al. (1998), Bastida et al. (2008) et Tabuchi et al. (2008) par analyse des acides gras phospholipidiques, Pérez-Piqueres et al. (2006) par une détermination des profils génétiques et Gomez et al. (2006) en étudiant la diversité fonctionnelle catabolique. Cependant, Saison et al. (2006) excluent l'effet des communautés microbiennes apportées par le compost et attribuent essentiellement les changements fonctionnels aux caractéristiques physico-chimiques des composts et aux communautés microbienne indigènes du sol.

Par ailleurs, l'ajout de compost favorise la suppression des microorganismes pathogènes des sols (Pérez-Piqueres et al., 2006). Cette amélioration peut être attribuée à la composition chimique mais aussi microbienne du sol à travers l'incorporation de microorganismes provenant du compost et la stimulation des microorganismes indigènes après l'apport de compost (Pérez-Piqueres et al., 2006).

De plus, comme mentionné précédemment, l'apport de compost augmenterait le pH du sol et provoquerait une immobilisation des éléments traces métalliques. Il réduit ainsi leurs toxicités et induit indirectement un accroissement de la biomasse microbienne **(**Alburquerque et al., 2011). Par conséquent, l'ajout de compost s'insèrerait dans les techniques de remédiation des sols acides contaminés par les métaux lourds **(**Alburquerque et al., 2011). Cependant, il est important de mentionner que les composts contiennent parfois des métaux lourds. Il faut donc être prudent sur la question d'utilisation des composts dans ces techniques de remédiation.

## 5. Conclusion : compost et qualité des sols

Au-delà de l'apport de nutriments nécessaires à la croissance des plantes, les amendements organiques permettent l'amélioration des propriétés du sol. Ainsi l'apport de composts aux sols est une pratique agronomique contribuant à l'amélioration de leur qualité. Le concept de qualité des sols est une notion subjective et très dynamique (Gros, 2002), cependant l'évaluation de la qualité des sols se base sur l'évaluation de ses propriétés physiques, chimiques et biologiques. Ces propriétés sont interconnectées entre elles, les propriétés chimiques et physiques d'un sol conditionnent les processus biologiques qui s'y déroulent et inversement (Albiach et al., 2001 ; Gomez et al., 2006). En résumé, les composts augmentent la quantité de carbone organique des sols, leur stabilité structurale et améliorent leur rétention d'eau et l'immobilisation des ETM. Cette amélioration des propriétés physico-chimiques se traduit par une augmentation de la biomasse microbienne tellurique et de l'activité microbienne et par un changement de la structure microbienne du sol. Il s'ensuit une amélioration du fonctionnement biologique du sol et une augmentation de sa diversité.

## Conclusion de l'étude bibliographique

Le sol est un patrimoine non renouvelable. Face aux changements climatiques actuels et à l'augmentation des populations, la vulnérabilité du sol et des services écosystémiques qu'il rend s'accroît. En particulier dans les zones climatiques Méditerranéennes, les modèles météorologiques prévoient une augmentation des sécheresses estivales et une augmentation des températures (Gibelin et Déqué, 2003) accompagnées par l'apparition plus fréquente d'évènements extrêmes de type canicule et sécheresse (IPCC, 2002 ; IPCC, 2012). Dans ce contexte Méditerranéen, les sols sont particulièrement vulnérables à l'appauvrissement de leur teneur en carbone organique et à l'appauvrissement de leur diversité biologique (Bastida et al., 1998a ; Ros et al., 2003). Les vagues de chaleur et de sécheresse ont des impacts directs sur les microorganismes du sol ainsi que des impacts indirects via les transformations des propriétés physico-chimiques des sols. En particulier, les températures élevées et la dessiccation ont un impact direct sur les biomasses microbiennes (Joergensen et al., 1990 ; Griffiths et al., 2003 ; Jensen et al., 2003 ; Bérard et al. 2011 ; Hamdi et al., 2011). L'augmentation de la température est aussi accompagnée par une augmentation de la vitesse de minéralisation du carbone des sols (Hamdi, 2010) et la sécheresse pourrait augmenter la mobilisation de certains métaux (Sardans et Penuelas, 2007). Ces phénomènes biologiques et physico-chimiques accentuent ainsi l'appauvrissement des sols en termes de ressources et de diversité. Cependant, les microorganismes développent des stratégies de protection allant de l'échelle cellulaire jusqu'à l'échelle communautaire mais ces stratégies peuvent s'avérer insuffisantes et se traduire in fine par une perte de stabilité des sols en terme de diversité structurelle et fonctionnelle microbienne (Bérard et al., 2011 ; Chowdhury et al., 2011).

Le maintien et l'amélioration de la qualité de ces sols sont essentiels pour assurer la durabilité de l'environnement et la biosphère (Bastida et al., 2008b). Il est donc primordial de s'attaquer aux problèmes liés aux changements climatiques susceptibles d'impacter la diversité biologique qui supporte le fonctionnement des écosystèmes, en particulier des sols, et de proposer des solutions de remédiation. Compte tenu de l'amélioration des propriétés physiques, chimiques et biologiques des sols après ajout de compost, cette technique a été utilisée pour restaurer la qualité de sols dégradés (Borken et al., 2002a;b ; Bastida et al., 1998a ; Ros et al., 2003) ou incendiés (Guerrero et al., 2001 ; Larchevêque et al., 2005 ; Guénon, 2010 ; Cordovil et al., 2011 ; Cellier et al., 2012). Les composts riches en matière organique stable permettraient aussi de remédier au grand besoin des sols Méditerranéens

pauvres en matière organique et en nutriments et de réduire les problèmes liés au stress hydrique et thermique de ces sols (Culot, 2005). L'apport de compost au sol pourrait donc constituer une solution d'un intérêt majeur pour prévenir et atténuer les effets des sécheresses et des canicules dans les agrosystèmes Méditerranéens en améliorant la résistance et la résilience de la structure et des fonctions des communautés microbiennes édaphiques conservant ainsi la diversité biologique du sol et son fonctionnement.

**Références**


ADEME, 2005. Impacts environnementaux de la gestion biologique des déchets-Bilan des connaissances. p331.

ADEME, 2011. Schéma territorial de gestion des déchets organiques p16.

Albiach, R., Canet, R., Pomares, F., Ingelmo, F., 2000. Microbial biomass content and enzymatic activities after the application of organic amendments to a horticultural soil.Bioresource Technology 75, 43-48.

Albiach. R., Canet. R., Pomares. F., Ingelmo. F., 2001. Organic matter components and aggregate stability after the application of different amendments to a horticultural soil. Bioresource Technology. 76, 125-129.

Alburquerque. J.A., de la Fuente. C., Bernal. M.P., 2011. Improvement of soil quality after "alperujo" compost application to two contaminated soils characterised by differing heavy metal solubility. Journal of Environmental Management. 92, 733-741.

Arrouays D., Balesdent J., Germon J.C., Jayet P.A., Soussana J.F., Stengel P., 2002. Stocker du carbone dans les sols agricoles de France, Expertise scientifique collective. Ed INRA, 332 p.

Arthur. E., Cornelis. W. M., Vermang. J., De Rocker. E., 2011. Amending a loamy sand with three compost types: impact on soil quality. Soil Use and Management. 27, 116–123.

Bastida, F., Kandeler, E., Moreno, J.L., Ros, M., Hernandez, T., 2008a. Application of fresh and composted organic wastes modifies structure, size and activity of soil microbial community under semiarid climate. Applied Soil Ecology 40, 318-329.

Bastida, F., Zsolnay, A., Hernández, T., García, C., 2008b. Past, present and future of soil quality indices: A biological perspective. Geoderma 147, 159–171.

Ben Ammar, S., 2006. Les enjeux de la caractérisation des déchets ménagers pour le choix de traitements adaptés dans les pays en développement. Résultats de la caractérisation dans le grand tunis. Mise au point d'une méthode adaptée. Institut National Polytechnique de Lorraine. 326p.

Bérard, A., Bouchet, T., Sévenier, G., Pablo, A.L., Gros, R., 2011. Resilience of soil microbial communities impacted by severe drought and high temperature in the context of Mediterranean heat waves. European Journal of Soil Biology 47, 333-342.

Borken. W., Muhs. A., Beese. F., 2002a. Changes in microbial and soil properties following compost treatment of degraded temperate forest soils. Soil Biology and Biochemistry 34, 403-412.

Borken. W., Muhs. A., Beese. F., 2002b. Application of compost in spruce forests: effects on soil respiration, basal respiration and microbial biomass. Forest Ecology and Management. 159, 49-58.

Bossio, D.A., Scow, K.M., Gunapala, N., Graham, K.J., 1998. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. Microbial Ecology 36, 1–12.

Bougnom. B.P., Knapp. B.A., Elhottovi. D., Koubovi. A., Etoa, F.X., Insam. H., 2010. Designer compost with biomass ashes for ameliorating acid tropical soils: Effects on the soil microbiota. Applied Soil Ecology 45, 319–324.

Bresson, L.M., Koch, C., Le Bissonnais, Y., Barriuso, E., Lecomte, V., 2001. Soil Surface Structure Stabilization by Municipal Waste Compost Application. Soil Science Society of America Journal 65, 1804–1811.

Caravaca, F., Barea, J.M., Figueroa, D., Roldán, A. 2002. Assessing the effectiveness of mycorrhizal inoculation and soil compost addition for enhancing reafforestation with *Olea europaea* subsp. *sylvestris* through changes in soil biological and physical parameters. Applied Soil Ecology 20, 107–118.

Cellier, A., Francou, C., Houot, S., Ballini, C., Gauquelin, T., Baldy, V., 2012. Use of urban composts for the regeneration of a burnt Mediterranean soil: A laboratory approach. Journal of Environmental Management 95, S238-S244

Chenu, C., Le Bissonais, Y, Arrouays, D., 2000. Organic matter on clay wettability and soil aggregate stability. 64, Soil Science Society of America Journal 1479-1486.

Chowdhury, N., Burns, R.G., Marschner, P. 2011. Recovery of soil respiration after drying. Plant and Soil 348, 269–279.

Cordovil, C.M.d.S., de Varennes, A., Pinto, R., Fernandes, R.C., 2011. Changes in mineral nitrogen, soil organic matter fractions and microbial community level physiological profiles after application of digested pig slurry and compost from municipal organic wastes to burned soils. Soil Biology and Biochemistry 43, 845-852.

Crecchio, C., Curci, M., Pizzigallo, M.D.R., Ricciuti, P., Ruggiero, P., 2004. Effects of municipal solid waste compost amendments on soil enzyme activities and bacterial genetic diversity. Soil Biology and Biochemistry 36, 1595–1605.

Culot, M., 2005. Filières de valorisation agricole des matières organiques. Document réalisé à la demande du Cabinet du Ministre Benoît Lutgen. Laboratoire d'Ecologie microbienne et d'Epuration des Eaux. Faculté universitaire des sciences agronomiques de Grembloux. 73p.

Drenovsky, R.E., Vo, D., Graham., K.J., Scow, K.M., 2006. Soil Water Content and Organic Carbon Availability Are Major Determinants of Soil Microbial Community Composition Microbial Ecology 48, 424–430.

Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology. 88, 1354-1364.

Gárcia-Gil, J.C., Plaza C., Soler-Rovira P., Polo A., 2000. Long-term effects of municipal solid waste compost application on soil enzyme activities and microbial biomass. Soil Biology and Biochemistry 32, 1173-1181.

Gibelin, A. L., and Déqué, M., 2003. Anthropogenic climate change over the Mediterranean region simulated by a global variable resolution model. Climate Dynamics 20, 327–339.

Gomez, E., Ferreras, L., Toresani, S. 2006. Soil bacterial functional diversity as influenced by organic amendment application. Bioresource Technology 97, 1484–1489.

Griffiths, R.I., Whiteley, A.S., O'Donnell, A.G., Bailey, M.J., 2003. Physiological and community responses of established grassland bacterial populations to water stress. Applied and Environmental Microbiology 69, 6961–6968.

Gros, R., 2002. Fonctionnement et qualité des sols soumis à des perturbations physiques et chimiques d'origine anthropique : réponses du sol, de la flore et de la microflore bactérienne tellurique. Thèse ADEME Université de Savoie, 252p.

Guénon, R., 2010. Vulnérabilité des sols méditerranéens aux incendies récurrents et restauration de leurs qualités chimiques et microbiologiques par l'apport de composts. Thèse Université Paul Cézanne Aix-Marseille III, 248p.

Guerrero, C., Gomez, I., Moral, R., Mataix-Solera, J., Mataix-Beneyto, J., Hernandez, T., 2001. Reclamation of a burned forest soil with municipal waste compost: macronutrient dynamic and improved vegetation cover recovery. Bioresource Technology 76, 221–227.

Hamdi, S., 2010. Vulnérabilité des services écosystémiques des sols Tunisiens face aux changements climatiques régionaux : Sensibilité de la respiration du sol à la température. Université Montpellier II. 168p.

Hamdi, S., Chevallier, T., Ben Aïssa N., Ben Hammouda, M., Gallali, T., Chotte, J.L., Bernoux, M. 2011. Short-term temperature dependence of heterotrophic soil respiration after one-month of pre-incubation at different temperatures. Soil Biology and Biochemistry 43, 1752–1758.

Harada, Y., Inoko, A., Tadaki, M., Izawa, T., 1981. Maturing process of city refuse compost during piling. Soil Science Plant Nutrition 27, 357-364.

Hassen. A., Belguith. K., Jedidi. N., Cherif. A., Cherif. M., Boudabous.A., 2001. Microbial characterization during composting of municipal solid waste. Bioressource technology 80, 217-225.

Houot, S., Francou, C., Vergé-Leviel, C., Michelin, J., Bourgeois, S., et al., 2003. Valeur agronomique et impacts environnementaux de composts d'origine urbaine: variation avec la nature du compost. *AGREDE: Agriculture et épandage des déchets urbains et agroindustriels*. Tercé M. (Ed), Paris. 107-123.

IPCC, 2002. Climate Change and Biodiversity. Technical paper V: 46pp. (http://www.ipcc.ch/pub/tpbiodiv.pdf)

IPCC, 2012. Summary for Policymakers, in *Managing the risks of extreme events and disasters to advance climate change adaptation*, Cambridge University Press., C. B. Field et al. , ed. Cambridge, UK, and New York, NY, USA, 19 p.

Jemai, I., Guirat, S.B., Aissa, N.B., Jedidi, N., Gallali, T., 2011. Effet de l'amendement par fumier de ferme et par compost d'ordures ménagères sur la restauration d'un sol argileux de plaine sous climat semi-aride tunisien. Etude et Gestion des Sols 18, 271-285.

Jensen, K. D., Beier, C., Michelsen, A., Emmett, B., 2003. Effects of experimental drought on microbial processes in two temperate heathlands at contrasting water conditions. Applied Soil Ecology 24, 165–176.

Joergensen, R.G., Brookes, P.C., Jenkinson, D.S., 1990. Survival of the soil microbial biomass at elevated temperatures. Soil Biology and Biochemistry 22, 1129-1136.

Klamer, M., and Bååth, E., 1998. Microbial community dynamics during composting of straw material studied using phospholipid fatty acid analysis. FEMS Microbiology Ecology 27, 9-20.

Lakhdar, A., Hafsi, C., Rabhi, M., Debez, A., Montemurro, F., Abdelly, C., Jedidi, N., Ouerghi, Z., 2008. Application of municipal solid waste compost reduces the negative effects of saline water in Hordeum maritimum L. Bioresource Technology 99, 7160–7167.

Larchevêque, M., Baldy, V., Korboulewsky, N., Ormeño, E., Fernandez, C., 2005. Compost effect on bacterial and fungal colonization of kermes oak leaf litter in a terrestrial. Mediterranean ecosystem Applied Soil Ecology 30 79–89.

Laudicina, V.A., Badalucco, L., Palazzolo, E., 2011. Effects of compost input and tillage intensity on soil microbial biomass and activity under Mediterranean conditions. Biology and Fertility of Soils 47, 63–70.

Leifeld, J., Siebert, S., Kogel-Knabner, I., 2012. Biological activity and organic matter mineralization of soils amended with biowaste composts. Journal of Plant Nutrition and Soil Science 165, 151-159.

Mustin M., 1987. Le compostage, gestion de la matière organique. Ed Dubusc: 954 P.

NF U 44-095. Amendements organiques: Composts contenant des matières d'intérêt agronomique, issues du traitement des eaux. AFNOR, 2004.

NF U44-051. Amendements organiques Dénominations, spécifications et marquage. AFNOR, 2006.

Ouédraogo, E., Mandob, A., Zombréc, N.P., 2001. Use of compost to improve soil properties and crop productivity under low input agricultural system in West Africa. Agriculture, Ecosystems and Environment 84, 259–266.

Paul, E.A. and Clark, F.E., 1996. Soil Microbiology and Biochemistry. Academic Press, San Diego, pp. 245–264.

Pérez-Piqueres, A., Edel-Hermann,V., Alabouvette, C., Steinberg, C., 2006. Response of soil microbial communities to compost amendments. Soil Biology and Biochemistry 38, 460–470.

Ros, M., Hernandez, M.T., Garcia, C., 2003. Soil microbial activity after restoration of a semiarid soil by organic amendments. Soil Biology and Biochemistry 35, 463-469.

Ros, M., Klammer, S., Knapp, B., Aichberger, K., Insam, H., 2006a. Long-term effects of compost amendment of soil on functional and structural diversity and microbial activity. Soil Use and Management 22, 209–218.

Ros, M., Pascual, J.A., Garcia, C., Hernandeza, M.T., Insam, H. 2006b. Hydrolase activities, microbial biomass and bacterial community in a soil after long-term amendment with different composts. Soil Biology and Biochemistry 38, 3443–3452.

Ryckeboer, J., Mergaert, J., Coosemans, J., Deprins, K., Swings, J., 2003b. Microbiological aspects of biowaste during composting in a monitored compost bin. Journal of Applied Microbiology 94, 127- 137.

Ryckeboer, J., Mergaert, J., Vaes, K., Klammer, S., De Clercq, D., Coosemans, J., Insam, H., Swings, J., 2003a. A survey of bacteria and fungi occurring during composting and self-heating processes. Annals of Microbiology 53, 349-410.

Saison, C., Degrange, V., Olivier, R., Millard, P., Commeaux, C., Montange, D., Le Roux, X., 2006. Alteration and resilience of the soil microbial community folllowing compost amendment: effects of compost level and compost-borne microbial community. Environmental Microbiology 8, 247-257.

Sardans, J., and Peñuelas, J., 2007. Drought changes the dynamics of trace element accumulation in a Mediterranean Quercus ilex forest. Environmental Pollution 147, 567-583.

Sharma, V.K., Canditelli, M., Fortuna, F. Cornacchia, G., 1997. Processing of urban and agro-industrial residues by aerobic composting: review. Energy Conversion and Management 38, 453-478.

Tabuchi, H., Kato, K., Nioh, K., 2008. Season and soil management affect soil microbial communities estimated using phospholipid fatty acid analysis in a continuous cabbage (*Brassica oleracea* var. *capitata*) cropping system. Soil Science and Plant Nutrition 54, 369–378.

Tejada, M. and Gonzalez, J.L., 2003. Effects of the application of a compost originating from crushed cotton gin residues on wheat yield under dryland conditions. European Journal of Agronomy 19, 357–368.

Tejada, M., Garcia, C., Gonzalez, J.L., Hernandez, M.T., 2006. Use of organic amendment as a strategy for saline soil remediation: influence on the physical, chemical and biological properties of soil. Soil Biology and Biochemistry 38, 1413–1421.

Tejada, M.A. and Gonzalez, J.L., 2005. Crushed cotton gin compost on soil biological properties and rice yield. European Journal of Agronomy 25, 22–29.

Xiao, Y., Zeng, G.M., Yang, Z.H., Ma, Y.H., Huang, C., Xu, Z.Y., Huang, J., Fan, C.Z., 2011. Changes in the actinomycetal communities during continuous thermophilic composting as revealed by denaturing gradient gel electrophoresis and quantitative PCR. Bioresource Technology 102, 1383–1388.

# CHAPITRE II

# MATERIEL ET METHODES

Ce chapitre est consacré à la présentation du matériel et des méthodes d'analyses utilisés au cours des différentes expériences. Nous avons développé et amélioré une méthode de microrespirométrie proposée par une équipe Ecossaise (Macaulay Institute). Ce chapitre inclut deux articles intitulés : "Improving the MicroRespTM substrate induced respiration method by a more complete description of $CO_2$ behaviour in closed incubation wells" soumis à *Geoderma* (Article 2) et "The FungiResp method : An application of the MicroResp$^{TM}$ method to assess fungi in microbial communities as soil biological indicators" publié dans *Ecological Indicators* (Article 3).

# Matériel et méthodes

Dans ce chapitre, nous présentons la zone d'étude et l'ensemble des matériels et méthodes mis en œuvre au cours des expérimentations réalisées dans ce travail de thèse. Notre étude s'est déroulée à deux échelles : (1) des observations *in situ* à l'échelle parcellaire couvrant une année d'étude et deux périodes d'été après ajout de composts issus ou non d'unité de méthanisation, et (2) et une simulation des conditions extrêmes de canicule/sécheresse à l'échelle des microcosmes au laboratoire pendant les périodes estivales du suivi (l'été 2010 et l'été 2011) à partir d'échantillons de sols prélevés sur les parcelles d'étude aux mois de Juin 2010 et 2011.

Nous décrirons, dans un premier temps la zone d'étude ainsi que les systèmes expérimentaux. Nous exposerons, ensuite les méthodologies analytiques utilisées pour la caractérisation physico-chimique des écosystèmes étudiés ainsi que les méthodologies analytiques utilisées, développées et améliorées dans le cadre de cette thèse pour la caractérisation biologique des sols.

## 1. Zone d'étude

### 1.1. Situation géographique

La zone d'étude se situe au Sud-Est de la France à l'INRA d'Avignon 43°9N/4°9E.

### 1.2. Climat

Le climat de la région d'étude est Méditerranéen. Les précipitations annuelles au cours de la période d'étude (Octobre 2009 à Octobre 2011) sont de 650mm. La température de l'air (calculée sur la base de moyennes mensuelles) minimale enregistrée est de 3,3°C et la température maximale est de 26°C. La figure 1 représente les variations mensuelles moyennes de la température et des précipitations enregistrées au cours de la période d'étude à la station météorologique d'Avignon.

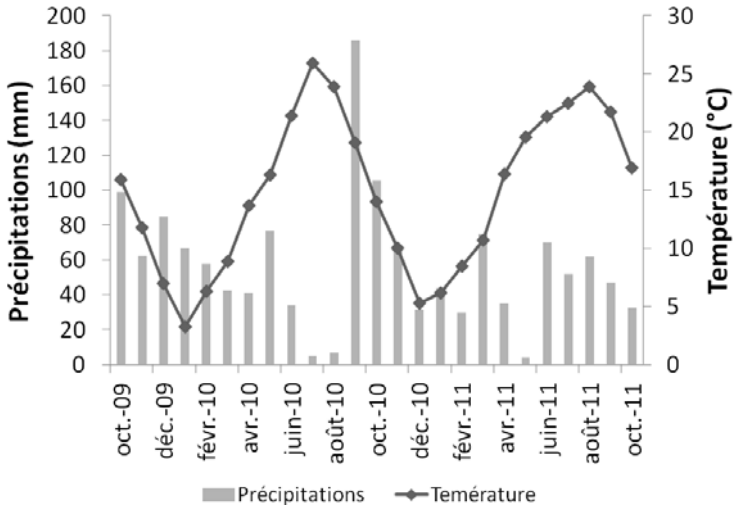


**Figure 1 :** Température et précipitations sur le site pendant la période d'étude.

## 2. Le sol

La caractérisation des paramètres physico-chimiques du sol de la parcelle d'étude a été réalisée par le Laboratoire d'Analyse de Sol à Arras. Les tableaux 1 et 2 présentent les principales caractéristiques granulométriques et physico-chimiques du sol respectivement. L'horizon (0-10 cm) est de texture argilo-limoneuse. Son pH KCl est élevé, ce sol est donc alcalin. Ceci peut être en partie expliqué par sa teneur en calcaire.

**Tableau 1 :** Caractéristiques granulométriques du sol dans l'horizon (0-10 cm)

| Profondeur | Argile 0-2 µm ($g\ kg^{-1}$) | Limon F 2-20 µm ($g\ kg^{-1}$) | Limon G 20-50 µm ($g\ kg^{-1}$) | Sable F 50-200 µm ($g\ kg^{-1}$) | Sable G 200-2000 µm ($g\ kg^{-1}$) |
|---|---|---|---|---|---|
| 0-10 cm | 323 | 259 | 41 | 19 | 6 |

**Tableau 2 :** Caractéristiques physico-chimiques du sol dans l'horizon (0-10 cm).

| pH (eau) | pH (KCl) | $CaCO_3$ ($cmol\ kg^{-1}$) | CEC ($cmol\ kg^{-1}$) | Ca* ($cmol\ kg^{-1}$) | Na* ($cmol\ kg^{-1}$) | Mg* ($cmol\ kg^{-1}$) | K* ($cmol\ kg^{-1}$) | $C_{org}$* ($g\ kg^{-1}$) | MO* ($g\ kg^{-1}$) | $N_{total}$* ($g\ kg^{-1}$) | C/N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 8,51 | 7,85 | 347 | 11,4 | 17,4 | 0,076 | 1,65 | 1,05 | 13,2 | 22,8 | 1,54 | 8,59 |

*Analyses faites après décarbonatation (et rapportées à la masse de sol décarbonatée).

## 3. Description du dispositif au champ

Une parcelle expérimentale (15 × 20 m) d'un sol précédemment cultivé (vesce en 2005, sorgho en 2006, blé en 2007 et pois en 2008) et nu durant la période d'étude a été subdivisée en quatre placettes (placettes de 15 × 5 m). Les différents scénarios d'épandage de l'amendement organique, réalisés dans le cadre du programme PRECCOD (Projet CleanWast, ANR PRECODD 2009-2011[2]), sont représentés dans le tableau 3.

**Tableau 3 :** Répartition des parcelles et planning d'épandage des composts issus (M) ou non issus (NM) d'unité de méthanisation. Le compost NMa a été fourni par l'usine de Launay-Lantic ; le compost M a été fourni par l'usine de Varennes-Jarcy et le compost NMb a été fourni par l'usine de Beaucaire.

| **Placette 1** | **Placette 2** | **Placette 3** | **Placette 4** |
|---|---|---|---|
| Epandage du compost NMa le 10/2009 | Référence R | Epandage du compost M 10/2010 | Epandage du compost NMb 01/2011 |

Les travaux ont été réalisés après épandage de composts de déchets urbains issus ou non d'unité de méthanisation. Le compost M issu d'unité de méthanisation provient de l'unité de compostage de Varenne-Jarcy (Essonne). Les composts NMa et NMb non issus d'unité de méthanisation proviennent, respectivement, des unités de compostage de Launay-Lantic (Côtes d'Armor) et de Beaucaire (Bouches du Rhône). Tous les composts ont été incorporés mécaniquement à 10 cm de profondeur à une dose de 30 t $ha^{-1}$ (dose préconisée). Les caractéristiques des matières organiques exogènes épandues sont données dans le tableau 4 Les essais ont débuté en 2009 sur les placettes NMa et R. Afin que les 4 placettes aient le même historique de travail mécanique, le labour du sol a été réalisé sur les 4 placettes à chaque date d'épandage. L'expérimentation *in situ* à l'échelle parcellaire a permis de réaliser

[2] Programme ANR PRECODD (Programme de Recherche Ecotechnologie et Développement Durable) N° ANR-08-ECOT-004. Développement d'un cadre méthodologique pour l'évaluation des performances environnementales des technologies de prétraitement des déchets. http://cleanwast.brgm.fr/

un suivi qui a couvert une année complète (à l'exception de la placette NMa 2 années de suivi) et deux périodes d'été.

**Tableau 4 :** Caractéristiques des composts épandus.

| | Compost NMa | Compost M | Compost NMb |
|---|---|---|---|
| pH* | 8,10 | | 8,18 |
| Matière sèche % MB | 62,00 | 57,80 | 48,60 |
| Matière organique % MB | 28,00 | 26,80 | 20,80 |
| Azote total % MB | 1,14 | 0,78 | 1,07 |
| Rapport (C/N) | <20 | 17,50 | 11,20 |
| Phosphore % p.s | 0,60 | 0,38 | 0,97 |
| Potassium % p.s | 0,70 | 0,58 | 1,77 |
| Magnésium % p.s | 0,60 | 0,44 | 0,76 |
| Calcaire total % p.s | 5,10 | 4,40 | 11,20 |
| Cuivre mg/kg p.s | 70,00 | 94,00 | 96,30 |
| Manganese mg/kg p.s | 238 | 290 | 375 |
| Fer mg/kg p.s | 10523 | 11610 | 15200 |
| Zinc mg/kg p.s | 247 | 366 | 295 |

*Selon la norme NF ISO 10390, 2005.

La teneur en matières sèches est supérieure à la valeur 30 % du brut. Les rapports C/N> 8 et les concentrations en ETM sont inférieures aux valeurs limites retenues dans la norme pour tous les composts. Les composts répondent donc aux exigences de la norme NF U 44-051.

### 4. Description du dispositif au laboratoire

La simulation des conditions extrêmes de canicule/sécheresse a été réalisée dans des microcosmes au laboratoire pendant les périodes estivales de l'étude (l'été 2010 et l'été 2011) à partir d'échantillons de sols prélevés sur les parcelles d'étude aux mois de Juin 2010 et 2011.

Parallèlement aux suivis saisonniers, des bio-essais simulant une perturbation de sécheresse et de température élevée de courte durée ont été réalisés après 3 campagnes de prélèvement (Printemps, Eté et Automne 2011).

### 4.1. Expérimentations réalisées l'été 2010

Les incubations en microcosmes ont été réalisées sur du sol prélevé (0-10 cm) à partir de la placette référence le 10 Juin 2010. Le dispositif expérimental détaillé par Bérard et al. (2011) a été appliqué. Après tamisage à 2-3 mm, le sol a été progressivement ré-humidifié et maintenu à -0,01 MPa sur une table à succion pendant 13 jours à 25°C ± 2. Le sol a ensuite été réparti dans des boites de pétri (microcosmes) à raison de 40 g de sol par boite. Trois perturbations ont été appliquées (Figure 2) : (i) une perturbation de température élevée "H" (50°C et un taux d'humidité d'environ 25% ≈ potentiel hydrique d'environ −0.01 MPa), (ii) une perturbation de sécheresse "D" (25°C et un taux d'humidité d'environ 5% ≈ potentiel hydrique d'environ −10 MPa), (iii) une perturbation de sécheresse et de température élevée "DH" (50°C et un taux d'humidité d'environ 5% ≈ potentiel hydrique d'environ −10 MPa). La situation contrôle "C" correspond à des microcosmes maintenus à 25°C et à un taux d'humidité d'environ 25% ≈ potentiel hydrique d'environ −0.01 MPa.

Pour les perturbations "D et DH", le potentiel hydrique de −10 MPa a été atteint en plaçant les microcosmes remplis de sol ouverts à l'air libre et à une température de 25°C ± 2. Ces microcosmes ont ensuite été placés dans des dessiccateurs en présence d'un sel de $KNO_3$ à saturation afin de maintenir le taux d'humidité à 5%. Ces dessiccateurs ont été placés dans des incubateurs à 25°C (D) ou 50°C (DH) en fonction de la perturbation appliquée. Pour les traitements "H et C", les microcosmes remplis de sol ont directement été placés dans des dessiccateurs (en présence de coupelle contenant de l'eau) à 50°C (H) ou 25°C (C).

Des durées de perturbation fixées à 2, 7, 14, 21 et 28 jours ont ensuite été simulées. Le taux d'humidité de tous les microcosmes a été régulièrement suivi par pesée.

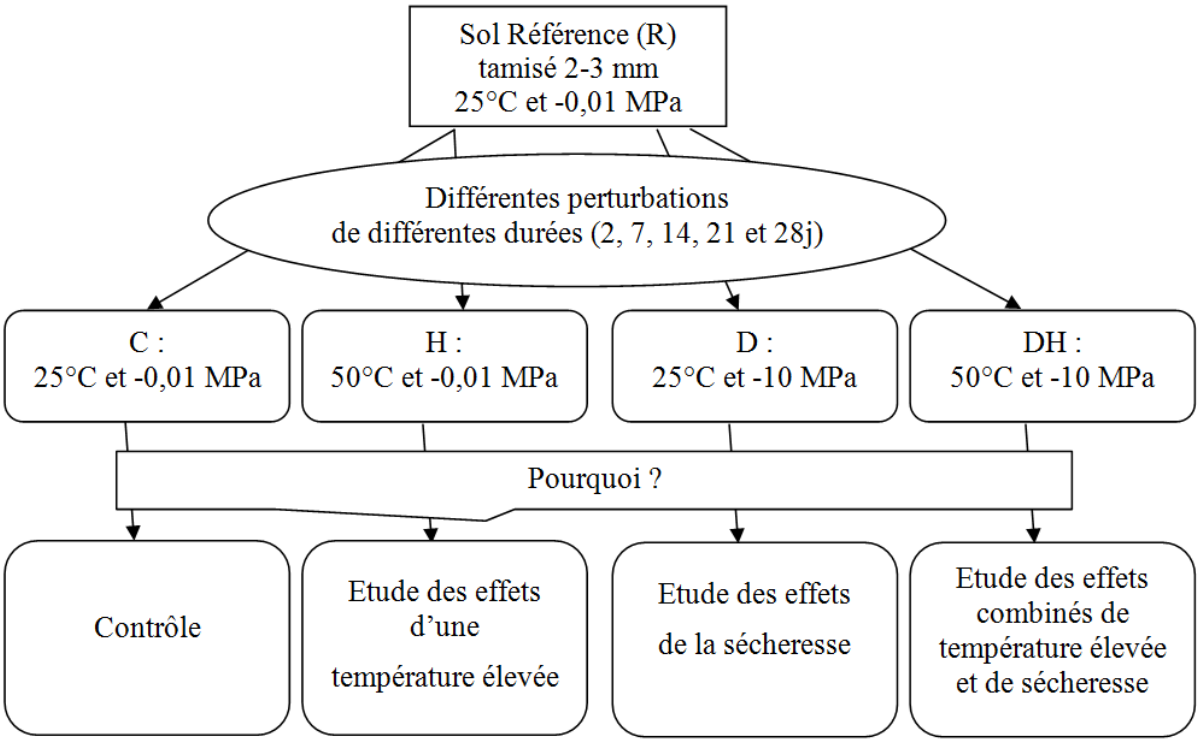


**Figure 2 :** Plan d'expériences : Etude des effets de perturbations de nature et de durées différentes sur les propriétés du sol.

A la fin de chaque durée de perturbation, les sols ont été ramenés aux conditions du contrôle "C" en les ré-humectant progressivement avec de l'eau milliQ au moyen d'un spray (taux d'humidité 25% ≈ −0.01 MPa) et en les plaçant dans des incubateurs à 25°C.

La résistance et la résilience ont été étudiées pour les différentes durées de perturbation (Tableau 5). Pour chaque durée de perturbation, les effets à court terme ont été étudiés 24 h après retour des sols aux conditions du contrôle. Les effets à plus long-terme ont été étudiés 7 jours (éliminer l'effet flush et étudier la résilience à court-terme) et 28 jours (durée maximale de la perturbation) après la fin de la perturbation et retour des sols aux conditions du contrôle et en comptant 2 fois la durée de la perturbation (étude de la résilience à plus long-terme).

**Tableau 5 :** Planification de l'étude de la résilience (les chiffres représentent les campagnes de mesure après la fin du traitement).

| Durées des parturbations (j) | 2 | 7* | 14 | 21* | 28 |
|---|---|---|---|---|---|
| Nombre de jours entre la fin de la perturbation et les analyses | 4 | 7 | 7 | 7 | 7 |
| | 7 | 14 | 14 | 28 | 28 |
| | 28 | 28 | 28 | 42 | 56 |

*Durées des perturbations pour lesquelles les résultats de la résilience ont été exploités dans ce manuscrit

### 4.2. Expérimentations réalisées l'été 2011

Les incubations en microcosmes ont été réalisées sur des sols prélevés (0-10 cm) à partir des 4 placettes expérimentales : la placette référence (R) nue, la placette amendée avec le compost non méthanisé fin 2009 (NMa), la placette amendée avec le compost non méthanisé début 2011 (NMb) et la placette amendée avec le compost méthanisé fin 2010 (M) le 10 Juin 2011. Les sols ont été tamisés à 2-3 mm et seuls le traitement "C" et le scénario de perturbation de sécheresse et de température élevée "DH", simulés en microcosmes sur le sol référence l'été 2010, ont étés appliqués (Figure 3). Les suivis à long terme de la communauté microbienne ont uniquement été réalisés pour une durée de perturbation de 21 j.

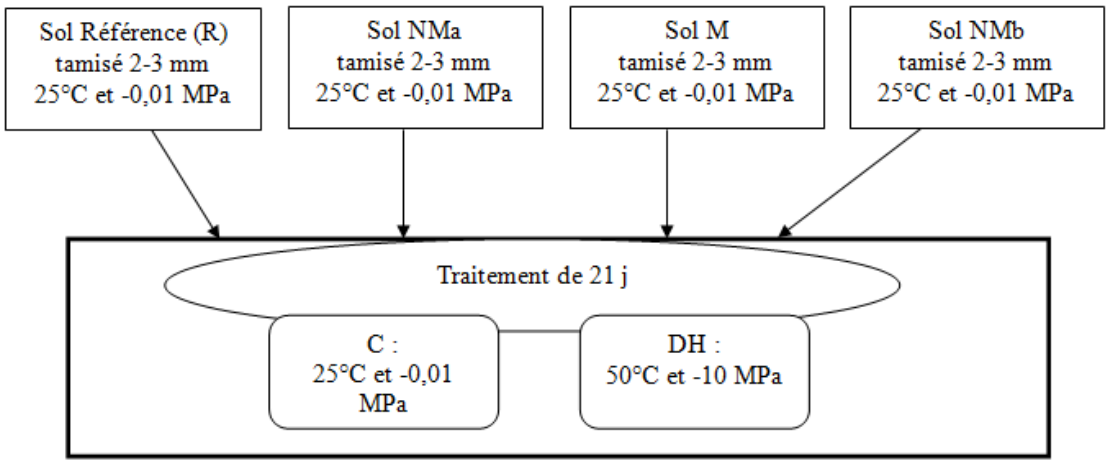


**Figure 3 :** Plan d'expériences : Etude des effets d'ajouts préalables de composts de différentes origines sur la résistance et la résilience de la communauté microbienne après une perturbation de canicule et de sécheresse (DH).

### 4.3. Bio-essais 2011

Les incubations en microcosmes ont été réalisées sur des sols prélevés (0-10 cm) à partir des 4 placettes expérimentales : la placette référence (R) nue, la placette amendée avec le compost non méthanisé fin 2009 (NMa), la placette amendée avec le compost non méthanisé début 2011 (NMb) et la placette amendée avec le compost méthanisé fin 2010 (M) le 10 Juin 2011. Trois campagnes de prélèvement (Printemps, Eté et Automne 2011) ont été réalisées. Les sols ont été tamisés à 2-3 mm. Après réajustement de l'humidité des sols à 19%, les sols ont été répartis à raison de 20g dans 32 microcosmes et placés dans un dessiccateur à 25°C pendant 7 jours afin de permettre un ré-équilibrage. Le taux d'humidité de tous les microcosmes a été régulièrement suivi par pesée. A la fin de cette période de ré-équilibrage, les échantillons séchés à 50°C ont été placés dans l'étuve à 50°C pendant 2j (4 microcosmes par type de sol soit un total de 16 microcosmes). Les échantillons contrôles ont été placées dans un dessiccateur à 25°C (4 microcosmes par type de sol soit un total de 16 microcosmes).

A la fin de la durée de 2j de perturbation, les sols ont été ramenés aux conditions du contrôle "C" en les ré-humectant progressivement avec de l'eau milliQ au moyen d'un spray et en les plaçant dans des incubateurs à 25°C. La résistance a été étudiée 24 h après retour des sols aux conditions du contrôle.

## 5. Analyses physico-chimiques

### 5.1. Humidité et capacité de rétention d'eau

L'humidité du sol a été obtenue en soustrayant la masse d'un échantillon de sol séché sur un module de dessiccation à rayons Infra Rouges. Le taux d'humidité a été exprimé en masse d'eau par masse de sol sec. La capacité de rétention d'eau (Water Holding Capacity) correspondant à la quantité d'eau qu'un sol peut retenir après drainage gravitaire a été obtenue en introduisant 50 g de sol frais dans un entonnoir contenant préalablement de la laine de verre. De l'eau distillée (50 ml) a ensuite été ajoutée et laissée 30 minutes de manière à ce que le sol soit saturé. Après ces 30 minutes, l'eau est drainée gravitairement pendant une durée de 30 minutes et la masse d'eau retenue par le sol est déterminée.

### 5.2. Carbone organique dissous (COD)

Sept grammes de sol ont été mélangés avec 35 ml d'eau ultra pure et agités pendant

1 h. Le mélange a ensuite été centrifugé à 7000 rpm et 5°C pendant 10 min (centrifugeuse SIGMA 6K15) et 5 ml de surnageant ont été microfiltrés à 0.2 µm et conservés à 4°C dans des tubes Venoject. Juste avant analyse, le carbone inorganique apporté principalement par les carbonates du sol a été éliminé par ajout de 100 µL de HCL (0.2 M). Les échantillons ont ensuite été analysés par un COT-mètre (Shimadzu TOC-5050A total organic C analyser).

### 5.3. Manganèse (Mn)

Sept grammes de sol ont été mélangés avec 35 ml de KCl (1M) et agités pendant 1 h. Le mélange a ensuite été centrifugé à 7000 rpm et 5°C pendant 10 min (centrifugeuse SIGMA 6K15) et 10 ml de surnageant ont été microfiltrés à 0.2 µm, additionnés de 50 µL de HCl (38%) et conservés à 4°C dans des tubes Venoject. La concentration de Manganèse a été déterminée par absorption atomique (SpectrAA 220, atomic absorption spectrometer, Varian, Austria).

## 6. Analyses biologiques

### 6.1. Analyse des acides gras membranaires

Afin de caractériser la structure des communautés microbiennes, nous avons opté pour l'étude des profils des acides gras membranaires. De nombreuses études utilisent les acides gras membranaires pour aborder les changements de structures taxonomiques microbiennes liés à des perturbations de type physique (e.g. Peterson and Klug, 1994 ; Bérard et al., 2011) et chimique (e.g. Olsson et al., 1997). Cette méthode permet de différencier les acides gras d'origine bactérienne de ceux d'origine fongique et de déterminer les biomasses des différents groupes microbiens (e.g. Klamer and Bååth, 1998 ; Bérard et al., 2011). Cependant, cette méthode n'avait pas été utilisée auparavant dans notre laboratoire. Un travail de mise au point a donc été effectué afin de choisir et d'adapter la technique d'extraction la mieux adaptée à nos besoins. Les méthodes d'extraction MIDI, EL-FAME (Shutter et Dick, 2000) et la méthode PLFA (Kaur et al., 2005) ont été testées. Pour cette dernière méthode l'extraction de Bligh and Dyer modifiée (White et al., 2009) et l'extraction par la méthode modifiée de Folch (1957) ont été testées. Nous avons aussi testé deux méthodes de dérivatisation: la "Mild Alkaline Methanolisis" (Dowling et al., 1986) et l'utilisation d'un agent de méthylation classique en chimie le BF3/MeOH (Scardino, 2010). Afin de garantir l'extraction des phospholipides de microorganismes vivants uniquement, l'utilisation de la méthode PLFA est

recommandée. Les PLFA représentent la partie active des microorganismes du sol car ils se dégradent rapidement après la mort des cellules (Frostegard et Bååth 1996). Cependant, pour des raisons matérielles et d'urgence analytique, la méthode PLFA n'a pu être utilisée que pour l'étude de la structure des communautés microbiennes extraites des expérimentations réalisées l'été 2011. Nous avons utilisé la méthode ELFAMEs pour les échantillons de sol durant la première expérimentation de 2010, mais le risque de « contamination » des biomarqueurs microbiens par des acides gras d'origine végétale était faible, puisque nous avons travaillé avec du sol nu et non amendé avec du compost.

### 6.1.1. Expérimentations été 2010

Dans cette étude, nous nous sommes basés sur les travaux de Gómez et al. (2010) qui ont comparé trois méthodes d'extraction (la méthode Folch modifiée, la méthode Bligh and Dyer modifiée et l'extraction assistée par micro-ondes) ainsi que deux méthodes de dérivatisation (la "mild alkaline methanolysis" et la dérivatisation par le TMSH) et ont montré que la méthode Folch modifiée permet d'obtenir les meilleurs rendements d'extraction et que les meilleurs taux de conversion des lipides en acides gras méthylés sont obtenus en présence de l'agent de dérivatisation TMSH. Nous avons alors opté pour l'utilisation de la méthode Folch modifiée suivie de la dérivatisation avec le TMSH.

L'extraction a été faite sur 5 g de sol mélangé avec 20 ml de chloroforme-méthanol, 2:1 (v/v). Après agitation, le mélange est décanté pendant 12 h. Le surnageant est ensuite évaporé à sec puis repris dans 500 µL de méthyl therbutyl éther. Cent microlitres de l'extrait sont alors transférés dans un vial et additionnés de 10 µL de standard interne méthyl nonadécanoate (19:0, 230 µg $mL^{-1}$) et 50 µL d'agent de dérivatisation. La solution est ensuite agitée pendant 30 s. Au bout de 30 min de réaction, les EL-FAMEs sont séparés dans une colonne capillaire Elite-5MS (30 m, 0.25 mm, 0.25 µm) et analysés par GC-Masse (GCMS-QP 2010, Shimadzu).

### 6.1.2. Expérimentations été 2011 et suivis saisonniers

La méthode Bligh and Dyer modifiée a été utilisée pour extraire les PLFA des échantillons de sol. L'extraction a été réalisée sur 5 g de sol mélangé avec 20 ml de solution de chloroforme, méthanol, tampon citrate (solution BD 1 :2 :0.8). Après 2 h d'agitation, le mélange est centrifugé à 2200 rpm pendant 10 min. Le surnageant est ensuite récupéré et le culot repris avec 5 ml de solution BD et les étapes précédentes sont répétées. Les surnageants

récupérés sont alors additionnés de 4 ml de tampon phosphate et 4 ml de chloroforme. Le mélange est ensuite placé au frigo pendant 12 h. La phase organique est récupérée et évaporée sous flux d'azote puis reprise avec 0.5 ml de chloroforme. Le fractionnement des acides gras a été réalisé par chromatographie liquide sur colonne de silice. En premier lieu, les lipides neutres ont été élués avec du chloroforme et les glycolipides avec de l'acétone. En dernier lieu, les phospholipides ont été élués avec du méthanol, évaporés sous flux d'azote puis repris avec 1ml de DCM. L'étape de méthanolyse est réalisée après évaporation du DCM, par ajout de 2 ml de mélange toluène/ MeOH (1 :1) et 2 mL d'une solution de KOH à 0.2 M. Le mélange est ensuite chauffé pendant 30 min à 37°C au bain marie puis refroidi. Les PLFA méthylés sont récupérés en ajoutant 4 ml d'une solution d'hexane/ chloroforme (4 :1), à 0,6 ml d'acide acétique (1 M) et 4 ml d'eau milliQ. Après mélange et centrifugation, la phase organique est évaporée puis reprise avec 0.5 ml de DCM. Les PLFA sont ensuite séparés dans une colonne capillaire Elite-5MS (30 m, 0.25 mm, 0.25 µm) et analysés par GC-Masse (GCMS-QP 2010, Shimadzu).

## 6.2. Analyses moléculaires de la communauté bactérienne

Elles permettent à partir de l'ADN extrait du sol, d'estimer la biomasse bactérienne et l'abondance de certains taxa *via* des PCR quantitatives et de déterminer la structure génétique de la communauté bactérienne *via* la méthode ARISA.

### 6.2.1. Extraction et purification de l'ADN (échantillons microcosmes 2010 étude à court-terme pour toutes les durées de perturbation et à long-terme pour la durée 21j de perturbation)

Les différents échantillons ont été conservés à -80°C puis extraits au Centre de Microbiologie du Sol et de l'Environnement (UMR MSE 1229, INRA Dijon, France). La norme ISO 11063 a été utilisée afin d'extraire l'ADN génomique du sol. La première étape consiste à extraire rapidement l'ADN total du sol. Pour cela, 250 mg de sol sont mélangés, en présence de billes de 106 µm et 2 mm de diamètre, avec 1 ml d'un tampon d'extraction contenant 0,1 M Tris-HCl (pH 8,0), 0,1 M EDTA (pH 8,0), 0,1 M de NaCl, 1% de polyvinylpyrrolidone et 2 % de sodium dodécyl-sulfate. Les cellules sont ensuite éclatées par lyse mécanique, chimique et thermique. Après centrifugation, la déprotéinisation a été réalisée en ajoutant aux surnageants 1/10$^{ème}$ du volume total d'une solution d'acétate de sodium (3M) et en incubant les échantillons dans de la glace pendant 10 minutes. Les échantillons ont

ensuite été centrifugés à 14000g pendant 5 min et à 4°C. Les surnageants recueillis, ont ensuite été additionnés de 600 µl d'une solution d'isopropanol pur préalablement stockée à -20°C. Les culots d'ADN obtenus après centrifugation (14000g, 30 min, 4°C) ont été lavés avec une solution d'éthanol à 70%. Après les avoir séchés à 37°C pendant 30 minutes, les culots ont additionnés de 100 µl de tampon TE (Tris-EDTA, pH 8,0). La seconde étape permet d'éliminer d'éventuels éléments polluants (e.g. des sucres, des protéines, des acides humiques). Cette étape de purification de l'ADN extrait du sol a été réalisée en utilisant deux types de colonnes de séparation: une en polyvinyl-polypyrrolidone et l'autre en Sépharose 4B. Enfin, la quantité d'ADN obtenue après purification a été estimée par quantification sur un gel d'agarose 1%.

### 6.2.2. PCR quantitative (échantillons microcosmes 2010 étude à court-terme pour toutes les durées de perturbation et à long-terme pour la durée 21 j de perturbation)

Afin de tester la présence d'inhibiteurs de la PCR quantitative (qPCR) dans les extraits d'ADN du sol (1 ng/µl), nous avons réalisé un test d'inhibition en présence de l'ADN plasmidique pGEM-T de concentration connue ($0{,}5.10^5$ copies/µl), des amorces universelles SP6 (5'-GGT GAC TAT TTA ACT ATA G-3') et T7 (5'-AAT ACG ACT CAC TAT AG-3') et soit 2 µl d'eau (contrôle positif), soit 2 µl de nos échantillons d'ADN du sol. Ce test d'inhibition permet de comparer la quantité de plasmide dans les témoins positifs et les échantillons de sol. Une inhibition a été détectée au niveau de la plupart des échantillons ayant subis une perturbation de sécheresse à température élevée 50°C (D50). Nous avons ensuite dilué tous les extraits d'ADN du sol des échantillons D50 à 0,1 ng/µl et 0,2 ng/µl et repris le test d'inhibition. L'absence d'inhibition a été confirmée pour les deux dilutions. L'analyse d'abondance a été ensuite réalisée sur des extraits d'ADN du sol à 0,2 ng/µl pour tous les échantillons.

L'analyse de l'abondance de la communauté bactérienne totale a été estimée par qPCR en utilisant le couple d'amorces universelles correspondant au gène ADNr 16S: 341F 5'-GGA CCT ACG GGC AGC AG-3' et 515R 5'-ATT ACC GCG GCT GCT GGC A-3'. Nous avons également testé l'abondance des taxons prédominants dans le sol en utilisant les couples d'amorces présentés dans le tableau 7.

**Tableau 7 :** Groupes et amorces utilisées dans cette étude

| Groupe | Amorce sens | Amorce Antisens | Références |
|---|---|---|---|
| α-proteobacteria | Eub338 | Alf685 | Muyzer et al., 1993 |
| Actinobacteria | Actino235 | Eub518 | Fierer et al., 2005 |
| Planctomycètes | Plancto352f | Plancto920r | Muhling et al., 2008 |
| Bacteriodetes | Cbf319f | Eub518 | Fierer et al., 2005 |
| Verrucomicrobiales | Verru349f | Eub518 | Fierer et al., 2005 ; Philippot et al., 2009 |
| β-proteobacteria | Eub338 | Bet680R | Fierer et al., 2005 |
| γ-proteobacteria | Gamma395f | Gamma871r | Muhling et al., 2008 |
| Acibacteria | Acid31 | Eub518 | Fierer et al., 2005 |
| Gemmatimonadetes | Gemmo440F | 534R | Philippot et al., 2009 |
| Firmicutes | Lgc353 | Eub518 | Fierer et al., 2005 |
| CrenArchae | Crenar771F | Crenar975R | Ochsenreiter et al., 2003 |

### 6.2.3. Analyse ARISA des régions 16S-23S (bactérie) (échantillons microcosmes 2010 étude à court-terme pour toutes les durées de perturbation)

La technique ARISA a été utilisée pour étudier la structure génétique de la communauté bactérienne dans les échantillons de sol. Les séquences intergéniques 16S-23S de l'ADNr bactérien ont été amplifiées dans un volume final de 25 µl contenant 0,4 µM des amorces universelles 1552F* (5′-TCG GGC TGG ATC ACC TCC TT-3′) et 132R (5'-GCC GGT TTC CCC ATT CGG-3') (Ranjard et al., 2001) ; 3,75U de Taq DNA polymerase et 5 ng d'ADN matrice. L'amplification PCR a été réalisée dans un thermocycleur PTC-200 selon les conditions d'amplification choisies et présentées dans le Tableau 8.

**Tableau 8 :** Paramètres de l'amplification par PCR pour l'ARISA.

| | | ARISA 16S-23S | |
|---|---|---|---|
| **Etape** | | *Température* | *Durée* |
| Dénaturation initiale | | 94°C | 3min |
| Dénaturation | Cycle | 94°C | 1min |
| Hybridation | | 55°C | 1min |
| Elongation | | 72°C | 2min |
| Elongation finale | | 72°C | 7min |
| Nombre total de cycles | | 30 | |

Les produits PCR ajustés à 12 ,5% de concentration et additionnés du colorant de migration bromophénol cyanol xylène bleu sont déposés sur un gel d'acrylamide à 3,7% (Li-Cor Biosciences). Leur migration est réalisée pendant 15 h à 2500 V/80 W dans Li-Cor 4300 DNA Analysis System (Biosciences). Les profils ARISA de diversité bactérienne obtenus ont été analysés avec le logiciel One-D-Scan software permettant d'avoir une matrice basée sur la quantification de la taille et des intensités relatives de chaque bande.

### 6.3. MicroResp™

L'étude de la respiration et de la diversité fonctionnelle de la communauté microbienne édaphique a été réalisée en utilisant la technique MicroResp™ développée par Campbell et al. (2003). Cette méthode colorimétrique combine les avantages du Biolog™ et de l'approche SIR. Elle consiste en un dispositif de mesure miniaturisé (Figure 4) permettant de mesurer la production de $CO_2$ de la communauté microbienne totale du sol, induite par l'ajout de différents substrats carbonés pendant une incubation de courte durée. Cette technique permet donc de déterminer le profil catabolique d'une communauté microbienne (Degens et Harris, 1997). Elle permet aussi d'estimer la biomasse microbienne du sol à travers la mesure de la respiration induite par le glucose, substrat facilement dégradable par les microorganismes (Anderson and Domsch, 1978). La respiration en présence d'eau

(respiration basale) divisée par la respiration induite par le glucose permet aussi d'estimer le quotient métabolique (qCO2 ; Anderson and Domsch, 1985).

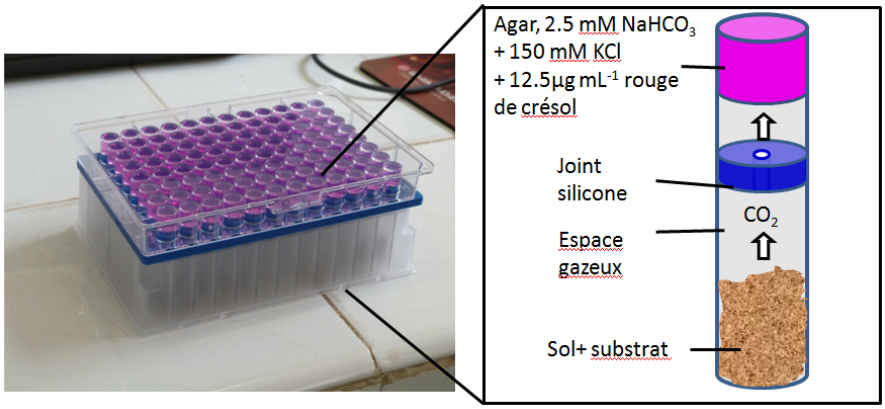


**Figure 4 :** Schéma du dispositif MicroResp™.

La méthode MicroResp™ initialement développée par Campbell et al. (2003) et utilisée pour des sols acides (pH <7). Une étude bibliographique sur l'utilisation de cette technique appliquée spécifiquement aux sols (Tableau 6), nous a permis de constater que la méthode MicroResp™ a été ultérieurement utilisée aussi bien avec des sols acides qu'avec des sols alcalins en supposant, dans la plupart des cas, que le $CO_2$ qui s'accumule dans l'espace gazeux est exclusivement d'origine microbienne et que tout le $CO_2$ microbien ne s'accumule que dans l'espace gazeux, alors qu'il peut s'accumuler dans la solution du sol (Oren et Steinberger, 2008) et dans le gel. Cependant, l'accumulation de $CO_2$ dans l'espace gazeux, la solution du sol et le gel varient selon les caractéristiques physico-chimiques du sol et du gel et une fraction de $CO_2$ dans ces trois compartiments peut ne pas être d'origine microbienne. Après avoir mis en évidence les limites de cette technique, un modèle géochimique pour estimer la production microbienne de $CO_2$, un modèle à base géochimique permet de prendre en compte la distribution du $CO_2$ microbien (sous toutes ses formes) entre solution du sol, espace gazeux et gel, ainsi que la fraction de $CO_2$ qui pourrait avoir une origine abiotique (due à la dissolution de calcite).

### 6.3.1. Correction des artéfacts des bioessais de microrespirométrie (Article 2)

Ce travail a fait l'objet d'un article présenté dans ce chapitre sous le titre "Improving the MicroResp$^{TM}$ substrate induced respiration method by a more complete description of $CO_2$ behaviour in closed incubation wells" et soumis à la revue "Geoderma".

### 6.3.2. Adaptation de la technique inhibiteurs à l'outil MicroResp$^{TM}$, contribution de la partie fongique à la communauté microbienne (Article 3)

Afin de déterminer les profils cataboliques de la communauté microbienne totale et de sa composante fongique, une adaptation de la technique inhibiteurs à l'outil MicroResp$^{TM}$ a été réalisée au cours de ce travail de thèse. Différentes concentrations d'oxytétracycline hydrochloride et de bronopol (Bailey et al., 2003 ; Rousk et al., 2009), inhibiteurs bactériens, ont été testées sur des sols variés dont celui de notre étude afin de choisir le bactéricide le plus efficace et la concentration permettant une inhibition spécifique optimale.

Ce travail a fait l'objet d'un article présenté dans ce chapitre sous le titre "The FungiResp method : An application of the MicroResp$^{TM}$ method to assess fungi in microbial communities as soil biological indicators" et publié dans la revue "Ecological indicators".

**Tableau 6 :** Récapitulatif de l'utilisation de la technique appliquée spécifiquement aux sols MicroResp $^{TM}$ (hiérarchisation selon le pH du sol et la date de publication).

| pH du sol | Durée d'incubation (h) | Substrats alcalins | Substrats acides | $CO_2$ mesuré pour | | | Références |
|---|---|---|---|---|---|---|---|
| | | | | Espace gazeux | Solution du sol | Gel | |
| 5.8 | 6 | + | + | + | | | Campbell et al., 2003 |
| 4.59-5.03 | 4 | +[a] | +[a] | + | | | Lalor et al., 2007 |
| 3.59-4.1 | 6 | +[b] | +[b] | + | | | Yan et al., 2008 |
| 4.3-7.4 | 6 | + | + | + | | | Wakelin et al., 2008 |
| Acidic burned and unburned soils | 6 et 24 | + | + | + | | | Campbell et al., 2008 |
| 4.5-7.5 | 6 | + | + | + | | | Creamer et al., 2009 |
| 5.19-5.64 | 6 | + | + | + | | | Macdonald et al., 2009 |
| 5.5-6.6 | Non spécifiée | + | + | + | | | Gonzalez-Quiňones et al., 2009 |
| 4.7-6.7 | 6 | + | + | + | | | Qu and Varennes 2010 |
| 5.2-6.6 | 6 | + | + | + | | | Burton et al., 2010 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 4.6-5.1 | 6 | + | + | + | | Zhou et al., 2011 |
| 5.1-7.3 | 24 à 4°C | + | + | + | | Stres et al ., 2010 |
| 3.23-7.29 | 6 | + | + | + | | Yao et al., 2011 |
| 5.5-7.2 | 6 | + | + | + | | Liu and Haynes 2011 |
| 4.6-5.1 | 6 | + | + | + | | Zhou et al., 2012 |
| 4.1-4.5 | 6 | + | + | + | | Jiang et al., 2012 |
| 5.89-8.37 | 6 | + | + | + | | Ben Sassi et al., 2012 |
| 7.9 | 6 et 24 | + | + | + | | Ginzburg et al., 2008 |
| 7.5-7.9 | 5[i] | + | + | +* | +* | Oren and Steinberger, 2008a. |
| 7.5-7.9 | 5[i] | + | + | +* | +* | Oren and Steinberger, 2008b. |
| 7.8 | 6 | + | + | + | | Saul-Tcherkas and Steinberger, 2009 |
| 8.5 | 6 | + | + | + | | Kohler et al., 2010 |
| Acidic soils (4.6-5.1) amended with compost (7.9-8.6) | 6 | + | + | + | | Bougnom et al., 2010 |

| | | | | | |
|---|---|---|---|---|---|
| Initial soil pH 6.7 + solutions of ammonium nitrate | 6 | + | + | + | Dalmonech et al., 2010 |
| 8.37 | 6 | +[c] | +[c] | + | Bérard et al., 2011 |
| 8.06-8.35 | 6[ii] et 24 | + | + | + | García-Palacios et al., 2011 |
| Burned and unburned soils with digested pig slurry and composted municipal solid waste (~8) | 6 | + | + | + | Cordovil et al., 2011 |
| 7.76 | 2 | + | + | + | Yu et al., 2012 |
| 8.37 | 6 | + | + | + | Bérard et al., 2012 |
| Non specified | 6 | + | + | + | Lagomarsino et al., 2007 |
| Non specified | 6 | + | + | + | Berg and Steinberger., 2008 |
| Non specified | 6 | + | + | + | Berg and Steinberger., 2010 |
| Non specified | 6 | + | + | + | Marshall et al., 2011 |
| Non specified | 6 | + | + | + | Curlevski et al., 2011 |
| Non specified | 6 | + | + | + | Whitford et al., 2012 |

*Utilisation de facteur de correction.

[a] pH des solutions de substrats ajusté entre 5.5–6.0.

[b] Utilisation de substrats marqués radioactivement.

[c] pH des solutions de substrats ajusté au pH du sol (pH 8.4).

[i] La durée de l'incubation est de 5 h, mais le suivi de l'émission du $CO_2$ a été réalisé entre 3 et 5 h d'incubation.

[ii] La durée de l'incubation est de 6 h, précédée de 2 h de "pré-incubation ouverte" pour éviter le dégagement abiotique de $CO_2$.

**Références**


Anderson, J.P.E., and Domsch, K.H., 1978. A physiological method for the quantitative measurement of microbial biomass in soil. Soil Biology and Biochemistry 10, 215–221.

Anderson, T.H., Domsch, K.H., 1985. Determination of ecophysiological maintenance carbon requirements of soil microorganisms in a dormant state. Biology and Fertility of Soils 1, 81–89.

Bailey, V.L., Smith, J.L, Bolton, H., 2003. Novel antibiotics as inhibitors for the selective respiratory inhibition method of measuring fungal: bacterial ratios in soil. Biology and Fertility of Soils 38, 154–160.

Ben Sassi, M., Dollinger, J., Renault, P., Tlili, A., Bérard, A., 2012. The FungiResp method: An application of the MicroResp$^{TM}$ method to assess fungi in microbial communities as soil biological indicators. Ecological Indicators 23, 482-490.

Bérard, A., Ben Sassi, M., Renault, P., Gros, R., 2012. Severe drought-induced community tolerance to heat wave. An experimental study on soil microbial processes. Journal of Soils and Sediments 12, 513-518.

Bérard, A., Bouchet, T., Sévenier, G., Pablo, A.L., Gros, R., 2011. Resilience of soil microbial communities impacted by severe drought and high temperature in the context of Mediterranean heat waves. European Journal of Soil Biology 47, 333-342.

Berg, N., and Steinberger, Y., 2008. Role of perennial plants in determining the activity of the microbial community in the Negev Desert ecosystem. Soil Biology and Biochemistry 40, 2686–2695.

Berg, N., and Steinberger, Y., 2010. Are Biological Effects of Desert Shrubs More Important than Physical Effects on Soil Microorganisms? Microbial Ecology 59, 121–129.

Bougnom, B.P., Knapp, B.A., Elhottová, D., Koubová, A., Etoa, F.X., Insam, H., 2010. Designer compost with biomass ashes for ameliorating acid tropical soils: Effects on the soil microbiota. Applied Soil Ecology 45, 319–324.

Burton, J., Chen, C., Xu, Z., Ghadiri, H., 2010. Soil microbial biomass, activity and community composition in adjacent native and plantation forests of subtropical Australia. Journal of Soils and Sediments 10, 1267–1277.

Campbell, C.D., Cameron, C.M., Bastias, B.A., Chen, C., Cairney, J.W.G., 2008. Long term repeated burning in a wet sclerophyll forest reduces fungal and bacterial biomass and responses to carbon substrates. Soil Biology and Biochemistry 40, 2246–2252.

Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S., Potts, J.M., 2003. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Applied and Environmental Microbiology 69, 3593–3599.

Cordovil, C.M.d.S., de Varennes, A., Pinto, R., Fernandes, R.C., 2011. Changes in mineral nitrogen, soil organic matter fractions and microbial community level physiological profiles after application of digested pig slurry and compost from municipal organic wastes to burned soils. Soil Biology and Biochemistry 43, 845-852.

Creamer, R.E., Bellamy, P., & Black, H.I.J., Cameron, C.M., Campbell, C.D., Chamberlain, P., Harris, J., Parekh, N., Pawlett, M., Poskitt, J., Stone, D., Ritz, K., 2009. An inter-

laboratory comparison of multi-enzyme and multiple substrate-induced respiration assays to assess method consistency in soil monitoring. Biology and Fertility of Soils 45, 623–633.

Curlevski, N.J.A., Artz, R.R.E., Anderson, I.C., Cairney, J.W.G., 2011. Response of soil microbial communities to management strategies for enhancing Scots pine (Pinus sylvestris) establishment on heather (Calluna vulgaris) moorland. Plant and Soil 339, 413–424.

Dalmonech, D., Lagomarsino, A., Moscatelli, M.C., Chiti , T., Valentini, R., 2010. Microbial performance under increasing nitrogen availability in a Mediterranean forest soil. Soil Biology and Biochemistry 42, 1596-1606.

Degens, B.P., Harris, J.A., 1997. Development of a physiological approach to measuring the catabolic diversity of soil microbial communities. Soil Biology and Biochemistry 29, 1309-1320.

Dowling, N.J.E., Widdel, F., White, D.C., 1986. Phospholipid ester-linked fatty acid biomarkers of acetate-oxidizing sulfate-reducers and other sulfide-forming bacteria. Journal of General Microbiology 132, 1815–1826.

Fierer, N., Jackson, J.A., Vilgalys, R., Jackson, R.B., 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Applied and Environmental Microbiology 71, 4117–4120.

Folch, J., Lees, M., Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226, 497-509.

Frostegard, A., and Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils 22, 59–65.

García-Palacios, P., Bowker, M.A., Chapman, S.J., Maestre, F.T., Soliveres, S., Gallardo, A., Valladares, F., Guerrero, C., Escudero, A., 2011. Early-successional vegetation changes after roadside prairie restoration modify processes related with soil functioning by changing microbial functional diversity. Soil Biology and Biochemistry 43, 1245-1253.

Ginzburg, O., Whitford, W.G., Steinberger, Y., 2008. Effects of harvester ant (Messor spp.) activity on soil properties and microbial communities in a Negev Desert ecosystem. Biology and Fertility of Soils 45, 165–173.

Gómez, B.M., Lores M., Domínguez J., 2010. A new combination of extraction and derivatization methods that reduces the complexity and preparation time in determining phospholipid fatty acids in solid environmental samples. Bioresource Technology 101, 1348-1354

Gonzalez-Quiñones, V., Banning, N.C., Ballesta, R.J., Murphy, D.V., 2009. Influence of cold storage on soil microbial community level physiological profiles and implications for soil quality monitoring. Soil Biology and Biochemistry 41, 1574–1576.

ISO-10390, 2005. Qualité du sol-Détermination du pH. International organization for Standardization (ISO).

ISO-11063, 2010. Soil quality—Method to directly extract DNA from soil samples. International organization for Standardization (ISO).

Jiang, Y.M., Chen, C.R., Xu, Z.H., Liu, Y.Q., 2012. Effects of single and mixed species forest ecosystems on diversity and function of soil microbial community in subtropical China. Journal of Soils and Sediments 12, 228–240.

Kaur, A., Chaudhary, A., Kaur, A., Choudhary, R., Kaushik, R., 2005. Phospholipid fatty acid- A bioindicator of environment monitoring and assessment in soil ecosystem. Current Science 89 1103-1112.

Klamer, M., and Bååth, E., 1998. Microbial community dynamics during composting of straw material studied using phospholipid fatty acid analysis. FEMS Microbiology Ecology 27, 9-20.

Kohler, J., Knapp, B.A., Waldhuber, S., Caravaca, F., Roldán, A., Insam, H., 2010. Effects of elevated CO2, water stress, and inoculation with Glomus intraradices or Pseudomonas mendocina on lettuce dry matter and rhizosphere microbial and functional diversity under growth chamber conditions. Journal of Soils and Sediments 10, 1585–1597.

Lagomarsino, A., Knapp, B.A., Moscatelli, M.C., De Angelis P., Grego, S., Insam, H., 2007. Structural and Functional Diversity of Soil Microbes is Affected by Elevated [CO2] and N Addition in a Poplar Plantation. Journal of Soils and Sediments 7, 399–405.

Lalor, B.M., Cookson, W.R., Murphy, D.V., 2007. Comparison of two methods that assess soil community level physiological profiles in a forest ecosystem. Soil Biology and Biochemistry 39, 454–462.

Liu, Y.Y., Haynes, R.J., 2011. Influence of land application of dairy factory effluent on soil nutrient status and the size, activity, composition and catabolic capability of the soil microbial community. Applied Soil Ecology 48, 133–141.

Macdonald, C.A., Thomas, N., Robinson, L., Tate, K.R., Ross, D.J., Dando, J., Singh, B.K., 2009. Physiological, biochemical and molecular responses of the soil microbial community after afforestation of pastures with *Pinus radiate.* Soil Biology and Biochemistry 41, 1642–1651.

Marshall, C.B., McLaren, J.R., Turkington, R., 2011. Soil microbial communities resistant to changes in plant functional group composition. Soil Biology and Biochemistry 43, 78-85.

Mühling, M., Woolven-Allen, J., Colin Murrell, J., Joint, I., 2008. Improved group-specific PCR primers for denaturing gradient gel electrophoresis analysis of the genetic diversity of complex microbial communities. International Society for Microbial Ecology 2, 379–392

Muyzer, G.A., de Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial population by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes coding for 16S rRNA. Applied and Environmental Microbialogy 59, 695-700.

Ochsenreiter, T., Selezi, D., Quaiser, A., Bonch-Osmolovskaya, L., Schleper, C., 2003. Diversity and abundance of Crenarchaeota in terrestrial habitats studied by 16S RNA surveys and real time PCR. Environmental Microbiology 5, 787-797.

Olsson, P.A., Bååth, E., Jakobsen, I., 1997. Phosphorus effects on the mycelium and storage structures of an arbuscular mycorrhizal fungus as studied in the soil and roots by analysis of fatty acid signatures. Applied and Environmental Microbiology 63, 3531–3538.

Oren, A., Steinberger Y., 2008b. Catabolic profiles of soil fungal communities along a geographic climatic gradient in Israel. Soil Biology and Biochemistry 40, 2578–2587.

Oren, A., Steinberger, Y., 2008a. Coping with artifacts induced by $CaCO_3$–$CO_2$–$H_2O$ equilibria in substrate utilization profiling of calcareous soils. Soil Biology and Biochemistry 40, 2569–2577.

Petersen, S.O., and Klug, M.J., 1994. Effects of Sieving, Storage, and Incubation Temperature on the Phospholipid Fatty Acid Profile of a Soil Microbial Community. Applied and Environmental Microbiology 60, 2421-2430.

Philippot, L., Bru, D., Saby, N.P.A., Cuhel, J., Arrouays, D., Simek, M., Hallin, S., 2009. Spatial patterns of bacterial taxa in nature reflect ecological traits of deep branches of the 16S rRNA bacterial tree. Environmental Microbiology 11, 3096-3104.

Qu, G., and de Varennes, A., 2010. Use of hydrophilic polymers from diapers to aid the establishment of Spergularia purpurea in a mine soil. Journal of Hazardous Materials 178, 956–962.

Ranjard, L.F.P., Lata, J.C., Muogel, C., Thioulouse, J., Nazaret, S., 2001. Characterization of bacterial and fungal soil communities by automated ribosomal intergenic spacer analysis fingerprints: biological and methodological variability. Applied and Environmental Microbiology 67, 4479–4487.

Rousk, J., Brookes, P.C., Bååth, E., 2009. Contrasting soil pH effects on fungal and bacterial growth suggests functional redundancy in carbon mineralisation. Applied and Environmental Microbiology 75, 1589–1596.

Saul-Tcherkas, V., Steinberger, Y., 2009. Substrate utilization patterns of desert soil microbial communities in response to xeric and mesic conditions. Soil Biology and Biochemistry 41, 1882–1893.

Scardino, L., 2010. Mise au point d'une technique d'extraction et d'une technique d'analyse des acides gras membranaires du sol. Rapport de stage, 25pp.

Shutter, A.E., Dick, R.P., 2000. Comparison of Fatty Acid Methyl Ester (FAME) Methods for Characterizing Microbial Communities. Soil Science Society American Journal 64, 1659-166.

Stres, B., Philippot, L., Faganeli, J., Tiedje, J.M., 2010. Frequent freeze-thaw cyclesyield diminishedyet resistant and responsive microbial communities in twotemperate soils: a laboratory experiment. FEMS Microbioloy Ecology 74, 323–335.

Wakelin, S.A., Macdonald, L.M., Rogers, S.L., Gregg, A.L., Bolger, T.P., Baldock, J.A., 2008. Habitat selective factors influencing the structural composition and functional capacity of microbial communities in agricultural soils. Soil Biology and Biochemistry 40, 803–813.

White, P. M., Potter, T.L., Strckland, T.C., 2009. Pressurized Extraction of Soil Microbial Phospholipid and Neutral Lipid Fatty Acids. Journal of Agricultural and Food Chemistry 57, 7171-7177.

Whitford, W.G., Ginzburg, O., Berg, N., Steinberger, Y., 2012. Do long-lived ants affect soil microbial communities? Biology and Fertility of Soils 48, 227–233.

Yan, W., Artz, R.R.E, Johnson, D., 2008. Species-specific effects of plants colonising cutover peatlands on patterns of carbon source utilisation by soil microorganisms. Soil Biology and Biochemistry 40, 544–549.

Yao, H., Campbell, C.D., Qiao, X., 2011. Soil pH controls nitrification and carbon substrate utilization more than urea or charcoal in some highly acidic soils. Biology and Fertility of Soils 47, 515–522.

Yu, J., Kidron, G.J., Pen-Mouratov, S., Wasserstrom, H., Barness, G., Steinberger, Y., 2012. Do development stages of biological soil crusts determine activity and functional diversity in a sand-dune ecosystem? Soil Biology and Biochemistry 51, 66-72.

Zhou, X., Liu, X., Rui, Y., Chen, C., Wu, H., Xu, Z., 2011. Symbiotic nitrogen fixation and soil N availability under legume crops in an arid environment. Journal of Soils and Sediments 11, 762–770.

Zhou, X.Q., Wu, H.W., Koetz, E., Xu, Z.H., Chen, C.R., 2012. Soil labile carbon and nitrogen pools and microbial metabolic diversity under winter crops in an arid environment. Applied Soil Ecology 53, 49– 55.

# Improving the MicroRespTM substrate-induced respiration method by a more complete description of CO2 behaviour in closed incubation wells

P. RENAULT[a*3], M. BEN-SASSI[a,b4], A. BERARD[a5]

[a]INRA, UMR 1114 EMMAH, Domaine St-Paul, Site Agroparc, 84914 Avignon Cedex 9, France

[b]ADEME, 20 avenue du Grésillé, BP 90406, F-49004 Angers Cedex 01, France

*****Corresponding author: Pierre Renault INRA, UMR 1114 EMMAH (Environnement Méditerranéen et Modélisation des Agro-Hydrosystèmes), Domaine Saint-Paul, Site Agroparc, 84914 Avignon Cedex 9, France.

Tel.: +33 (0)4 32 72 22 23; fax: +33 (0)4 32 72 22 12.

E-mail address: pierre.renault@avignon.inra.fr (P. Renault)

**Article 2 soumis à *Geoderma***

[3] Fax: +33 (0)4 32 72 22 12, E-mail: pierre.renault@avignon.inra.fr

[4] Fax: +33 (0)4 32 72 22 12, E-mail: meriem.bensassi@avignon.inra.fr

[5] Fax: +33 (0)4 32 72 22 12, E-mail: annette.berard@avignon.inra.fr

**Abstract**

The MicroResp$^{TM}$ method couples microwell format with colorimetric $CO_2$ measurement to measure soil respiration and yield microbial community physiological profiles. Initially considering only $CO_2$ in the well air space, it was improved by including $CO_2$ in the soil solution without considering its effects on calcite dissolution and on pH of the solution. Our objectives were to improve the method by including $CO_2$ in the gel carrying the colorimetric indicator, explaining the effect of microbial $CO_2$ on pH of the soil solution and calcite dissolution, and checking whether $CO_2$ distribution between calcite, soil solution, air and gel is near-balanced after incubation. We propose a thermodynamic equilibrium model describing (a) distribution of $CO_2$ between calcite, soil solution, gel and air, (b) dissociations of water, carbonic acid, cresol red, and substrates in the gel and soil solution, (c) exchange of adsorbed cations with $H_3O^+$ in the gel, and (d) calcite dissolution in soil. On-gel experiments were designed to calibrate the model, quantify the rate of $CO_2$ exchange with air, and compare conservation modalities. On-soil experiments were designed to check whether calcite dissolution is near equilibrium and whether the model predicts the effect of $CO_2$ on pH of the solution. On-microplate experiments were designed to assess the effects of incubation period and soil quantity on estimated microbial respiration. The model can describe $CO_2$ fate in the wells. Initial properties of the gel vary with storage: soda lime partly extracts $CO_2$ supplied as $NaHCO_3$ and dries out the gel, which can skew the calibration. When incubation is over, the proportion of microbial $CO_2$ in the gel is higher at lower microbial respiration. Incubations of less than 4 h underestimate microbial respiration due to the slow transfer of $CO_2$ in the gel. $CO_2$ in the soil solution cannot be overlooked, but it decreases soil pH and may promote calcite dissolution in calcareous soil. It is important to finely estimate initial $CO_2$ air fraction and to control temperature, which affects both thermodynamic constants and microorganisms.

**Keywords:** soil, substrate-induced respiration, carbon dioxide, geochemistry, agar gel, cresol red

## 1. Introduction

Soil microbial respiration drives $CH_4$ and $N_2O$ greenhouse gas emissions and reductions of metals as it consumes $O_2$ (Lahlah et al., 2009; Parry et al., 2000) and drives organic matter turnover as it mineralizes organic C (Schlesinger and Andrews, 2000), as well as driving acid-base, complexation and precipitation/dissolution reactions (Lahlah et al., 2009; Dassonville et al., 2004). Profiling this multi-substrate respiration using methods like substrate induced respiration opens insights into microbial functional diversity (Chapman et al., 2007; Degens and Harris, 1997).

Microbial respiration has been assessed by $O_2$ consumption (Garland et al., 2003), by $CO_2$ production (Cheng and Coleman, 1989), and by coupling the two (Sierra and Renault, 1995). Although $O_2$ is the terminal electron acceptor of aerobic respiration while $CO_2$ is not involved in respiratory chains (Pelmont, 1993), respiration is more often characterized by $CO_2$ measurements which are easier and sensitive (Dilly, 2001). Oxygen fraction is measured by gas chromatography (Bertrand et al., 2007), dynamic fluorescence quenching (Garland et al., 2003; Klimant et al., 1995) or polarography (Revsbech et al., 1983), whereas $CO_2$ fraction is measured by gas chromatography (Degens and Harris, 1997), infrared radiation absorption (Alavoine et al., 2008; Stevenson et al., 2004; Degens et al., 2001) or colorimetric methods (Chapman et al., 2007; Campbell et al., 2003; Rowell, 1995). $CO_2$ can also be trapped in alkaline solutions (Alavoine et al., 2008; Bertrand et al., 2007) that can be titrated *a posteriori* (Hoffman et al., 2010), making it possible to monitor $O_2$ consumption based on the drop in air space volume or pressure during incubation (Hoffman et al., 2010). Many characterizations suffer common limitations (long gas analysis, large volumes required, complex set-up).

The MicroResp™ method is a miniaturized substrate-induced respiration method that overcomes these limitations (Campbell et al., 2003) and offers a wide range of applications (Ben Sassi et al., 2012; Tlili et al., 2011): it couples the microplate format of the Biolog™ test restricted to cultivable micro-organisms (Stefanowicz, 2006; Garland and Mills, 1991) with the measurement of $CO_2$ air fraction according to the work of Rowell (1995) on indicator dyes in agar gel. In each closed well of a 96-well microplate, moist soil enriched or not in substrate is incubated for 6 h in presence of an agar gel carrying cresol red as indicator dye (Campbell et al., 2003). The method only accounts for $CO_2$ in the well air space, which it assumes is of microbial origin (Campbell et al., 2003). The $CO_2$ air fraction is estimated from gel absorbance at 570 nm (Rowell, 1995). The MicroResp™ method has been widely used on

soils with pH<7 (Zhou et al., 2011; Yao et al., 2011; Burton et al., 2010; Gonzalez-Quiňones et al., 2009; Macdonald-et-al, 2009; Wakelin et al., 2008; Lalor et al., 2007; Campbell et al., 2003) and occasionally used on soils with higher pH and calcite (Bérard et al., 2011, 2012; Saul-Tcherkas and Steinberger, 2009; Wakelin et al., 2008), although the technical manual recommends to restrict to soil with pH<7 (Cameron, 2007). The method was modified by Oren and Steinberger (2008a) to include $CO_2$ in the soil solution and the effects of substrates on soil solution pH and calcite dissolution, but without considering the effects of $CO_2$ on calcite dissolution and on the pH of the solution (Stumm and Morgan, 1996). Their improvements, which require measurements on sterilized soil and evaluation of substrate impact on soil pH, have gained little adoption to date (Oren and Steinberger, 2008b). To our knowledge, $CO_2$ in the agar gel has never been accounted for.

Thus, the MicroResp$^{TM}$ method still suffers from limitations. First, the volume of in-well gel (0.15 mL) cannot be neglected given the volumes of soil solution (about 0.12 mL) and air space (about 1 mL). The pH of the gel (from 7 to 9 or more) magnifies the problem with more $HCO_3^-$ and sometimes $CO_3^{2-}$ than $H_2CO_3$* in the gel solution (Stumm and Morgan, 1996) as well as in solutions of calcareous soils (Ström et al., 2001). Second, failing to account for the fact that increasing the $CO_2$ air fraction decreases the pH of the soil solution (Stumm and Morgan, 1996) ultimately overestimates the amount of $CO_2$ in the solution. For calcareous soils, increasing the $CO_2$ air fraction may also induce calcite dissolution (Tamir et al., 2011; Stevenson and Verburg, 2006; Stumm and Morgan, 1996), leading to an abiotic production of $CO_2$, even without acidic substrates. Third, it has never been checked whether the transfers between calcite, soil solution, air and gel can be considered at equilibrium. Failing to approach equilibrium would make calibration impossible. In addition, although tackled by Oren and Steinberger (2008a), it remains important to know whether most of the calcite dissolution caused by acidic substrate occurs before or after microplate clamping.

Therefore, the objectives of this study were to improve the MicroResp$^{TM}$ method by (i) including $CO_2$ in the gel carrying the colorimetric indicator, (ii) refining microbial $CO_2$ in soil by explaining its effects along with those of substrates and calcite on pH of the solution and quantifying calcite dissolution, and (iii) checking whether $CO_2$ distribution between calcite, soil solution, air space and the gel is near-balanced after 6 h incubation.

## 2. Materials and methods

The study combines a modelling of $CO_2$ distribution during MicroResp$^{TM}$ incubations based on geochemical equilibrium with measurements performed on the gel, on a calcareous soil, and on both combined in the MicroResp$^{TM}$ experimental design. For certain incubations, the soil was sterilized and/or supplied with substrate (e.g. glucose, glucosamine-HCl and $Na_2$-malate as neutral, acid and alkaline substrates, respectively).

### 2.1. Background on the MicroResp$^{TM}$ method

The MicroResp$^{TM}$ experimental design consists of two 96-well microtiter plates placed face-to-face (Campbell et al., 2003). One of these plates with a capacity of 300 µl.well$^{-1}$ holds 150 µL.well$^{-1}$ of an agar gel (1 g.L$^{-1}$) enriched in KCl (0.15 mol.L$^{-1}$), $NaHCO_3$ (2.5 mmol.L$^{-1}$) and cresol red dye (32.7 µmol.L$^{-1}$) to estimate $CO_2$ air fraction based on gel absorbance at 570 nm. After preparation, this plate is generally conserved 7 d in a closed environment with soda lime and water, without protective Parafilm lab film during the first 24 h. The other plate with a capacity of 1.2 mL.well$^{-1}$ holds about 0.45 g.well$^{-1}$ of moist soil with or without substrate. Just before incubation, the plate containing the gel is read with an absorbance microplate reader. The two plates are then sealed together with a silicone rubber gasket with interconnecting holes. After 6 h of incubation, the plates are separated and the plate containing the gel is immediately re-read. We have adapted the method to 24-, 12- and 6-well microplates with silicone rubber gaskets manufactured in our lab to seal identical plates.

### 2.2. Modelling of in-well $CO_2$ distribution and its effect on gel absorbance

The modelling study aimed to describe the relationships between gel absorbance, $CO_2$ air fraction, amounts of total $CO_2$ in both gel and soil solution ($H_2CO_3$* (i.e. aqueous $CO_2$ and actual $H_2CO_3$), $HCO_3^-$, $CO_3^{2-}$), and calcite dissolution. Thermodynamic equilibrium was considered for transfers between soil, air space and gel, for acid-base reactions in solutions, for exchange of adsorbed cations with $H_3O^+$ in the gel solution, and for calcite dissolution. For the gel and the soil solution, mass action laws were combined with equations relating the balance of ionic charges and the total quantity of either cresol red or substrate. Mass action laws were written with $K'$ constants combining $H_3O^+$ activity and concentrations of other species. In soil solution where ionic strength $I \approx 15$ mmol.L$^{-1}$ (Table 3), activities are almost equal to concentrations, so $K'$ constants were assumed to be equal to $K$ constants combining

activities only. In gel solution where $I \approx 150$ mmol.L$^{-1}$, $K'$ constants were estimated from $K^*$ constants combining concentrations only or $K$ constants using specie activities only. Deviations between $K$ and $K^*$ are consistent with independent estimates of activity coefficients.

$CO_2$ distribution between air space, gel and soil solution was described by Henry's law. Henry constants, $k_H$ for water and soil solution and $k_H'$ for saline solution and gel, were estimated from Harned and Davis (1943) and Weiss (1974) equations, respectively (Table 1). Deviation between $k_H$ and $k_H'$ is consistent with the increase in $H_2CO_3^*$ activity coefficient in the gel calculated by the Pytkowicz (1975) equation (Table 1). The fugacity coefficient of $CO_2$ in air was set to 1 (DOE, 1994). $H_2O$ activity in gel and soil solution was set to 1 (Stumm and Morgan, 1996). $K_w$ and $K_w^*$ constants for $H_2O$ ion product were estimated from Harned and Owen (1958) and Millero (1995) equations, respectively (Table 1). $K_{a1}$ and $K_{a2}$ constants for the first and second dissociations of $H_2CO_3^*$, respectively, were estimated from Weiss (1974) equations (Table 1), and $K_{a1}^*$ and $K_{a2}^*$ constants were estimated from Millero et al. (2007) equations for NaCl solutions (Table 1) since they differ from those for seawaters (Millero et al, 2006). In the gel, cresol red symbolized by $H_2CR$ dissociates into $HCR^-$ and $CR^{2-}$. The first dissociation is complete, as its $pK_{CR1}$ constant is about 1.1 (El Nahhal et al., 2012; Heger et al., 2006; French et al., 2002; Smith and Matachek, 2002), and the $K_{CR2}$ constant of the second dissociation was estimated by the French et al. (2002) equation (Table 1). $K_{CR2}'$ was deduced from $K_{CR2}$ and $HCR^-$ and $CR^{2-}$ activity coefficients. Exchange of adsorbed cations with protons in the gel was accounted for empirically (see equation (7) below). Activity coefficients of ions in the gel solution were calculated by the Davies equation (Table 1) (Pankow, 1991), except for $CR^{2-}$ from which the two negative charges have to be considered independently, as is the case with other diprotic acids used as indicator dyes (Salvatore et al., 1986). We thus extended the relationship that uses specific interaction theory for bromophenol blue (Salvatore et al., 1986) in order to assess realistic variations in the $pK_{CR2}$ of the second dissociation of cresol red at 25°C (Table 1). In soil, dissociations of acid and alkaline substrates were taken into account: retained reactions for substrates containing more than one acid or alkaline functional group were chosen based on their $pK$ and the pH of the soil (Table 2). For calcareous soils, the solubility constant $K_S$ for calcite was set to 8.3, mirroring data reported by Stumm and Morgan (1996) at 25°C. The simulated increase of $[Ca^{2+}]$ during incubations makes it possible to assess the release of abiotic $CO_2$. Geochemical simulations were performed on soils at various $CO_2$ air fractions for different

substrates and amounts of substrates. pH was adjusted so as to cancel the sum of charges of ions in solution. The variation $\Delta[CO_2]_{tot}$ in total $CO_2$ concentration in the solution ($H_2CO_3$*, $HCO_3^-$ and $CO_3^{2-}$) minus the increase $\Delta[Ca^{2+}]$ in $Ca^{2+}$ amount for calcareous soils between the considered and 0.04% $CO_2$ air fractions was then fitted by empirical functions:

$$\Delta[CO_2]_{tot} - \Delta[Ca^{2+}] = a \times ([CO_2]_a)^b - c \quad (1)$$

where *a*, *b* and *c* are 3 parameters specific to each "soil – substrate type – substrate amount" combination. Considering initial and final $CO_2$ air fractions eliminates the reference to 0.04% $CO_2$ that was introduced to facilitate the adjustment of empirical functions.

The measured dimensionless absorbance $A_{pH}$ is the sum of the absorbances of empty microplate $A_e$, agar gel without cresol red $A_g$, and cresol red $A_{CR}$:

$$A_{pH} = A_e + A_g + A_{CR} \quad (2)$$

$A_{CR}$ may be expressed as a function of $HCR^-$ and $CR^{2-}$ concentrations:

$$A_{CR} = \varepsilon_{HCR^-} \times l_g \times [HCR^-] + \varepsilon_{CR^{2-}} \times l_g \times [CR^{2-}] \quad (3)$$

where $l_g$ is the path length of light through the gel (m), and $\varepsilon_{HCR-}$ and $\varepsilon_{CR2-}$ the molar absorptivities of $HCR^-$ and $CR^{2-}$ ($L.mol^{-1}.m^{-1}$), respectively. Combining equations (2) and (3) with a mass action law for the second dissociation of cresol red and a related equation for the stability of total cresol red amount leads to the following estimate of gel solution pH:

$$pH = pK_{CR2}' + \log_{10}\left(\frac{A_{pH} - (A_e + A_g) - \left(A_{max} \times \left(\frac{\varepsilon_{HCR^-}}{\varepsilon_{CR^{2-}}}\right)\right)}{A_{max} + (A_e + A_g) - A_{pH}}\right) \quad (4)$$

where $A_{max}$ ($= \varepsilon_{CR^{2-}} \times l_g \times [CR_{tot}]$) is the absorbance of cresol red when it is in $CR^{2-}$ form only. The ratio $\varepsilon_{HCR^-} / \varepsilon_{CR^{2-}}$ was set to 0.0019, in line with Smith and Matachek (2002) at 574 nm. The concentration of $H_2CO_3$* in the gel solution may then be estimated as:

$$[H_2CO_3^*] = \frac{\Delta q_g + \left(\frac{10^{-pH}}{\gamma_{H_3O^+}}\right) + 10^{pH - pK_w'}}{10^{pH - pK_{a1}'} + \left(2 \times 10^{2pH - (pK_{a1}' + pK_{a2}')}\right)} \quad (5)$$

where $\Delta q_g$ is the net charge of ions other than $H_3O^+$, $HO^-$, $HCO_3^-$ and $CO_3^{2-}$ in the gel solution (mol $c^+.L^{-1}$). It would be equal to the concentration $\left[Na^+\right]_{NaHCO_3}$ of $Na^+$ supplied as $NaHCO_3$ (mol.$L^{-1}$) if agar did not affect the solution, which is not the case (Ferreira et al., 2012; Scholten and Pierik, 1998; Lahaye and Rochas, 1991):

$$\Delta q_g = \left[Na^+\right]_{NaHCO_3} + \Delta c^+ \tag{6}$$

where $\Delta c^+$ is the net concentration of charges (mol $c^+.L^{-1}$) of ions released by agar. $\Delta c^+$ increases with decreasing pH of the gel, as in other media (Renault et al., 2009), so we assumed the following equation:

$$\Delta c^+ = \Delta c_{min}^{+}{}^+ + \left( \Delta c_{max}^+ \times \left( 1 - \frac{\left( \frac{pH}{pH_{1/2}} \right)^\alpha}{1 + \left( \frac{pH}{pH_{1/2}} \right)^\alpha} \right) \right) \tag{7}$$

where $\Delta c_{min}^+$ is the concentration of charges released by agar at a high pH (mol $c^+.L^{-1}$), $\Delta c_{max}^+$ the maximum concentration of charges released during acidification (mol $c^+.L^{-1}$), $pH_{1/2}$ the pH at which one half of the initially-adsorbed charges are released, and $\alpha$ an empirical constant related to the brutal or progressive release of charges around $pH \approx pH_{1/2}$. $Q_g(CO_2)$, i.e. the amount of $CO_2$ stored in the gel (mol) as $H_2CO_3$*, $HCO_3^-$ and $CO_3^{2-}$ is then easily estimated:

$$Q_g(CO_2) = V_g \times \left[H_2CO_3*\right] \times \left(1 + 10^{pH - pK_{a1}'} + \left(2 \times 10^{2pH - (pK_{a1}' + pK_{a2}')}\right)\right) \tag{8}$$

where $V_g$ is the volume of the gel (L). While the $CO_2$ air fraction is initially the lab $CO_2$ air fraction not in equilibrium with the gel, its final value is assumed in equilibrium with the gel and estimated from Henry's law (Table 1):

$$\left[CO_2\right]_a = k_H' \times \left[H_2CO_3*\right]_g \tag{9}$$

where a and g subscripts are for air and gel, respectively. The amount of $CO_2$ stored in the air space is estimated as:

$$Q_a(CO_2) = V_a \times \left[CO_2\right]_a \tag{10}$$

where $V_a$ is volume of the air space (L). Thus, variations in $CO_2$ quantities in both gel and air space are estimated from the amounts of $CO_2$ in these compartments at the start and the end of

incubations, assuming that the gel is not in equilibrium with the air space or the soil solution at the start. In contrast, empirical functions (equation (1)) are used to estimate the variations $\Delta Q_s(\mathrm{CO_2})$ of amount of $CO_2$ in soil solution (mol) minus $CO_2$ released by calcite dissolution:

$$\Delta Q_s(\mathrm{CO_2}) = V_s \times \left(\Delta[\mathrm{CO_2}]_{\mathrm{tot},t=6\mathrm{h}} - [\mathrm{CO_2}]_{\mathrm{tot},t=0}\right) \tag{11}$$

where $V_s$ is the volume of the soil solution (L). A negative value means that abiotic $CO_2$ production exceeds the total amount of $CO_2$ stored in the soil solution.

In addition, we assume that the variability in $A_{p\mathrm{H}}$ between wells of a given plate results from the variability in light path length $l_g$. Initial and final absorbances $A_{p\mathrm{H}}$ of each well were therefore replaced by absorbances $A_{\mathrm{pH}}$' by accounting for the initial absorbance of the target well $A_{p\mathrm{H}}(t=0)$ and the mean absorbance $\overline{A_{p\mathrm{H}}}(t=0)$ across all the wells in a plate:

$$A_{p\mathrm{H}}' = A_e + \left(\left(\frac{\overline{A_{p\mathrm{H}}}(t=0) - A_e}{A_{p\mathrm{H}}(t=0) - A_e}\right) \times \left(A_{p\mathrm{H}} - A_e\right)\right) \tag{12}$$

In this way, $A_{p\mathrm{H}}$, $A_g$ and $A_{\max}$ can be replaced by $A_{p\mathrm{H}}'$, $\overline{A_g}$ and $\overline{A_{\max}}$, respectively, in equation (4). When $A_e \approx 0$, equation (12) approaches the correction of Campbell *et al.* (2003):

$$A_{p\mathrm{H}}' \approx \left(\frac{\overline{A_{p\mathrm{H}}}(t=0)}{A_{p\mathrm{H}}(t=0)}\right) \times A_{p\mathrm{H}} \tag{13}$$

At high T, the gel is distributed in greater amounts than desired, i.e. 0.20, 0.94, 1.74 and 4.09 mL instead of 0.15, 0.664, 1.314 and 3.27 mL for 96-, 24-, 12- and 6-well plates, respectively, and mean gel thickness $\overline{l_g}$ (mm) varies with plates, i.e. 5.51, 4.68, 4.38 and 4.13 mm, for 96-, 24-, 12- and 6-well plates, respectively. Therefore, $\overline{A_g}$ and $\overline{A_{\max}}$ were estimated from measured values on 96-well microplates according to the following equations:

$$\overline{A_g}(n_w = i) = \left(\frac{\overline{l_g}(n_w = i)}{\overline{l_g}(n_w = 96)}\right) \times \overline{A_g}(n_w = 96) \tag{14a}$$

$$\overline{A_{\max}}(n_w = i) = \left(\frac{\overline{l_g}(n_w = i)}{\overline{l_g}(n_w = 96)}\right) \times \overline{A_{\max}}(n_w = 96) \tag{14b}$$

where $n_w$ is the number of wells of the considered microplate.

Thus, estimating the amount of microbial $CO_2$ produced during MicroResp$^{TM}$ incubations hinges on knowing the values of 3 parameters relative to absorbance ($A_e$, $\overline{A_g}$ and $\overline{A_{max}}$) and 4 parameters relative to the exchange properties of the gel ($\Delta c^+_{min}$, $\Delta c^+_{max}$, $p\mathrm{H}_{1/2}$, and $\alpha$). All other parameters may be estimated from the literature.

**Table 1:** Thermodynamic parameters involved in the calculation of geochemical equilibrium. R is the gas constant (8.31441 $J.mol^{-1}.K^{-1}$), T the temperature (K), P the air pressure (Pa), and S the salinity of the agar gel solution (expressed as equivalent g $NaCl.kg^{-1}$).

| Constant | Equation | References |
|---|---|---|
| $k_H$: Henry's constant for $CO_2$ solubilization in pure water | $\frac{1}{k_H} = 10^{\left(-\left(\left(\frac{-2622.38}{T}\right)\right)+\left(-0.0178471\times T\right)+15.5873\right)} \times \frac{1000\times R\times T}{P}$ | Harned and Davis (1943) |
| $k_H'$: Henry's constant $CO_2$ solubilization in saline water | $\frac{1}{k_H'} = \exp\left(\begin{array}{l}\frac{9345.17}{T} + 23.3585\times\ln\left(\frac{T}{100}\right) - 60.2409 + \\ S\times\left(0.023517 + 0.00470356\times\left(\frac{T}{100}\right)^2 - 0.023656*\left(\frac{T}{100}\right)\right)\end{array}\right) \times \frac{1000\times R\times T}{P}$ | Weiss (1974) |
| $\gamma_{H_2CO_3*}$: Activity of $H_2CO_3$* in saline water | $\gamma_{H_2CO_3*} = 10^{0.00035863+\left(0.00196297\times S\right)}$ | Pytkowicz (1975) |
| $K_w$: Ion product of water in pure water | $pK_w = -\log_{10}\left(K_w\right) = \frac{4470.99}{T} - 6.0875 + 0.01706\times T$ | Harned and Owen (1958) |

| | | |
|---|---|---|
| $K_w$': Ion product of water in saline water | $$pK_w^* = -\log(K_w^*) = -\log\left(\exp\left(\begin{array}{l} 148.9802 + \left(\frac{-13847.26}{T}\right) + (-23.6521 \times \ln(T)) \\ \quad + \left(\left(-5.977 + \frac{118.67}{T} + 1.0495 \times \ln(T)\right) \times \left(S^{0.5}\right)\right) \\ \quad + (-0.01615 \times S) \end{array}\right)\right)$$ | Millero (1995) |
| $K_{a1}$: First dissociation constant of carbonic acid in pure water | $$pK_{a1} = -\log(K_{a1}) = -114.3106 + \left(\frac{5773.67}{\mathrm{T}}\right) + (17.779524 \times \ln(T))$$ | Weiss (1974) |
| $K_{a1}$*: First dissociation constant of carbonic acid in saline water | $$pK_{a1}^* = -\log(K_{a1}^*) = pK_{a1} + A_1 + \frac{B_1}{T} + (C_1 \times \ln(T)) \quad \text{with:}$$ $$A_1 = 35.2911 \times m^{0.5} + 0.8491 \times m - 0.32 \times m^{1.5} + 0.055 \times m^2$$ $$B_1 = -1583.09 \times m^{0.5}$$ $$C_1 = -5.4366 \times m^{0.5}$$ | Millero et al. (2007) |
| $K_{a2}$: Second dissociation constant of carbonic acid in pure water | $$pK_{a2} = -\log(K_{a2}) = -83.2997 + \left(\frac{4821.38}{T}\right) + (13.5962 \times \ln(T))$$ | Weiss (1974) |

| | | |
|---|---|---|
| $K_{a2}$*: Second dissociation constant of carbonic acid in saline water | $pK_{a2}* = -\log(K_{a2}*) = pK_{a1} + A_2 + \frac{B_2}{T} + (C_2 \times \ln(T))$ with:<br>$A_2 = 38.2746 \times m^{0.5} + 1.6057 \times m - 0.647 \times m^{1.5} + 0.113 \times m^2$<br>$B_2 = -1738.16 \times m^{0.5}$<br>$C_2 = -6.0346 \times m^{0.5}$ | Millero et al. (2007) |
| $K_{CR2}$: Second dissociation constant of cresol red in pure water | $pK_{a2} = -\log(K_{a2}) = 2.049 + \left(\frac{913.4}{T}\right) + (1.266 \times \log(T))$ | French et al. (2002) |
| $\gamma_{CR2}$*: Activity coefficient for cresol red anion $CR^{2-}$ with 2 $e^-$ charges | $\gamma_{CR^{2-}} = \left(2 \times \left(-0.5107 \times \left(\frac{\sqrt{I}}{1+(1.5 \times \sqrt{I})}\right)\right)\right) - (-0.178 * m)$ | Empirical equation deduced from |
| $\gamma_i$*: Davis equation of Activity coefficients for ionic compound i, having a charge $z_i$, except for $CR^{2-}$ | $\log(\gamma_i) = -A \times z_i^{\,2} \times \left(\left(\frac{\sqrt{I}}{1+\sqrt{I}}\right) - 0.2 \times I\right)$ | Pankow (1991) |

**Table 2:** Retained acid-base reactions for substrates used in MicroResp™ measurements for soils whose pH (water) is about 7.5-8.5.

| Substrate supplied | Acid form | Charge of the acid form | Charge of the alkaline form | pKa |
|---|---|---|---|---|
| Glucosamine-HCl | $C_6H_{13}NO_5$-HCl | +1 | 0 | 11.5 |
| $Na_2$-Malate | $Na_2$-$C_4H_4O_5$ | -1 | -2 | 5.1 |
| Alanine | $C_3H_7NO_2$ | 0 | -1 | 9.71 |
| Glycine | $C_2H_5NO_2$ | 0 | -1 | 9.58 |

## 2.3. Experimental approach

### 2.3.1. The soil

Measurements were performed on a calcareous cambisol (FAO classification) from the INRA's Saint-Paul experimental station (43°91' N, 4°88' E) near Avignon. It was cultivated with peas in 2008, but since summer 2008 the soil laid bare. Annual rainfall is about 650 mm. About 20 kg of this soil was sampled in the first 10 cm depth at the edge of a 0.0075 ha experimental field on 8 December 2011. Its moisture was about 21.3% wt/wt. The soil was air-dried for 4 d in the lab (14% wt/wt residual moisture), mechanically crushed, sieved at 2 mm for soil analyses and at 2 to 3 mm for experiments, and stored at 4°C in hermetically-sealed bags until the beginning of the experiments. Its properties, measured at the *Laboratoire d'Analyse des Sols* (LAS-INRA) in Arras (France), were as follows: 347 g $kg^{-1}$ $CaCO_3$ and, after decarbonation, 323 g $kg^{-1}$ clay; 259 g $kg^{-1}$ silt; 41 g $kg^{-1}$ sand; 13.2 g $kg^{-1}$ organic C; 1.54 g $kg^{-1}$ total N; 1.4 mg $kg^{-1}$ $N\text{-}NH_4^+$; 101 mg $kg^{-1}$ $N\text{-}NO_3^-$. Soil pH(water) and pH(KCl 1 M) were 8.51 and 7.85, respectively. Physicochemical properties of solutions for soil-to-solution mass ratios of 1, 2.5 and 5 (Table 3) were used to assess composition of the soil solution in MicroResp™ incubations. For geochemical simulations on this soil, we have assumed that the sum of charges of ions other than $H_3O^+$, $HO^-$, $HCO_3^-$ and $CO_3^{2-}$ is equal to 4.6 mmol $c^+.L^{-1}$, including the initial concentration of $Ca^{2+}$ equal to 6 mmol $c^+.L^{-1}$.

**Table 3:** Physicochemical properties of the solutions for mixtures of soil-to-solution mass ratios of 1, 2.5 and 5.

| Dilution | pH(water) | $Ca^{2+}$ | $Mg^{2+}$ | $K^+$ | $NO_3^-$ | $Cl^-$ |
|---|---|---|---|---|---|---|
| | | ---------- mMol.$L^{-1}$ ---------- | | | | |
| 5 | 7.56 | 0.47 | 0.049 | 0.212 | 0.28 | 0.019 |
| 2.5 | 7.84 | 0.71 | 0.078 | 0.252 | 0.54 | 0.025 |
| 1 | 7.88 | 1.38 | 0.173 | 0.360 | 1.12 | 0.057 |
| Limit for ω=31%* | 7.97 | 2.96 | 0.426 | 0.523 | 3.06 | 0.120 |

*: linear regression of Ln values

### 2.3.2. Experiments on the gel

The agar product used in this work was the Merk 1614 product. Its properties are presented and compared to those of other market products in Scholten and Pierik (1998).

Calibration and assessment of the relationship between $A_{pH}$' and $CO_2$ air fraction: We used an EL800 absorbance microplate reader (BioTek Instruments, Winooski, VT) to measure (i) absorbance of empty microplates ($A_e$), (ii) mean absorbance of microplates filled with gel prepared as in the MicroResp™ method (Campbell et al., 2003) but excluding cresol red ($A_e + \overline{A_g}$), and (iii) mean absorbance of microplates filled with gel containing or not cresol red and in which $NaHCO_3$ 2.5 mmol.$L^{-1}$ was replaced by NaOH 10 mmol.$L^{-1}$ to ensure a pH>11. The mean maximum absorbance of cresol red as $CR^{2-}$ ($\overline{A_{max}}$) was estimated by the difference in absorbances at pH>11. Just after reading the absorbances, gel pH was measured in 8 wells of each microplate using a 16-gauge micro-combination needle pH probe (MI-414B, Microelectrodes Inc., Bedford, NH) gently touching the bottom of the wells and connected to a Bioblock Scientific 93327 pH-EH meter (HANNA Instruments, Smithfield, RI). Additional measurements were performed to assess the relationship between $A_{pH}$' and the pH of the gel solution: for agar gel containing or not cresol red and buffered at $p$H≈p$K_{CR2}$ by replacing $NaHCO_3$ 2.5 mmol.$L^{-1}$ with $H_3BO_3$ 0.50 mol.$L^{-1}$ and NaOH 0.65 mol.$L^{-1}$, and for microplates prepared according to the MicroResp™ method after 24 h in sealed bags containing either dry soda lime and water in distinct capsules or air enriched with 1.5% $CO_2$.

To estimate $\Delta c^{+}_{min}$, $\Delta c^{+}_{max}$, $pH_{1/2}$ and $\alpha$, we attempted to obtain the relationship between $\Delta q_g$ and gel pH after equilibrating the gel with air enriched by 0.5%, 1% and 1.5% $CO_2$. To better circumvent the effect of the gel, we simultaneously measured the pH of a solution of the same composition, but without agar, equilibrated with $CO_2$-enriched air. However, the calculations are highly sensitive to gel pH, and 0.5% or more $CO_2$ bars insight into pHs higher than 7.6. Therefore, parameters $\Delta c^{+}_{min}$, $\Delta c^{+}_{max}$, $pH_{1/2}$ and $\alpha$ were empirically refined in order to ensure that (i) the calculated $CO_2$ disappearing from the air space of empty wells over 6 h balances against the $CO_2$ stored in the gel of these wells in 96-well microplates, and (ii) the respiration rate estimated for 6 h incubation in wells of 24-well microplates filled with either 0.6, 1.2, 1.8 or 2.4 g of moist soil does not depend on the final $CO_2$ air fraction in the wells. The relationship between gel absorbance and $CO_2$ air fraction was checked for microplates filled with gel previously equilibrated 5 d with $CO_2$ air fractions of 0.5, 1, 1.5 and 2% at about 22.5°C after having been stored uncovered in sealed plastic bags with soda lime and water during 6 d.

Kinetics of $CO_2$ exchange between the air space and the gel: Just after being filled with gel, two 96-well microplates were stored for 1 d in sealed plastic bags that contained dry soda lime and water in distinct capsules, and two other microplates in sealed plastic bags with air enriched by 1.5% $CO_2$. Just after opening a bag, one of the two microplates was used for absorbance measurements every 2 to 5 min for 6 h at about 22.5°C, while the other was used to record the pH of the gel every 1 to 5 min in a single well, using the 16-gauge micro-combination needle pH probe. Beyond 6 h, drying led to cracks in the gel of some wells.

Effect of microplate storage on the initial state of the gel: Since soda lime partly dries the gel and extracts $CO_2$ supplied as $NaHCO_3$, we checked whether absorbance varied between microplates prepared simultaneously and kept for 7 d either in a poorly-closed desiccator with dry soda lime and water in separate capsules, in a sealed plastic bag with dry soda lime and water in separate capsules, and in a sealed plastic bag with soda lime and water in the same capsule. In addition, we monitored the kinetics of gel desiccation in microplates stored for 7 d in sealed plastic bags with dry soda lime and water in separate capsules, one of them being covered with a plastic film after 1 d of storage, the other remaining exposed.

### 2.3.3. Experiment on the soil

Ability of the model to simulate the pH of soil solutions for various $CO_2$ air fractions: Since retained reactions greatly simplify the complex geochemistry of soils, we checked whether they permit to simulate the pH of a soil solution and whether the calcite dissolution that results from an increase in $CO_2$ air fraction or the supply of an acid substrate could be described by a thermodynamic equilibrium. The relationship between $CO_2$ air fraction and pH of the soil solution was assessed on soil slurries of a water-to-soil mass ratio of 1, 2.5 and 5 after being equilibrated with either lab air (between 0.04 and 0.12% $CO_2$) or air enriched by 0.5, 1, 1.5 and 2% $CO_2$ , respectively, by sparging with air and stirring the slurry.

Abiotic $CO_2$ emissions after supplying acid substrate to the soil: Soil was autoclaved at 121°C and 0.1 MPa for 1 h, incubated for 2 d, then autoclaved a second time for 1 h at same temperature and pressure to eliminate any microorganisms that were not destroyed in the initial autoclaving (Skipper et al., 1996). Then, 24 wells of a 96-well microplate were filled with sterilized soil while 24 other wells were filled with non-sterilized soil. For each modality, 6 wells each were supplemented with either 0.05 mL of water or with a mixture of 0.025 mL water plus 0.025 mL of a 120 $mg.mL^{-1}$ solution of either glucose, glucosamine-HCl or $Na_2$-malate, respectively. Abiotic emissions due to glucosamine-HCl supply were estimated from 6-h incubation and compared to biotic emissions on unsterilized soil.

### 2.3.4. MicroResp™ incubations of various times and for various amounts of soil

Three MicroResp™ incubation sets were performed to assess and illustrate the new MicroResp™ data analysis. In a first set of incubations, the wells of four 24-well microplates were filled with either 0.6, 1.2, 1.8 or 2.4 g of the same soil at 19% weight soil moisture supplemented with 0.033, 0.066, 0.099 or 0.133 mL of water, respectively, and 0.033, 0.066, 0.099 or 0.133 mL of a 120 $mg.mL^{-1}$ solution of glucose. The microplates were then incubated at 22.5°C for 6 h. Results served to estimate the $\alpha$ and $pH_{1/2}$ parameters and to assess the relative contributions of gel, air space and soil solution to $CO_2$ storage. In a second set of incubations, the wells of five 96-well microplates were either left empty or filled with about 0.38 g of soil initially at 19% weight soil moisture supplemented with 0.05 mL of water or 0.025 mL of water and 0.025 mL of a 120 $mg.mL^{-1}$ solution of either glucose, $Na_2$-malate or glucosamine-HCl. The microplates were then incubated at 22.5°C for 1, 2, 4, 6 and 8 h. Results served to check whether estimated microbial respiration is incubation time-dependent.

A third set of experiments was performed solely to illustrate variations in microbial $CO_2$ production with changes in substrates. Only one 96-well microplate was used; the wells were filled with about 0.38 g of dry soil initially at 19% weight soil moisture and supplemented with 0.05 mL of water or with 0.025 mL of water and 0.025 mL of a 120 $mg.mL^{-1}$ solution of either glucose, sucrose, trehalose, mannose, cellobiose, dextrin, glucosamine-HCl, alanine, glycine or $Na_2$-malate.

## 3. Results and discussion

### 3.1. Calibration of the relationship between $A_{pH}$' and the $CO_2$ air fraction

The means and standard deviations of the absorbances of empty microplates ($A_e$) were 0.0296 and 0.0012, respectively. The means and standard deviations of the absorbances of the wells filled with gel without cresol red ($A_e$+$A_g$) were 0.17076 and 0.0088, respectively. Thus, an estimate of $\overline{A_g}$ is 0.1411, the variability in $A_e$ can be neglected given its low contribution to $A_{pH}$, and the standard deviation of $A_g$ is nearly 0.0088, leading to a variation coefficient of $A_g$ of about 6.2%. Similar estimates for $A_g$ mean and standard deviation were obtained for agar gel without cresol red and for pH≈11.7 and pH=p$K_{aCR2}$, although slightly lower when borate was used to buffer the gel solution. The means and standard deviations of the absorbances of microplates with gel carrying cresol red and having a pH>11 were 0.987 and 0.0302, respectively, while the means and standard deviations of absorbances of microplates with gel without cresol red and having a pH>11 were 0.162 and 0.0078, respectively. Thus, an estimate of $\overline{A_{max}}$ is 0.825. Although an exact estimation of the $A_{max}$ variation coefficient is impossible, it is probably lower than that of $A_g$ in our experiment. Using these values and the calculated $pK_{CR2}$ to simulate variations in the absorbance $A_{pH}$ as a function of the pH of the gel solution correctly reflects experimental data (Fig. 1).

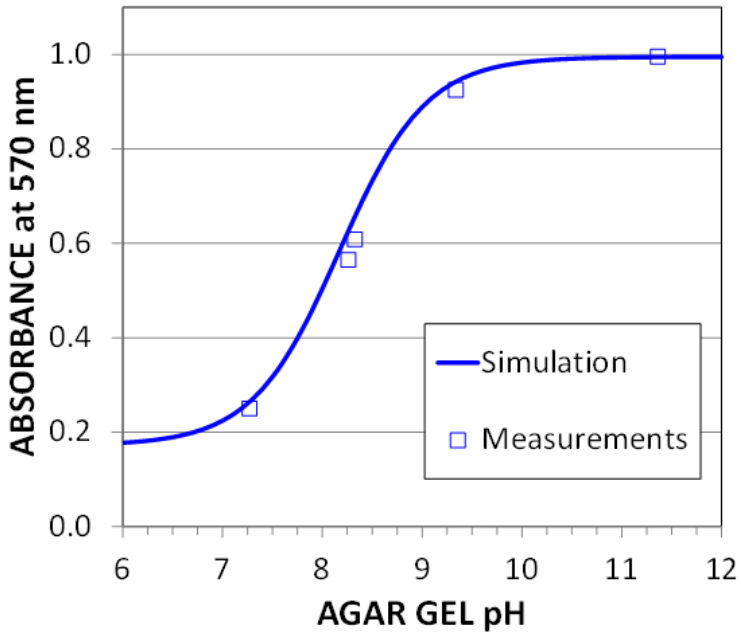


**Fig.1.** Relationship between pH of the agar gel and mean of the measured absorbance $A_{pH}$ ($A_{pH} = A_e + A_g + A_{CR}$) over the 96 wells of a microplate. The X coordinates of the experimental points are directly-measured pH. The Y coordinates of the experimental points are directly-measured absorbances when the gel matched the composition used in the MicroResp$^{TM}$ method, whereas for non-matched gels they were recalculated to replace the absorbance of the modified gel by the actual absorbance of the gel matched to the composition used in the MicroResp$^{TM}$ method.

The initial *vs* final absorbances of empty wells (i.e. $A_{pH}$(t=0) and $A_{pH}$(t=6 h), respectively) strongly suggest that $\Delta c^+_{min}$ is either nearly equal to or slightly lower than zero, and that there is no exchange of adsorbed cations with $H_3O^+$ in the gel solution at pH≥8: the opposite (i.e. $\Delta c^+_{min} \geq 0$ and/or $\Delta c^+ >> 0$) would require an unrealistically high initial $CO_2$ air fraction in the air space of the wells to explain the change in absorbance measured for these wells (results not shown). The pH of the solution of the same composition as the gel in the MicroResp$^{TM}$ method but without agar and in equilibrium with 0.5%, 1% and 1.5% $CO_2$ was 7.49, 7.16 and 6.98, respectively. These values correspond to a net $\Delta q_g$ of about 3.05, 2.85 and 2.82 mmol $c^+.L^{-1}$, respectively, which is near the 2.5 mmol.$L^{-1}$ of $Na^+$ supplied as $NaHCO_3$. In contrast, the pH of the agar gel in equilibrium with 0.5%, 1% and 1.5% $CO_2$ was 7.66, 7.41 and 7.22, respectively. These values correspond to a net $\Delta q_g$ of about 4.50, 5.03

and 4.89 mmol $c^{+}.L^{-1}$, respectively, suggesting that $\Delta q_g$ increases with a decrease in pH in this pH range with a $\Delta c^{+}_{max}$ probably higher than 2. We set $\Delta c^{+}_{max}$ to 3.0 mmol $c^{+}.L^{-1}$ in order to have a good fit of the relationship between the air $CO_2$ fraction and the absorbance at 570 nm on experimental data for 0.5%, 1%, 1.5% and 2% $CO_2$ (Fig. 2). Parameters $pH_{1/2}$ and $\alpha$ were set to 7.7 and 80, respectively, by minimizing differences in the respiration rates of various amounts of the same soil (see below), making it possible to simulate a majority of cation exchange for pH values between 7.4 and 8.

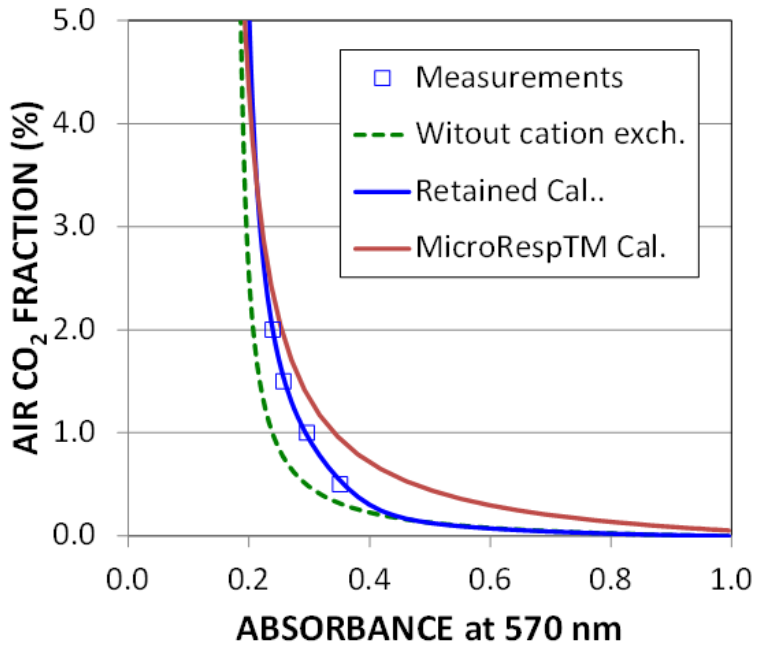


**Fig.2.** Relationship between means of measured absorbance $A_{pH}$ ( $A_{pH} = A_e + A_g + A_{CR}$ ) over the 96 wells of a microplate and $CO_2$ air fraction in equilibrium with the microplates.

### 3.2. Effects of soil alkalinity, substrate and calcite on soil pH and microbial $CO_2$ in solution

While solute concentrations were highly dependent on water-to-soil mass ratio (Table 3), pH measured on soil slurries in equilibrium with a range of $CO_2$ air fractions was only slightly dependent on water-to-soil mass ratio (1, 2.5 and 5 in this work) but highly dependent on $CO_2$ air fraction (Fig. 3). Simulations performed for soil solutions in equilibrium with calcite and having an initial $[Ca^{2+}]$ near the value extrapolated for soil solutions at the start of

MicroResp$^{TM}$ incubations were used to simulate slurry behaviour. Although this was not the objective of these measurements, the simulations confirmed that pH(water) (8.51 for our soil) differs from pH of the soil solution that would be in equilibrium with lab air (pH slightly lower than 8 here) since alkaline soil solution can trap a large amount of gaseous $CO_2$ but only very slowly in the absence of facilitated transfer (vigorous stirring and bubbling).

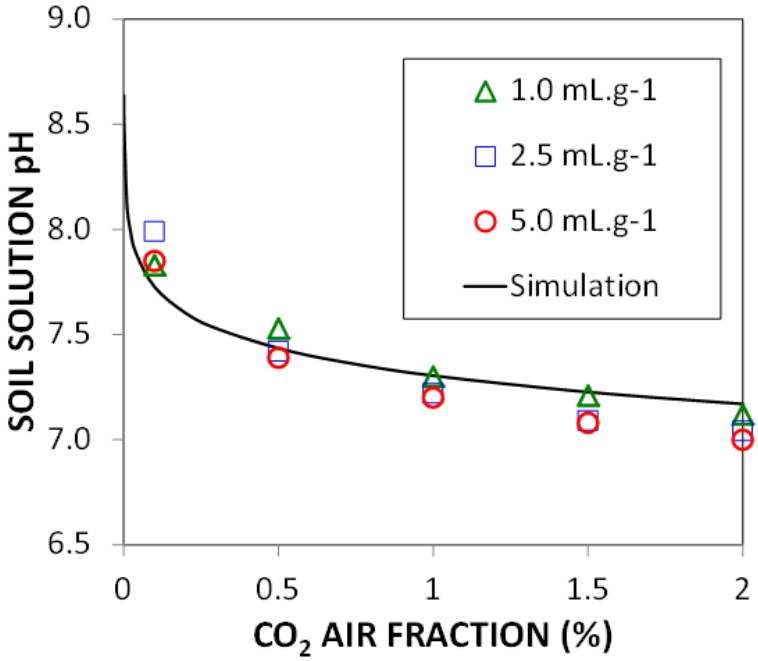


**Fig.3.** Measured pH of soil slurry as a function of $CO_2$ air fraction in equilibrium with it by simultaneous air bubbling and slurry stirring. *Simulations were performed for soil at weight moistures as in MicroResp$^{TM}$ incubations*.

Empirical functions were then easily obtained from geochemical modelling to estimate the amount of microbial $CO_2$ stored in the soil solution ($H_2CO_3$*, $HCO_3^-$ and $CO_3^{2-}$) minus, for calcareous soils, the amount of total abiotic $CO_2$ emitted by calcite dissolution. The variation of $\Delta[CO_2]_{tot}$ in total $CO_2$ concentration in the solution ($H_2CO_3$*, $HCO_3^-$ and $CO_3^{2-}$) minus the increase $\Delta[Ca^{2+}]$ in $Ca^{2+}$ concentration for calcareous soils between the considered $CO_2$ air fraction and the 0.04% $CO_2$ air fraction was then fitted by empirical functions. An example of these functions for several amounts of $Na_2$-malate is proposed in Fig. 4, and empirical coefficients defined in equation (1) for these functions are presented in Table 4 for the relevant soils.

**Table 4:** Retained coefficients *a*, *b* and *c* used in equation (1) to describe variations in amount of microbial C in the soil solution with regard to microbial $CO_2$ at 0.04% $CO_2$ air fraction

| Substrate | Concentration | *a* | *b* | *c* |
|---|---|---|---|---|
| - | | $1.83\ 10^{-3}$ | $6.45\ 10^{-1}$ | $2.28\ 10^{-4}$ |
| glycine | | $5.77\ 10^{-2}$ | $2.57\ 10^{-2}$ | $5.25\ 10^{-2}$ |
| alanine | | $5.20\ 10^{-3}$ | $2.50\ 10^{-1}$ | $2.28\ 10^{-3}$ |
| glucosamine-HCl | | $1.85\ 10^{-3}$ | $6.40\ 10^{-1}$ | $2.33\ 10^{-4}$ |
| $Na_2$-malate | 10 | $1.93\ 10^{-3}$ | $6.38\ 10^{-1}$ | $2.46\ 10^{-4}$ |
| | 20 | $2.03\ 10^{-3}$ | $6.31\ 10^{-1}$ | $2.64\ 10^{-4}$ |
| | 30 | $2.13\ 10^{-3}$ | $6.24\ 10^{-1}$ | $2.83\ 10^{-4}$ |
| | 42 | $2.25\ 10^{-3}$ | $6.17\ 10^{-1}$ | $3.28\ 10^{-4}$ |

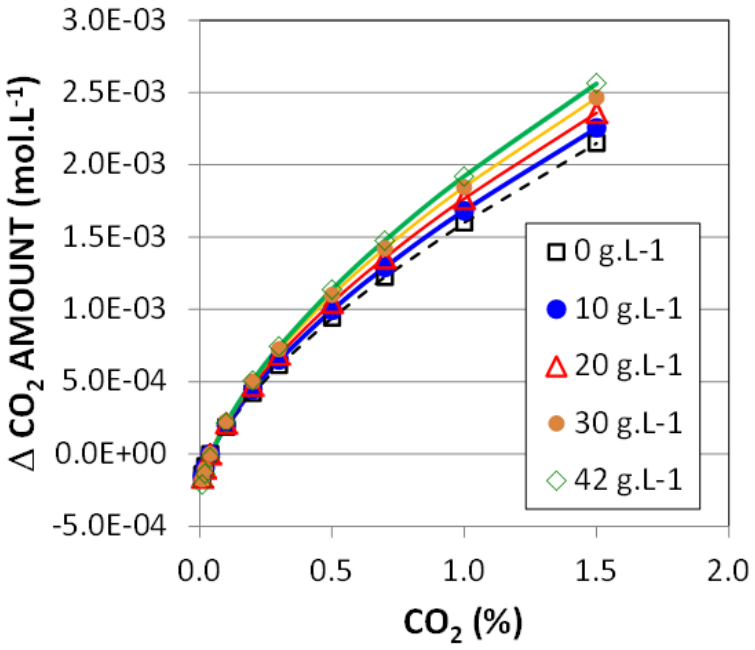


**Fig.4.** Simulated amount of total $CO_2$ ($mol.L^{-1}$) minus $CO_2$ derived from calcite dissolution as a function of air-space $CO_2$ fraction for a soil solution in equilibrium with calcite and initially having a sum of charges other than $H_3O^+$, $HO^-$, $HCO_3^-$ and $CO_3^{2-}$ equal to $-4.60\ 10^{-3}$ mol $c^+.L^{-1}$ and $+6.00\ 10^{-3}$ mol $c^+.L^{-1}$ $Ca^{2+}$, and for different amounts of $Na_2$-malate substrate (0, 10, 20, 30 and 42 $g.L^{-1}$). *Zero values indicate that total $CO_2$ in the soil solution is equal to $CO_2$ release by calcite dissolution. Negative values mean that calcite dissolution releases more $CO_2$ than was initially contained in the soil solution, indicating that $CO_2$ is partly transferred to the air and agar gel in the well.*

### 3.3. Kinetics of transfer and calcite dissolution and the effects on MicroResp™ estimation

Microbial respiration rate was assessed in a soil sterilized and subsequently supplied with a slightly acidic substrate (glucosamine-HCl) to indirectly check whether calcite dissolution can be described by a thermodynamic equilibrium system. The results showed that abiotic CO2 microbial respiration can be neglected, as absolute values were small (9.73 $10^{-5}$ µg C-$CO_2$.$g^{-1}$ soil.$h^{-1}$) compared to unsterilized soil enriched with the same substrate (8.96 $10^{-4}$ µg C-$CO_2$.$g^{-1}$ soil.$h^{-1}$), and positive or negative sign was dependent on the initial in-lab $CO_2$ air fraction.

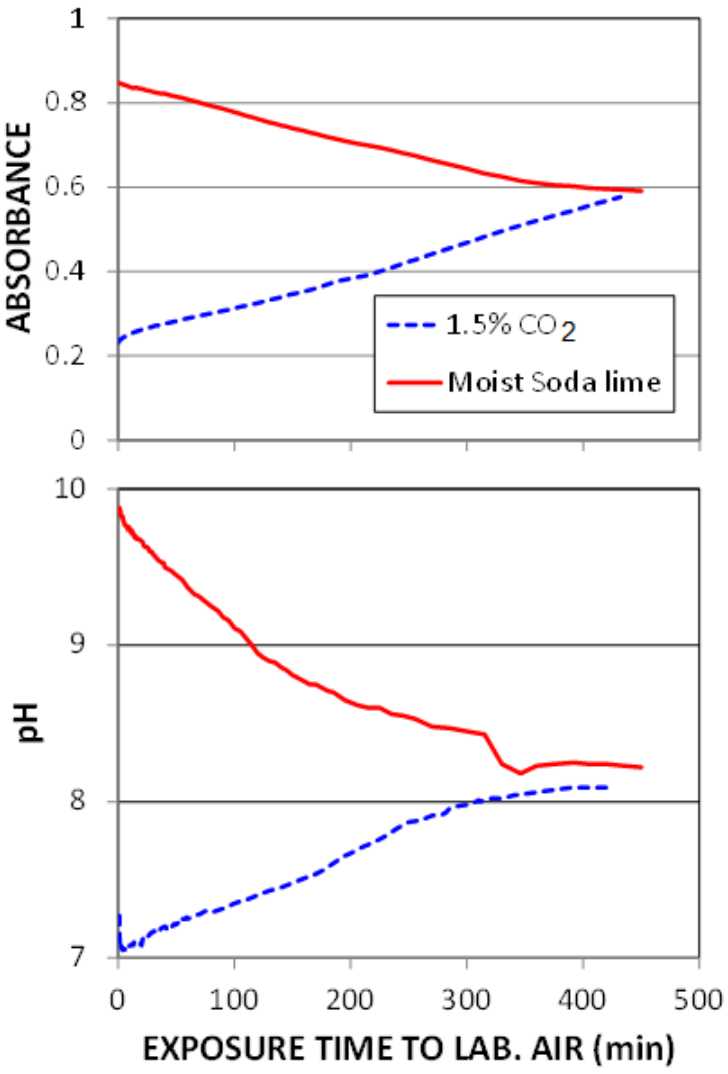


**Fig.5.** Changes in the pH of the gel solution and in absorbance $A_{pH}$ of microplates initially stored in sealed plastic bags enriched with either 1.5% $CO_2$ or with soda lime and water in separate capsules, and exposed to lab air.

Figs. 5a-b illustrate the changes in pH of the gel solution and in the absorbance $A_{pH}$ of microplates initially stored in sealed plastic bags either enriched with 1.5% $CO_2$ or containing soda lime and water in distinct capsules and exposed to lab air. Superposed yellow and pink colour layers (corresponding to $CO_2$-enriched air and $CO_2$-free air, respectively) in microplate well gel were clearly visible for long periods of these experiments. These observations indicate that $CO_2$ transfer between the air space and the gel is low and that equilibrium is only reached after about 4 h. This is mainly because low $CO_2$ transfer in the gel prevents a quick redistribution of $CO_2$ when it accumulates or disappears in the gel near the interface with air. For MicroResp™ microplate wells where microbial $CO_2$ production was continuous over the 6 h of incubation, it is reasonable to consider that equilibrium is never reached and that the estimated microbial respiration *a priori* underestimates actual respiration. However, the relative bias resulting from the equilibrium hypothesis will decrease with increasing incubation times. Staggered incubations (1, 2, 4, 6 and 8 h) of soil enriched with glucose suggest that 4 hours is the minimum time requirement for incubations and that the 6 h incubation proposed in the MicroResp™ method is reasonable (Fig. 6).

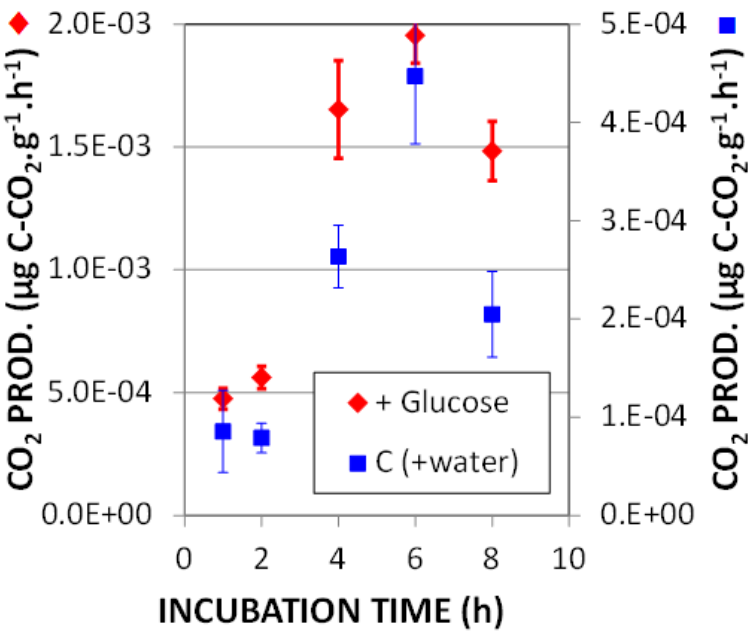


**Fig.6.** Microbial respiration rates (µg C-$CO_2$.$g^{-1}$ dry soil.$h^{-1}$) estimated from soil incubation in 96-well microplates for 1, 2, 4, 6 and 8 h, without substrate (Y coordinates on the right) and with glucose (Y coordinates on the left).

Initial state of the gel may vary with initial storage time and conditions. For example, the initial absorbance of 96-well microplates varies between simultaneously-prepared microplates stored for 7 d in either a poorly-closed desiccator with dry soda lime and water in separate capsules ($\overline{A_{p\mathrm{H}}}(t=0)$=0.90), a sealed plastic bag with dry soda lime and water in separate capsules ($\overline{A_{p\mathrm{H}}}(t=0)$=1.04), and a sealed plastic bag with soda lime and water in the same capsule ($\overline{A_{p\mathrm{H}}}(t=0)$=0.97). For plates stored in sealed plastic bags, differences in $\overline{A_{p\mathrm{H}}}(t=0)$ between dry soda lime and water in separate capsules and in the same capsule may have resulted from differences in gel desiccation, which would explain why $\overline{A_{p\mathrm{H}}}(t=0)$ exceeds the maximum simulated absorbance ($A_\mathrm{e}+\overline{A_\mathrm{g}}+\overline{A_\mathrm{max}}$ =0.996). The kinetics of gel desiccation in microplates stored 7 d in sealed plastic bags with dry soda lime and water in separate capsules varies greatly with presence or absence of a plastic film covering the wells (Fig. 7). The procedure proposed in the MicroResp$^{\mathrm{TM}}$ method therefore appears to have been adapted to protect the gel from desiccation. However, the initial absorbance may still vary with the amount of $CO_2$ extracted by soda lime during plate storage. This amount has to be accounted for in microbial respiration estimates. However, the worst-case scenario would be the gel drying out, which would ultimately modify the value of the sum $\Delta q_\mathrm{g}$ and skew the calibration. For this reason, it is essential to take all feasible precautions to protect the gel from partial drying. Repeated use of the same microplate gels "regenerated" between consecutive incubations by exposure to soda lime should be ruled out. Finally, we note that microplates with an initial absorbance $\overline{A_{p\mathrm{H}}}(t=0)$ of about 0.90 would correspond to a gel of about pH 9.1 and about 0.0073% $CO_2$ air fraction, indicating that the NaOH is partially replace by substitutes to $NaHCO_3$ within the gel during storage due to $CO_2$ extraction by the soda lime.

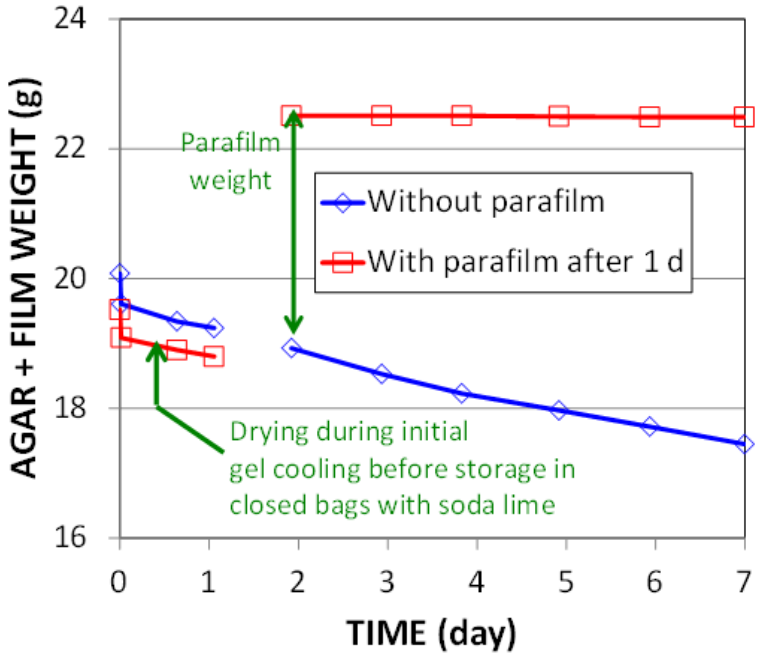


**Fig.7.** Change in the weight of microplates filled with agar gel during its initial 7-d storage in sealed bags with or without Parafilm anti-desiccant.

### 3.4. The effects of soil mass and substrate on respiration estimated from 6-h incubation

The 24-well microplates that were filled with 0.6, 1.2, 1.8 and 2.4 g of the same soil at 19% weight soil moisture and supplemented with 0.033, 0.066, 0.099 and 0.133 mL of water and 0.033, 0.066, 0.099 and 0.133 mL of a 120 $mg.mL^{-1}$ solution of glucose, respectively, were then incubated at 22.5°C for 6 h. Although the results partly contributed to calibration ($pH_{1/2}$ and $\alpha$ were estimated so as to minimize differences in estimated microbial respirations between these 4 soil weights), they show that estimated soil microbial respiration is not dependent on soil weight and therefore not dependent on $CO_2$ air fraction at the end of the incubations (Fig. 8). This is all the more interesting given that there is variation in the relative proportions of total $CO_2$ in gas phase in the soil solution and in the gel (Fig. 9). Variation in quantity of $CO_2$ during the 6 h of incubation is always stronger in the gel than in the air space and the soil solution, especially when final $CO_2$ content is low. Low final levels of $CO_2$ air fraction are common for soils incubated without substrates or with inefficient poorly-used substrates. These results clearly demonstrate that we cannot neglect variations in the amount of $CO_2$ in the gel. The fraction of $CO_2$ stored in the soil solution cannot be neglected, but it

can be estimated with less precision in view of its low contribution to the total accumulation of microbial $CO_2$.

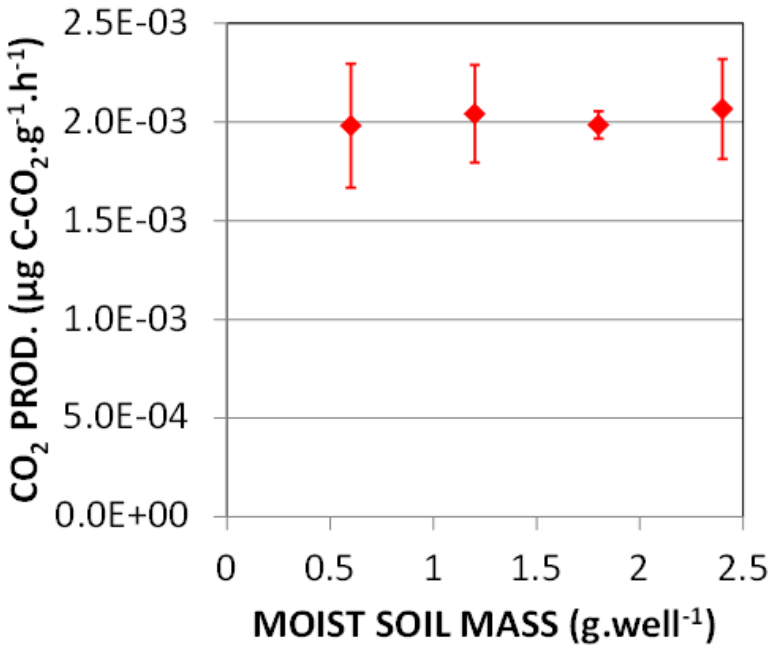


**Fig.8.** Microbial respiration rates (µg C-$CO_2$.$g^{-1}$ dry soil.$h^{-1}$) estimated for different amounts of soil (0.6, 1.2, 1.8 and 2.4 g of fresh soil at 19% weight soil moisture) supplied with water and glucose.

We then tested this new mathematical procedure for estimating microbial respiration from MicroResp™ incubation on various substrates (Fig. 10). Microbial respiration rates decreased in the following order of added substrate: sucrose≥glucose≥malate≥glucosamine-HCl≥cellobiose≥alanine≥mannose≥dextrin≥trehalose, and microbial respiration rates were between 5 and 15-fold stronger in the soil with substrate than in the soil with water only. The coefficients of variation of individual measurements (i.e. in a well) ranged from 6.3% (soil with water) to 53% (soil+trehalose).

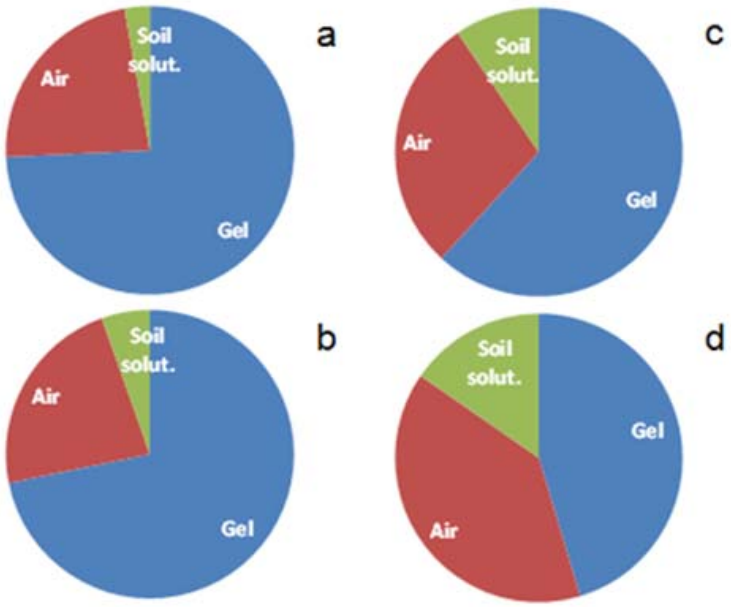


**Fig.9.** Distribution of microbial production of $CO_2$ in microplate well gel, air space and soil solution as a function of (a) 0.6 g, (b) 1.2 g, (c) 1.8 g and (d) 2.4 g of fresh soil (19% weight soil moisture) supplied with water and glucose, corresponding to estimated final $CO_2$ air fractions equal to 0.32, 0.57, 0.99 and 1.74% $CO_2$. *For the calculations, abiotic $CO_2$ originating from calcite $CO_2$ was assumed to be in the soil solution only. This slightly minimizes the contribution of soil solution to $CO_2$ storage based on calculations assuming even $CO_2$ distribution between soil solution, air space and gel.*

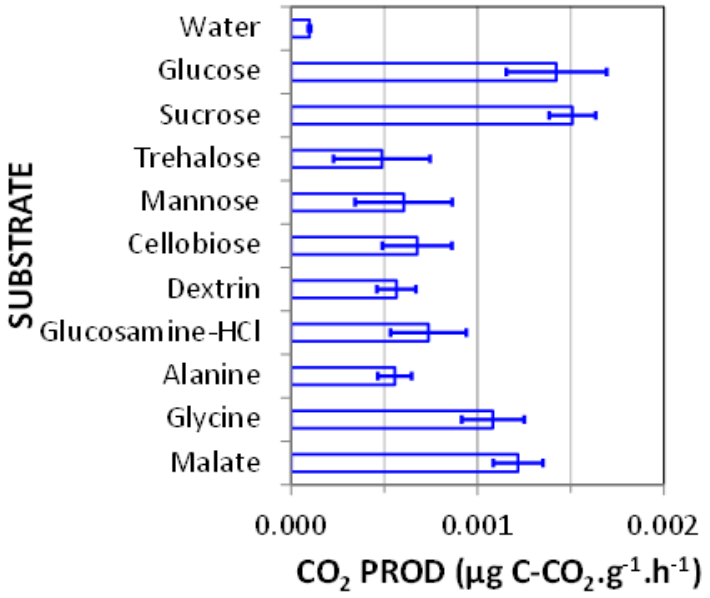


**Fig.10.** Example illustrating microbial respiration rate.

## 4. Conclusion

Here we propose a novel and more complete description of the fate of $CO_2$ during soil incubation in the wells of microplates used in the MicroResp$^{TM}$ method; for the first time, the storage of $CO_2$ (as $H_2CO_3$*, $HCO_3^-$ and $CO_3^{2-}$) in the gel carrying the colorimetric indicator and the effects of $CO_2$ on the pH of the soil solution and, in calcareous soils, the dissolution of calcite are now accounted for. The comparison between experimental data and simulations shows that this improved model makes it possible to simulate the main geochemical processes involved in the fate of $CO_2$. It leads to relevant estimates of microbial $CO_2$ production, as shown by 6 h-plus incubations of 0.6 to 2.4 g of soil (in 24-well microplates). The final proportion of $CO_2$ stored in the indicator gel is far from negligible, especially when final $CO_2$ air fraction is low, as is the case for incubation of soil without substrate or with substrates that are difficult to consume. Incubation for less than 4 h may lead to underestimate microbial respiration, since $CO_2$ dissolution and transfer within the gel are both slow processes. The amount of $CO_2$ in the gel at the start of incubation depends on the level of $CO_2$ (supplied as $NaHCO_3$) extraction by soda lime while the gel is in storage. Extracted $CO_2$ varies with microplate distance to soda lime, duration of the exposure to soda lime, etc. Storage may partially desiccate the gel and modify the relationship between $CO_2$ partial pressure and gel absorbance, and so repeated use of the same microplates "regenerated" between consecutive incubations by exposure to soda lime should be avoided. $CO_2$ stored in the soil cannot be neglected, but an increase in $CO_2$ partial pressure decreases the pH of the soil solution and, in the case of calcareous soils, generally promotes calcite dissolution. Neglecting this decrease in pH or the dissolution of calcite would lead to an overestimation of biotic $CO_2$ production. Since microbial $CO_2$ production in soils without substrate often leads to a final $CO_2$ fraction of less than 0.4%, it is important to have a good estimate of the initial $CO_2$ partial pressure (varying between 0.04 and more than 0.1% in lab air), possibly by having empty wells with gel. It is also vital to keep firm control over temperature, which can affect all the thermodynamic constants as well as microbial activity.

**Acknowledgements**

This work received support from the CleanWast Project that features as part of the French ANR [National Research Agency] PRECODD Programme. We thank the INRA's *Département Environnement et Agronomie* and the ADEME for providing support through M.

Ben-Sassi's PhD grant. We also thank Ghislain Sévenier and Cécile Péru for valuable technical assistance and Glen McCulley for English editing and proofreading.

## References

Alavoine, G., Houlbert, J.C., Nicolardot, B., 2008. Comparison of three methods to determine C decomposition of organic materials in soils under controlled conditions. Pedobiologia 52, 61–68.

Ben Sassi, M., Dolinger, J., Renault, P., Tlili, A., Bérard, A., 2012. The FungiResp method: an application of the MicroResp™ method to assess fungi in microbial communities as soil biological indicators. Ecological Indicators 23, 482–490.

Bérard, A., Ben Sassi, M., Renault, P., Gros, R., 2012. Severe drought-induced community tolerance to heat wave. An experimental study on soil microbial processes. Journal of Soils and Sediments 12, 513–518.

Bérard, A., Bouchet, T., Sévenier, G., Pablo, A.L., Gros, R., 2011. Resilience of soil microbial communities impacted by severe drought and high temperature in the context of Mediterranean heat-waves. European Journal of Soil Biology 47, 333-342.

Bertrand, I., Delfosse, O., Mary, B., 2007. Carbon and nitrogen mineralization in acidic, limed and calcareous agricultural soils: Apparent and actual effects. Soil Biology and Biochemistry 39, 276–288.

Burton, J., Chen, C., Xu, Z., Ghadiri, H., 2010. Soil microbial biomass, activity and community composition in adjacent native and plantation forests of subtropical Australia. Journal of Soils and Sediments 10, 1267–1277.

Cameron, C., 2007. MicroResp™ technical manual – a versatile soil respiration system. Macaulay Institute, Craigiebuckler, Aberdeen, Scotland, UK.

Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S., Potts, J.M., 2003. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Applied and Environmental Microbiology 69, 3593-3599.

Chapman, S.J., Campbell, C.D., Artz, R.R.E., 2007. Assessing CLPPs using MicroReSp™ – A comparison with biolog and multi-SIR. Journal of soils and sediments 7, 406–410.

Cheng, W., Coleman, D.C., 1989. A simple method for measuring $CO_2$ in a continuous air-flow system: modifications to the substrate-induced respiration technique. Soil Biology and Biochemistry 21, 385–388.

Dassonville, F., Renault, P., Vallès, V., 2004. A model describing the interactions between anaerobic microbiology and geochemistry in a soil amended with glucose and nitrate. European Journal of Soil Science 55, 29–45.

Degens, B.P., Harris, J.A., 1997. Development of a physiological approach to measuring the catabolic diversity of soil microbial communities. Soil Biology and Biochemistry 29, 1309–1320.

Degens, B.P., Schipper, L.A., Sparling, G.P., Duncan, L.C., 2001. Is the microbial community in a soil with reduced catabolic diversity less resistant to stress or disturbance? Soil Biology and Biochemistry 33, 1143–1153.

Dilly, O., 2001. Microbial respiratory quotient during basal metabolism and after glucose amendment in soils and litter. Soil Biology and Biochemistry 33, 117–127.

DOE, 1994. Handbook of methods for the analysis of the various parameters of the carbon dioxide system in sea water; version 2, A.G. Dickson & C. Goyet, eds., ORNL/CDIAC-74.

El Nahhal, I.M., Zourab, S.M., Kodeh, F.S., Babonneau, F., Hegazy, W., 2012. Sol–gel encapsulation of cresol red in presence of surfactants. Journal of Sol-Gel Science and Technology, DOI 10.1007/s10971-012-2693-y.

Ferreira, L.G., Noseda, M.D., Gonçalves, A.G., Ducatti, D.R.B., Fujii, M.T., Duarte, M.E.R., 2012. Chemical structure of the complex pyruvylated and sulfated agaran from the red seaweed Palisada flagellifera (Ceramiales, Rhodophyta). Carbohydrate Research 347, 83–94.

French, C.R., Carr, J.J., Dougherty, E.M., Eidson, L.A.K., Reynolds, J.C., De Grandpre, M.D., 2002. Spectrophotometric pH measurements of freshwater. Analytica Chimica Acta 453, 13–20.

Garland, J.L., Mills, A.L., 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Applied and Environmental Microbiology 57, 2351–2359.

Garland, J.L., Roberts, M.S., Levine, L.H., Mills, A.L., 2003. Community-level physiological profiling performed with an oxygen-sensitive fluorophore in a microtiter plate. Applied and Environmental Microbiology 69, 2994–2998.

Gonzalez-Quiňones, V., Banning, N.C., Ballesta, R.J., Murphy, D.V., 2009. Influence of cold storage on soil microbial community level physiological profiles and implications for soil quality monitoring. Soil Biology and Biochemistry 41, 1574–1576

Harned, H.S., Davis Jr., R.D., 1943. The ionization constant of carbonic acid in water and the solubility of carbon dioxide in water and aqueous salt solutions from 0 to 50°C. Journal of the American Chemical Society 65, 2030–2037.

Harned, H.S., Owen, B.B., 1958. The physical chemistry of electrolytic solutions, 3rd ed. Van Nostrand Reinhold, New York.

Heger, D., Klánová, J., Klán, P., 2006. Enhanced protonation of cresol red in acidic aqueous solutions caused by freezing. The Journal of Physical Chemistry B 110, 1277–1287.

Hoffmann, B., Müller, T., Joergensen, R.G., 2010. Carbon dioxide production and oxygen consumption during the early decomposition of different litter types over a range of temperatures in soil-inoculated quartz sand. Journal of Plant Nutrition and Soil Science 173, 217–223.

Klimant, I., Meyer, V., Kuhl, M., 1995. Fiber-optic oxygen microsensors, a new tool in aquatic biology. Limnology and Oceanography 40, 1159–1165.

Lahaye, M., Rochas, C., 1991. Chemical structure and physico-chemical properties of agar. Hydrobiologia 221, 137–148.

Lahlah, J., Renault, P., Cazevieille, P., Buzet, A., Hazemann, J.L., Womes, M., Cambier, P., 2009. Geochemical resilience of a ferralsol subjected to anoxia and organic matter amendment. Soil Science Society of America Journal 73, 1958–1971.

Lalor, B.M., Cookson, W.R., Murphy, D.V., 2007. Comparison of two methods that assess soil community level physiological profiles in a forest ecosystem. Soil Biology and Biochemistry 39, 454-462.

Macdonald, C.A., Thomas, N., Robinson, L., Tate, K.R., Ross, D.J., Dando, J., Singh, B.K., 2009. Physiological, biochemical and molecular responses of the soil microbial community after afforestation of pastures with Pinus radiata. Soil Biology and Biochemistry 41, 1642–1651.

Millero, F., Huang, F., Graham, T., Pierrot, D., 2007. The dissociation of carbonic acid in NaCl solutions as a function of concentration and temperature. Geochimica et Cosmochimica Acta 71, 46–55.

Millero, F.J., 1995. Thermodynamics of the carbon dioxide system in the oceans. Geochimica et Cosmochimica Acta 59, 661–677.

Millero, F.J., Graham, T.B., Huang, F., Bustos-Serrano, H., Pierrot, D., 2006. Dissociation constants of carbonic acid in seawater as a function of salinity and temperature. Marine Chemistry 100, 80–94.

Oren, A., Steinberger, Y., 2008a. Coping with artifacts induced by $CaCO_3$–$CO_2$–$H_2O$ equilibria in substrate utilization profiling of calcareous soils. Soil Biology and Biochemistry 40, 2569–2577.

Oren, A., Steinberger, Y., 2008b. Catabolic profiles of soil fungal communities along a geographic climatic gradient in Israel. Soil Biology and Biochemistry 40, 2578–2587.

Pankow, J.F., 1991. Thermodynamic principles, in: Aquatic chemistry concepts, Lewis Publishers, Inc. Chelsea, Michigan, 17–50.

Parry, S., Renault, P., Chadoeuf, J., Chenu, C., Lensi, R., 2000. Particulate organic matter as a source of variation in denitrification in clods of soil. European Journal of Soil Science 51, 271–281.

Pelmont, J., 1993. Respiration sur oxygène, in: Bactéries et environnement: adaptations physiologiques, Presses Universitaires de Grenoble (Eds), 669–703.

Pytkowicz, R.M., 1975. Activity coefficients of bicarbonates and carbonates in seawater. Lymnology and Oceanography 20, 971–975.

Renault, P., Cazevieille, P., Verdier, J., Lahlah, J., Clara, C., Favre, F., 2009. Variations in the cation exchange capacity of a ferralsol supplied with vinasse, under changing aeration conditions. Comparison between CEC measuring methods. Geoderma 154, 101–110.

Revsbech, N.P., Ward, D.M., 1983. Oxygen microelectrode that is insensitive to medium chemical composition: use in an acid microbial mat dominated by cyanidium caldarium. Applied and Environmental Microbiology 45, 755–759.

Rowell, M.J., 1995. Colorimetric method for $CO_2$ measurement in soils. Soil Biology and Biochemistry 27, 373–375.

Salvatore, F., Ferri, D., Palombari, R., 1986. Salt effect on the dissociation constant of acid-base indicators. Journal of Solution Chemistry 15, 423–431.

Saul-Tcherkas, V., Steinberger, Y., 2009. Substrate utilization patterns of desert soil microbial communities in response to xeric and mesic conditions. Soil Biology and Biochemistry 41, 1882–1893.

Schlesinger, W.H., Andrews, J.A., 2000. Soil respiration and the global carbon cycle.

Biogeochemistry 48, 7–20.

Scholten, H.J., Pierik, R.L.M., 1998. Agar as a gelling agent: chemical and physical analysis. Plant Cell Reports 17, 230–235.

Sierra, J., Renault, P., 1995. Oxygen consumption by soil microorganisms as affected by oxygen and carbon dioxide levels. Applied Soil Ecology 2, 175–184.

Skipper, H.D., Arthur, G., Wollum, I.I., Turco, R.F., Wolf, D.C., 1996. Microbiological aspects on environmental fate studies of pesticides. Weed Technology 10, 174–190.

Smith, E.T., Matachek, J.R., 2002. A colorful investigation of a diprotic acid: a general chemistry laboratory exercise. The Chemical Educator 7, 359–363.

Stefanowicz, A., 2006. The Biolog plates technique as a tool in ecological studies of microbial communities. Polish Journal of Environmental Studies 15, 669–676.

Stevenson, B.A., Sparling, G.P., Schipper, L.A., Degens, B.P., Duncan, L.C., 2004. Pasture and forest soil microbial communities show distinct patterns in their catabolic respiration responses at a landscape scale. Soil Biology and Biochemistry 36, 49–55.

Stevenson, B.A., Verburg, P.S.J., 2006. Effluxed $CO_2$-$^{13}C$ from sterilized and unsterilized treatments of a calcareous soil. Soil Biology and Biochemistry 38, 1727–1733.

Ström, L., Godbold, D.L., Jones, D.L., 2001. Procedure for determining the biodegradation of radiolabeled substrates in a calcareous soil. Soil Science Society of America Journal 65, 347–351.

Stumm, W., Morgan, J.J., 1996. Dissolved carbon dioxide, in: Shnoor, J.L., Zehnder, A.J.B., John Wiley & Sons (Eds.), Aquatic chemistry. Chemical Equilibra and Rates in Natural Waters, New York (3rd Edition), 148–205.

Tamir, G., Shenker, M., Heller, H., Bloom, P.R., Fine, P., Bar-Tal, A., 2011. Can soil carbonate dissolution lead to overestimation of soil respiration? Soil Science Society of America Journal 75, 1414–1422.

Tlili, A., Marechal, M., Montuelle, B., Volat, B., Dorigo, U., Bérard, A., 2011. Use of the Microresp™ method to assess pollution-induced community tolerance for lotic biofilms. Environmental Pollution 159, 18–24.

Wakelin, S.A., Macdonald, L.M., Rogers, S.L., Gregg, A.L., Bolger, T.P., Baldock, J.A., 2008. Habitat selective factors influencing the structural composition and functional capacity of microbial communities in agricultural soils. Soil Biology and Biochemistry 40, 803–813.

Weiss, R.F., 1974. Carbon dioxide in water and seawater: the solubility of a non-ideal gas. Marine Chemistry 2, 203–215.

Yao, H., Campbell, C.D., Qiao, X., 2011. Soil pH controls nitrification and carbon substrate utilization more than urea or charcoal in some highly acidic soils. Biology and Fertility of Soils 47, 515–522. DOI 10.1007/s00374-011-0554-4

Zhou, X., Liu, X., Rui, Y., Chen, C., Wu, H., Xu, Z., 2011. Symbiotic nitrogen fixation and soil N availability under legume crops in an arid environment. Journal of Soils and Sediments 11, 762–770.

# The FungiResp method. an application of the MicroResp™ method to assess fungi in microbial communities as soil biological indicators

Meriem Ben Sassi[a,b], Jeanne Dollinger[a,b], Pierre Renault[a,b], Ahmed Tlili[c], Annette Bérard[a,b]*

[a] INRA, UMR1114 EMMAH, F-84914 Avignon, France

[b] UAPV, UMR1114 EMMAH, F-84914 Avignon, France

e-mail: annette.berard@paca.inra.fr

[c] Institute of Freshwater Ecology and Inland Fisheries, Alte Fischerhütte 2, Neuglobsow, Germany

**Article 3: Ecological Indicators 23 (2012) 482-490**

**Abstract**

Soil ecosystem services need to monitor soil quality in terms of soil functions. This need in turn requires functional indicators. Microbial functional diversity offers a way to characterize soil quality and any changes to it. Among soil microbial communities, fungi play a critical role in organic matter decomposition and nutrient cycling in soil by decomposing complex substrates, but only a few studies have focused on the function of soil fungal communities. We have developed a protocol based on substrate-induced respiration using the MicroResp™ technique combined with a selective inhibition (SI) procedure to characterize the fungal biomass and catabolic profiles for soil microbial and fungal communities: the FungiResp method. After comparisons with oxytetracycline, we chose bronopol as a bactericide to extend the FungiResp protocol. An optimal bronopol concentration of 78 μg $g^{-1}$ soil was selected for the four soils tested to minimize the risk of inhibiting non-target communities (fungi). We used this convenient, miniaturized method to compare different soils and different perturbations (drought and heat). The FungiResp method gave further data on the fungal part of the microbial substrate-induced respiration in these different contexts. Also, the catabolic structure of microbial and fungal communities measured as pattern of substrate utilization (CLPPs) enabled us to contrast the functional contributions of the decomposer groups in the different soils studied and highlight the functional impacts of the different perturbations applied to them.

**Keywords:** Substrate-induced respiration, Selective inhibition, MicroResp™, Soil microbial catabolic structure, Soil fungal catabolic structure

## 1. Introduction

Soil faces a broad range of threats from human activities and global change. These pressures may degrade it through an increase in erosion, contamination, sealing, compaction, salinization, and loss of organic matter, structure and biodiversity (Commission of the European Communities, 2002). Soil ecosystem services (Millennium Ecosystem Assessment, 2005), need to monitor soil quality in terms of soil functions, which demands functional indicators (Romaniuk et al., 2011; Ritz et al., 2009). Doran and Zeiss (2000) suggested complementary criteria of soil bioindicators: They should be sensitive to variations in management and climate change, well correlated with beneficial soil functions, useful for elucidating ecosystem processes, comprehensible and useful to land managers and finally easy and inexpensive to measure.

Soil microbial communities are extremely diverse in their composition and play an essential role in nutrient cycling functions such as organic matter decomposition and mineralization, nutrient mobilization and carbon sequestration (Reynolds et al., 2003; Strickland and Rousk, 2010). Recent studies have sought to assess the importance of microbial diversity, microbial community composition and interactions between microbial species for soil functioning (Bell et al., 2005). Also, the loss of microbial functions is an indicator of decrease in soil quality (Chapman et al., 2007). Hence it is useful to assess microbial functional diversity to characterize soil quality and any decrease it may undergo. Community-level physiological profiles (CLPPs), usually assessed by carbon substrate utilization, are good tools to evaluate the microbial functional diversity of a soil, and have been widely implemented with the Biolog method (Calbrix et al., 2005). Alternative microplate methods derived from Biolog were devised for fungi (Buyer et al., 2001; Dobranic and Zak, 1999). However, these techniques are limited to the assessment of the extractable and cultivable fraction of soil microbial communities, and need long incubation times to induce microbial selection and growth. A respirometric technique based on the analysis of the substrate-induced respiration (SIR) response of whole-soil samples was investigated with a multiple carbon-source substrate for CLPP by Degens and Harris (1997). However, this method is still tedious, especially when assaying many microbial samples simultaneously in a multitude of bottles that have to be processed separately to measure the amount of released $CO_2$ (Chapman et al., 2007). Campbell et al. (2003) developed the MicroResp$^{TM}$ technique, an alternative method that combines the advantages of the Biolog$^{TM}$ technique, using the

microplate system, and those of the SIR approach with ability to measure $CO_2$ production during short-term incubation from a whole soil microbial community. This method was successfully implemented in a wide range of situations, including aquatic ecosystems (Tlili et al., 2011).

In most soil microbial communities, fungi play a critical role in organic matter decomposition and nutrient cycling in soil by decomposing complex substrates, but only a few studies have focused on the function of soil fungal communities (Dobranic and Zak, 1999; Buyer et al., 2001; Oren and Steinberger, 2008). However, changes in the relative numbers of fungi and bacteria may have significant effects on the soil ecosystem, such as carbon sequestration in agroecosystems (Six et al., 2006; Strickland and Rousk, 2010). In addition, fungi and bacteria are differently affected by environmental factors such as toxicants, nutrient status, drought, temperature or physical-chemical conditions (see the review of Strickland and Rousk, 2010; Rousk et al., 2010; Rajapaksha et al., 2004; Klamer and Bååth, 1998; Uhlirova et al., 2005).

The selective inhibition (SI) procedure, first proposed by Anderson and Domsch (1973), has been used in several studies to estimate bacterial and fungal biomass in different soils and environmental stressing conditions (e.g. Bardgett et al. 1996; Lin and Brookes, 1999[ab]; Bailey and al., 2002; Nakamoto and Wakahara, 2004). Methodological studies have assessed different inhibitors for measuring fungal/bacterial biomass ratios (Bailey and al., 2003; Nakamoto and Wakahara, 2004; Rousk et al., 2009b), and recently Oren and Steinberger (2008) used the Microresp™ procedure and a bactericide to assess fungal catabolic profiles along a geographic climate gradient. However, no studies have simultaneously used microbial and fungal biomass and their catabolic profiles to assess soil quality in microrespirometric bioassays.

The objectives of our study were thus (i) to assess the MicroResp™ technique using a selective inhibition (SI) procedure (the "FungiResp" method), (ii) to obtain a catabolic fingerprint of the microbial and fungal communities of different soils (MicroResp™ and FungiResp methods), and (iii) to apply these methods to soils subjected to different treatments (organic matter amendment, drought and heat) that could differently impact fungi in microbial communities.

## 2. Materials and methods

### 2.1. Selective inhibition procedure

#### 2.1.1. Soils

Four different soils were used in this study. The bulk soils were collected from the upper 10 cm of agricultural or grassland soils, sieved (0-2 mm) and stored at 4°C before use. Some soil characteristics are presented in Table 1. Before microrespirometric measurements, soils were pre-incubated at 30-60% of WHC for one week at ambient temperature (25°C +/- 2) in the dark (Campbell et al., 2003).

**Table 1:** Selected physical and chemical characteristics of the soils.

| Soil | Geographical context | Agronomical context | Water content[a] ($g\ g^{-1}$) | pH (water) | Texture | Total C ($mgg^{-1}$) | Total N ($mg/g^{-1}$) | C/N |
|---|---|---|---|---|---|---|---|---|
| Avignon | Mediterranean (43°9N/4°9E) | agricultural soil | 18.2 | 8.37 | fine calcareous silty clay | 11.6 | 1.42 | 8.1 |
| Theix | Central upland France (45°4N/3°0E) | Grassland (not cultivated) | 39.9 | 5.89 | sandy clay loam | 36.1 | 3.6 | 10.1 |
| Montpellier | Mediterranean (43°6N/3°8E) | Grassland (cultivated) | 15.0 | 7.64 | clay loam | 16.9 | 1.61 | 10.5 |
| Lusignan | South-west France (46°4N/0°1E) | Grassland (cultivated) | 5.5 | 6.49 | silty loam | 10.2 | 0.995 | 10.3 |

[a] Soil water content measured at the time of sampling

#### 2.1.2. Antibiotics

The inhibition potential of two bacterial inhibitors (oxytetracycline and bronopol, Rousk et al., 2009a, Bailey et al., 2003) was tested on glucose-induced soil respiration at 1.25, 2.5, 5, 10, 20, 39, 78, 156, 312, 625, 1250, 2500, 5000 and 10000 $\mu g\ g^{-1}$ soil.

Dilution plate cultures (bacteria and fungi) were run to confirm the efficiency and selectivity of the bactericide bronopol. Colony-forming units (CFUs) of bacteria from a diluted soil were counted on Trypticase Soy Agar (TSA) and that of fungi on Malt extract agar plus 78 or 1250 ppm of bronopol. Control plates contained diluted soil and bronopol-free agar. Concentrations of inhibitors were based on the wet weights of the culture media (Anderson and Domsch, 1975; Nakamoto and Wakahara, 2004). Plates were incubated at 25°C for 5 or 14 day, and CFUs were counted on three plates at appropriate dilution levels.

### 2.1.3. The micro-respirometry method

The MicroResp $^{TM}$ technique is a colorimetric method that allows the measurement of $CO_2$ from whole soils resulting from the utilization of different carbon sources (Campbell et al., 2003). We applied this technique to obtain a catabolic fingerprint of the microbial and fungal communities of different soils. The respiratory response to nine dissolved carbon sources was tested: six carbohydrates (glucose, sucrose, trehalose, mannose, dextrin and D+cellobiose); two amino acids (glycine and alanine) and one carboxylic acid (malic acid). The carbon substrates were selected for ecological relevance: Relevant to soil agroecosystems (i.e. plant residues, root exudates etc.), involved in desiccation tolerance (osmolytes like sucrose, trehalose and glycine), and differentially mineralized (Campbell et al., 1997). Water was also added to assess basal respiration. A stock solution was made from 25 µl of each substrate that was added to the 96-deep-well plate to deliver 30 mg of C per mL of soil water to each one (corresponding to 6.7 mg $g^{-1}$dw soil). To obtain the catabolic fingerprint of the fungal communities, we added 25 µL of the bacterial inhibitor to the deep-well microplate (Nunc 278012 1.2 mLvolume). However, to obtain the catabolic fingerprint of the whole microbial communities we added 25 µL of water. Soil (≈ 40% of WHC) was then added using a volumetric dispenser system (300 µl, corresponding to 0.35 g dry soil) in each deep well of the microplate, following Campbell et al. (2003). After 1 h preincubation with the bacterial inhibitor (to let the toxicant penetrate the cells and induce maximal inhibition; Tlili et al., 2011; Rousk et al., 2009b; Bailey et al., 2003), each deep-well microplate was sealed to the $CO_2$-trap microplate *via* a silicone seal (MicroResp$^{TM}$, UK) and incubated in the dark at 23°C (±2) for 6 h. $CO_2$-trap absorbance was measured at 570 nm (Bioteck L-800 spectrophotometer) immediately before sealing to the soil deep-well plate, and after 6 h incubation. Absorbance values were converted to $CO_2$ concentration after the construction of a calibration curve of absorbance *versus* headspace equilibrium $CO_2$ (measured on a GC,

Bérard et al., 2011). Glucose-induced respiration (SIR-glucose) was assumed to be proportional to active microbial and fungal biomasses (Anderson and Domsch, 1978, 1985; Chapman et al. 2007; Saul-Tcherkas and Steinberger, 2009). We then used the conversion factor of 40 proposed by Anderson and Domsch (1978).

### 2.2. Microbial and fungal biomasses assessed by EL-FAME analysis

Microbial fatty acids of 11 soil samples (Avignon soil samples characterized one day after subjected to different physical (drought, heat, heat-drought) and duration disturbances in laboratory conditions as presented in section 2.3) were analyzed for estimation of microbial and fungal biomasses and comparison to FungiResp data. We performed the ester-linked fatty acid methyl esters (EL-FAMEs) method modified from Schutter, and Dick, 2000. Total fatty acids were extracted from 5 g of each soil sample with 20 mL of chloroform-methanol, 2:1 (v/v) shaken vigorously. The phases allowed separating overnight. The supernatant was then reduced by evaporation. Lipids were dissolved in 500 µL of methyl *tert*-butyl ether. One hundred microliters of this solution was placed in a vial and added with 10 µL of the internal standard methyl nonadecanoate (19:0, 230 µg $mL^{-1}$) and 50µL of the derivatization agent. This solution was then vortex-mixed for 30 s and allowed to react for 30 min (Gómez et al., 2010). The fatty acid methyl esters (FAMEs) were separated on an Elite-5MS capillary column (30 m, 0.25 mm, 0.25 µm) in gas chromatograph equipped with a mass spectrometer (GCMS-QP 2010, Shimadzu). The injector temperature was 250°C. The FAMEs peaks were identified by comparing retention times with peaks of standard mixture and specific fatty acid markers. The relative abundance of FAMEs is expressed in µg $g^{-1}$ dry soil and specific lipid indicators were used to assess fungal biomass (18:2ω6,9) and total microbial biomass (18:2ω6,9; 18:1ω9; 16:1ω7; 16:1ω5; 17:1ω8; 18:1ω7; cyclo17:0 and cyclo19:0; i14:0; 14:0; i15:0; a15:0; 15:0; i16:0; i17:0 and a17:0, 10Me16:0; 10Me17:0 and 10Me18:0) (Bérard et al., 2011).

### 2.3. Application of the "FungiResp" method to a physical context of soil perturbation

A drought-heat laboratory experiment was conducted on the "Avignon soil" (Avignon bare soil sampled on June 2010), prepared following Bérard et al. (2011) and subjected to four sets of conditions: (i) control soil incubated at constant conditions ("C", 25% of gravimetric water content ≈ −0.01 MPa, 25°C), (ii) soil subjected to heating ("H", 25% of

gravimetric water content ≈ −0.01 MPa, 50°C), (iii) soil subjected to drying ("D", 5% of gravimetric water content ≈ −10 MPa, 25°C) and (iv) soil subjected to drying and heating ("D-H", of gravimetric water content ≈ −10 MPa, 50°C). For each treatment a disturbance of 7 days was applied.

All the treatments were conducted in darkness and at a constant temperature by placing each microcosm in a desiccator kept in a temperature-controlled incubator. For treatments C and H, the desiccators held a beaker containing free water to maintain high humidity. For treatments D and DH, the desiccators held a beaker containing a saturated aqueous solution of potassium nitrate (Bérard et al., 2011). Soil water contents were regularly checked by gravimetric measurements throughout the course of the experiment.

At the end of 7-day treatment, soils were rewetted by adding sterilized water and adjusted to achieve an average water gravimetric content of 24% (approximately −0.01 MPa). The microcosms were then placed in desiccators (with a beaker containing free water) in an incubator set at 25°C +/- 2. For each treatment, microbial measurements were made 1 day, 14 days and 28 days after the restoration of environmental soil conditions (rewetting and lowering of temperature).

### 2.4. Data processing

To compare soil microbial parameters (biomasses, % SIR fungal/microbial) we applied non-parametric statistical Kruskal-Wallis tests and *post hoc* paired comparisons tests (Bonferroni, the significance level was set to 5%, $n = 4$). Values (four replicates) of each substrate-induced respiration measurement (corresponding to the CLPP data) were normalized to the sum of all SIR measurements, and then underwent detrended correspondence analysis (DCA). Maximum length of the gradient was always below three, indicating that linear methods were appropriate. Accordingly, we carried out principal component analyses (PCA). Non-parametric partial redundancy analysis (RDA), followed by a Monte Carlo permutation test (999 unrestricted permutations) were then used on CLPP data to test the statistical significance of each explanatory variable (factor): (i) tillage, pH, total C and C/N for comparison of soils from different origins, and (ii) disturbance treatment-types (humidity and temperature) for the drought-heat experiment. Statistical Kruskal-Wallis tests and *post hoc* paired comparison tests were performed with XLSTAT® (Addinsoft, Paris, France) under EXCEL 7® (Microsoft Corporation, Redmond, WA). Ordination methods

(DCA, PCA and RDA) were implemented using CANOCO version 4.5 (ter Braack and Smilauer 1998).

## 3. Results

### 3.1. Antibiotics

Fig. 1 presents the dose/response curves for the glucose-induced respiration of the soils over a range of concentrations of the bactericides oxytetracycline and bronopol. Oxytetracycline did not significantly depress respiration at any of the concentrations tested in either the Avignon or the Theix soils. At some concentrations of oxytetracycline, respiration was increased above the respiration of the control (Fig. 1-a, 1-b). Bronopol inhibited respiration of the four studied soils (Fig. 1-c, 1-d, 1-e, 1-f). CFU measurements performed on Avignon bare soil showed that a concentration of 78 ppm of bronopol inhibited most of the bacteria growth but less of the fungal growth in the agar-plate culture, whereas a concentration of 1250 ppm of bronopol inhibited the growth of both bacteria and fungi (Table 2).

**Table 2:** Effects of bronopol on CFU of fungi and bacteria (standard deviation in brakets, n=3).

| CFU | Control | Bronopol (78 ppm) | Bronopol (1250 ppm) |
|---|---|---|---|
| Fungi ($\times 10^4$ $g^{-1}$ wet soil) | 13.33 (± 9.43) | 1.67 (± 2.36) | 0.0 (± 0.0) |
| Bacteria ($\times 10^5$ $g^{-1}$ wet soil) | 267.78 (± 42.86) | 0.0 (± 0.0) | 0.0 (± 0.0) |

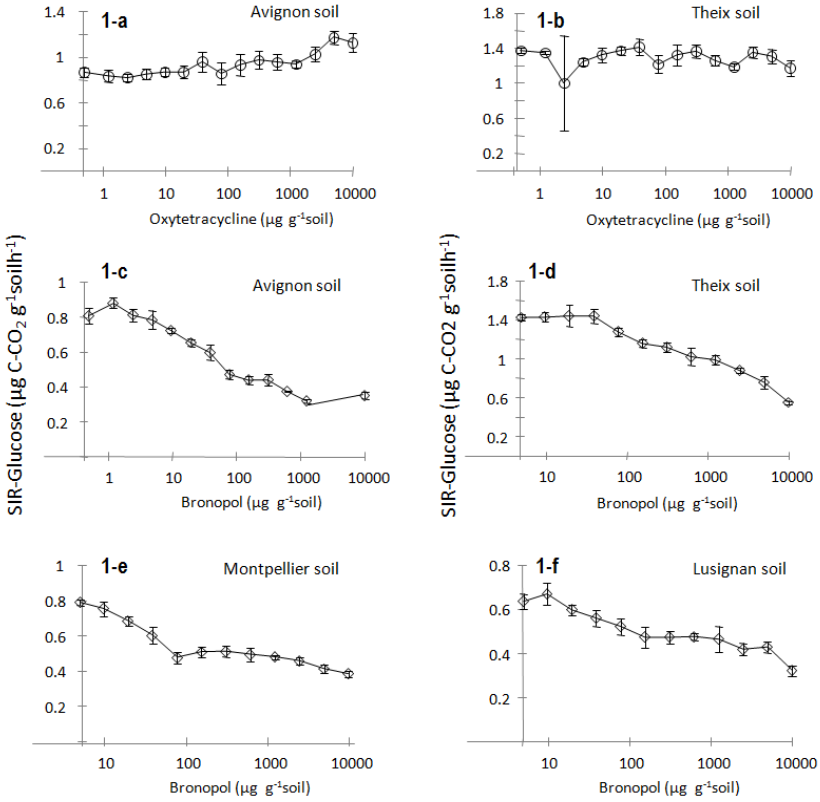


**Fig.1.** Inhibition of SIR-glucose respiration (µg C-$CO_2$ $g^{-1}$soil$h^{-1}$). 1-a in Avignon soil and 1-b Theix soil by the bactericide oxytetracycline. 1-c in Avignon soil, 1-d Theix soil, 1-e Montpellier soil and 1-f Lusignan soil by the bactericide bronopol. Error bars represent the SD from a mean of four replicates.

### 3.2. Comparison between ELFAMEs and FungiResp measurments

There were significant linear correlations between Fungi ELFAMEs and Fungal biomass measured by FungiResp method and between Microbial ELFAMEs and microbial biomass measured by FungiResp method (Pearson correlation test, R2=0.84, $p<0.001$; R2=0.46, $p<0.05$ respectively, Fig. 2).

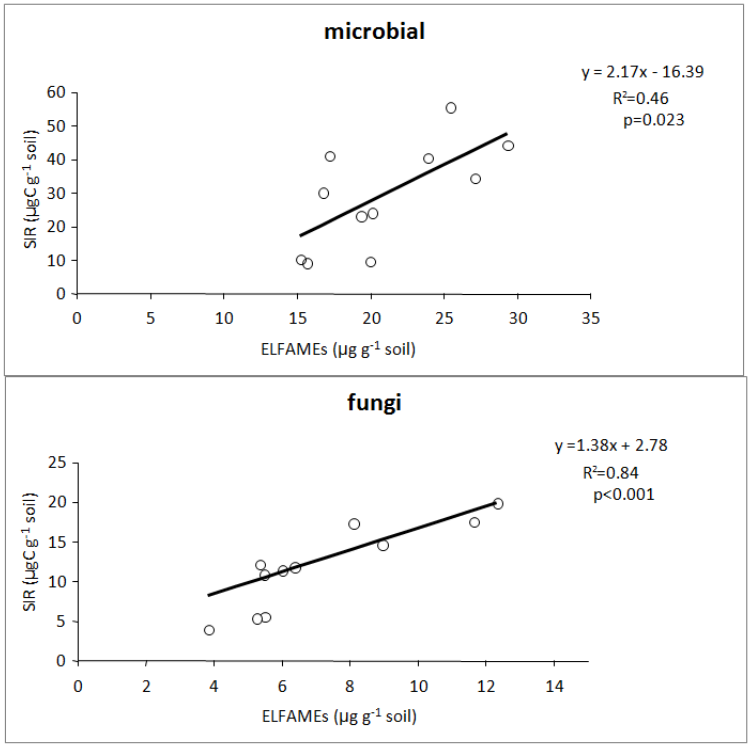


**Fig.2.** Correlation (Pearson) of microbial and fungal biomasses measured by SIR-glucose ("FungiResp") with total microbial and fungal ELFAMEs. 2-a microbial biomasses, 2-b fungal biomasses. Points are means of 3 replicates.

### 3.3. Comparing soils of different origins

The agricultural Mediterranean bare soil of Avignon presented the lowest BR and biomasses (microbial and fungal) and the lowest SIR fungi/microbial percentage. Conversely, biomasses were higher in the grassland soils of the Montpellier, Lusignan and especially Theix sites, and fungal activities were dominant, especially in the Lusignan and Theix sites (Table 3).

**Table 3:** Microbial Basal Respiration, microbial and fungal biomasses (SIR-glucose), and % SIR Fungal/SIR Microbial for the four soils studied. In each row, values with different letters are significantly different (Kruskal Wallis test, n=4, $p<0.05$).

| Soils | Avignon | Montpellier | Lusignan | Theix |
|---|---|---|---|---|
| Basal Respiration (µg C-CO2 $g^{-1}$soil$h^{-1}$) | 0.13 (a) | 0.34 (ab) | 0.59 (b) | 0.33 (ab) |
| Microbial biomass (µgbiomass-C$g^{-1}$soil) | 70.6 (a) | 114.6 (ab) | 110.7 (ab) | 161.2 (b) |
| Fungal biomass (µgbiomass-C$g^{-1}$soil) | 32.9 (a) | 69.7 (ab) | 83.2 (ab) | 120.3 (b) |
| SIR fungal/microbial (%) | 46.6 (a) | 60.9 (a) | 75.2 (b) | 74.7 (b) |

The principal component analysis performed on both microbial and fungal CLPP measurements (axes PC1 and PC2 of the PCA accounted for more than 70% of the total variability, Fig. 3), confirmed the marked difference between the agricultural bare soil of Avignon and the three other grassland soils, separated principally with the PCA1 axis. Also, the Avignon soil presented the greatest difference between microbial and fungal CLPPs, whereas the grassland soils presented the smallest difference between microbial and fungal CLPPs. PC2 axis, particularly for the fungal communities seemed to discriminate between the tilled soils (Avignon, Montpellier and Lusignan soils) and the untilled soil of Theix. Partial redundancy analysis (RDA) performed on microbial and fungal CLPPs, and followed by the Monte Carlo test, showed that pH, total C, C/N and historic tillage had significant effects on the catabolic functions of the soil microbial and fungal communities ($F$ = 8.7; 4.6; 7.2; 6.4 and $p$ = 0.001; 0.007; 0.001; 0.001 respectively). Avignon soil was characterized for microbial CLPP by the mineralization of sucrose and glucose, and for fungal CLPP by the mineralization of glycine. Montpellier soil was characterized for microbial CLPP by the mineralization of glycine and for fungal CLPP by the mineralization of D+manose. Lusignan soil was characterized for microbial CLPP by the mineralization of trehalose and D+cellobiose and for fungal CLPP by the mineralization of dextrin. Microbial and fungal Theix soil communities were characterized by the mineralization of malic acid.

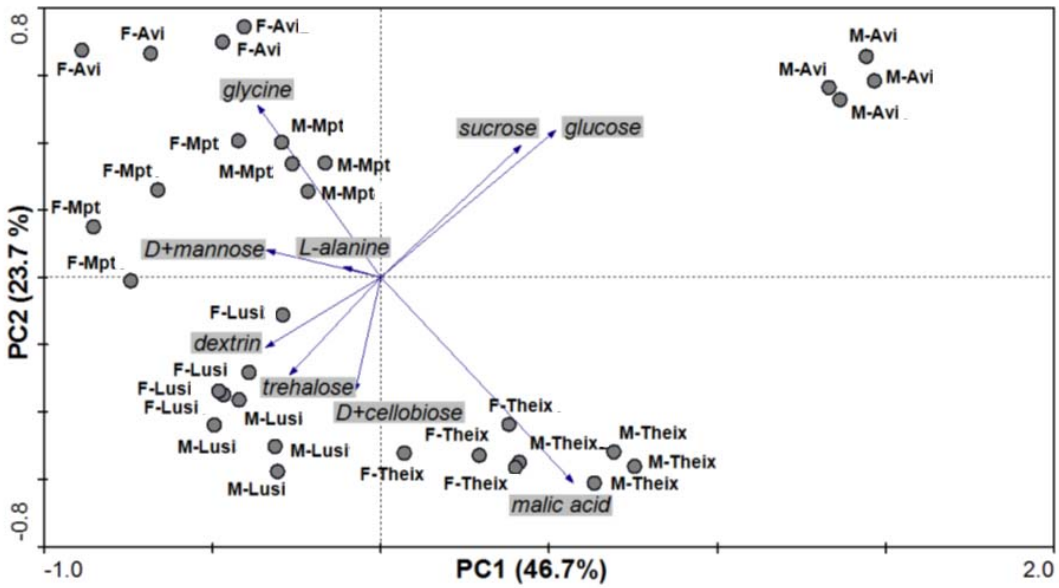


**Fig.3.** Principal component analysis of the microbial (M) and the fungal (F) CLPP measurements from the four different soils studied. Avi = Avignon, Mpt = Montpellier, Lusi = Lusignan, Theix = Theix (four replicates per sampling soil).

### 3.4. Avignon Soil subjected to drought and heat treatments

Just after the end of disturbance and during the recovery time after the end of disturbance, heat-treated soils (H) presented the lowest microbial and fungal biomasses, whereas control (C) and especially drought-treated (D) soils presented the highest biomasses (Table 4). However, fungal activities of the heated soils (H and DH), even just after the end of the disturbance, were significantly highest (Table 4). All soils presented a significant increase in their microbial and fungal biomasses during the recovery time (Kruskal Wallis tests, $n = 4$, $p<0.05$). However, at the end of the experiment the increase in fungal biomass was lower than the total microbial biomass increase for all soils except for the H soil, which remained constant in its SIR fungal/microbial percentage.

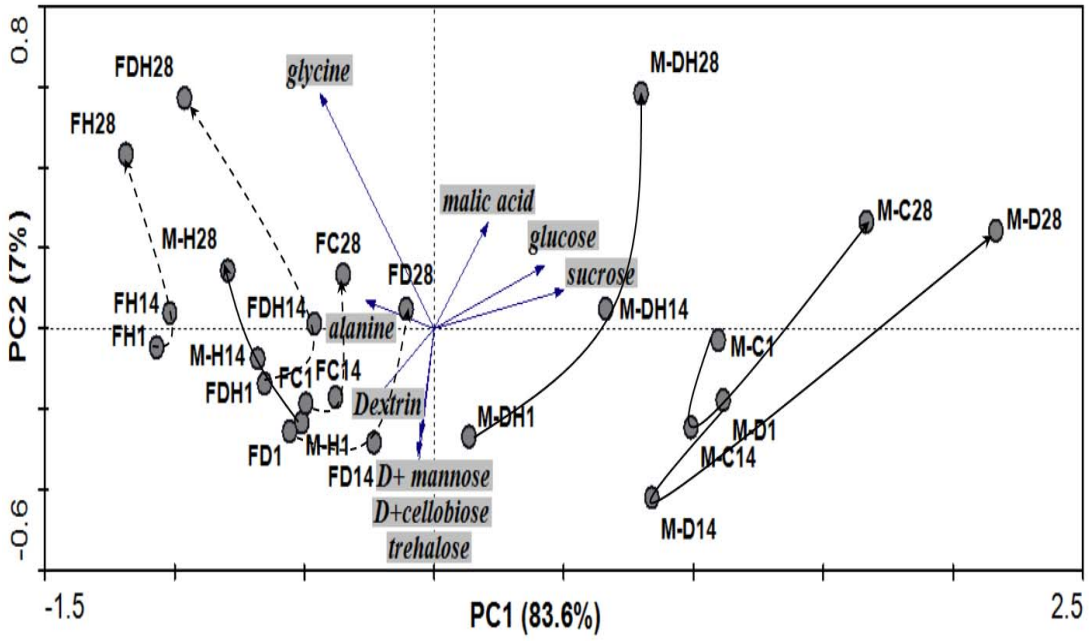


**Fig.4.** Principal component analysis of the microbial (M) and the fungal (F) CLPP measurements from microbial communities during the recovery time after a disturbance of 7 days (1: one day, 14: 14 days and 28: 28 days after the end of disturbance). Treatments (C: control, H: heat treatment, D: drought treatment, DH: drought-heat treatment).

**Table 4:** Microbial Basal Respiration, microbial and fungal biomasses (SIR-glucose), and % SIR Fungal/SIR Microbial for the control soil (C) the heated soil (H), the dried soil (D) and the dried heated soil (DH). Measurements were performed after the end of the perturbations applied (resilience days). Kruskal Wallis tests were performed to compare between control and treatments for each resilience day (values with different letters are significantly different, n=4, $p<0.05$).

| Resilience days | 1 day after the end of perturbation | | | | 14 days after the end of perturbation | | | | 28 days after the end of perturbation | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Soil treatments | C | H | D | DH | C | H | D | DH | C | H | D | DH |
| Basal microbial Respiration (µg C-CO2 $g^{-1}soilh^{-1}$) | 0.10 (ab) | 0.08 (a) | 0.11 (ab) | 0.14 (b) | 0.13 (ab) | 0.11 (a) | 0.15 (b) | 0.13 (ab) | 0.12 (b) | 0.09 (a) | 0.11 (ab) | 0.10 (ab) |
| Microbial biomass (µgbiomass-$Cg^{-1}$ soil) | 34.24 (b) | 9.49 (a) | 40.42 (b) | 23.02 (ab) | 36.95 (ab) | 11.54 (a) | 44.52 (b) | 33.33 (ab) | 74.79 (ab) | 10.08 (a) | 91.01 (b) | 35.35 (ab) |
| Fungal biomass (µgbiomass-$Cg^{-1}$ soil) | 14.57 (ab) | 7.6 (a) | 17.27 (b) | 11.75 (ab) | 20.95 (ab) | 9.72 (a) | 22.53 (b) | 18.24 (ab) | 22.15 (b) | 8.79 (a) | 22.86 (b) | 15.01 (ab) |
| SIR fungal/microbial (%) | 42.57 (a) | 80.45 (c) | 43.43 (ab) | 51.36 (b) | 56.81 (a) | 84.38 (c) | 50.87 (a) | 54.76 (a) | 30.04 (ab) | 87.19 (c) | 25.15 (a) | 42.76 (bc) |

The principal component analyses performed on both microbial and fungal CLPP measurements (axes PC1 and PC2 of the PCA accounted for more than 90% of the total variability, Fig. 4) during the recovery time, separated microbial fungal communities and treatments on PC1. The heat treatment initially induced a microbial catabolic structure comparable to fungal catabolic structures of control and dried soil. Also, the PCA showed an opposite directional change over time in the catabolic microbial and especially fungal structures of the heated soils (H and DH) compared with the control and dried soils (C and D) along the first axis. The partial redundancy analyses (RDA) performed on microbial and fungal CLPPs, and followed by the Monte Carlo test, confirmed the significant impacts of heat but not of drought on the catabolic functions of the soil microbial and fungal communities ($F = 7.28$; $p = 0.008$ and $F = 1.32$; $p = 0.26$ respectively). Microbial CLPPs seemed to separate dried-heated treatments (DH) from control and drought treatment (C and D) more than fungal CLPPs. However, at the end of the experiment (28 days after the end of

the disturbances) DH28 soil presented a fungal CLPP more closely similar to that of H28 soil (Fig.4). During the recovery time, H treatment was characterized for both microbial and fungal CLPPs by the mineralization of amino acids and dextrin, whereas Control and D treatment were characterized by the mineralization of malic acid and glucose and sucrose.

## 4. Discussion

### 4.1. MicroResp™ applied to fungal communities: the FungiResp method

To determine appropriate inhibitors and their concentrations, we examined plots of the respiration response of soils to a range of concentrations of both oxytetracycline and bronopol (Fig. 1). The low short-term inhibition of soil respiration by oxytetracycline observed in our study was also observed in other recent studies (Rousk et al., 2009b; Thiele-Bruhn and Beck, 2005), and bronopol bacteria-inhibiting efficiency was also reported elsewhere (Bailey et al., 2003; Rousk et al., 2009b). The different efficiency levels may be due to different modes of action of the two antibacterial agents. Oxytetracycline is known to affect bacterial growth and activity by inhibiting protein synthesis: This substance is thought to bind to the 30S subunit of the microbial 70S ribosomes inhibiting protein synthesis by blocking the attachment of aminoacyl-tRNA to the acceptor (Hardman et al., 2001). According to Shepherd et al. (1988) the inhibition action of bronopol may result from an oxidative stress: the interaction of bronopol with the thiol compounds in the cell is thought to lead to the oxidation of thiols through a radical anion intermediate.

We thus chose bronopol as a bactericide to extend the FungiResp protocol. Bronopol may impact fungi, as it is used to prevent fungal diseases in fish farming (Branson, 2002). Lin and Brookes (1999[b]) showed in a 25 days experiment that Bronopol at 200 µg $g^{-1}$ soil has both antibacterial and antifungal activity in soil. Fore that, optimal bronopol concentration was selected to minimize the risk of inhibiting non-target communities (fungi) (Bailey et al., 2003). Dose-response curves with bronopol present a classical shape (Welp and Brümmer, 1997): a first decrease in the SIR, a plateau in the middle, and then another decrease in SIR at higher doses of the bactericide. For bacterial inhibition efficiency and fungi safety tests, we thus selected two concentrations. The first one (78 µg.$g^{-1}$ soil) corresponded to the first decrease for the four soils tested (suggesting a bacterial inhibition) and the second one (1250 µg.$g^{-1}$ soil) corresponded to the second decrease for the Avignon soil (suggesting bacteria and fungi inhibition). CFU measurements on Avignon soils confirmed bacterial inhibition (but in

a lower extend fungi inhibition) for the first bronopol concentration and bacteria and fungi inhibition for the second concentration. CFU measurements on Avignon soils confirmed bacterial inhibition (but in a lower extend fungi inhibition) for the first bronopol concentration and bacteria and fungi inhibition for the second concentration. The CFU method was not of course in the environmental context of soil, and it is possible that the contaminant bioavailability in plate cultures was different from its bioavailability in soils because of soil dilutions to perform the bioassay. Moreover CFU method is based on several days' microbial culture (culturable microorganisms) with bronopol into media, whereas FungiResp protocol implies short-term (6+1 hours) soil incubation containing the whole microbial community (culturable and also unculturable microbes that may be less susceptible to the biocide impacts, Piotrowska-Seget et al., 2008) with the selective inhibitor. These differences of bioassay context may explain the fungi inhibition into the plates even at the first concentration of SIR decrease. Moreover our results are consistent with the results of Rousk et al. (2009b) showing that only 80 $\mu g.g^{-1}$ arable soil was needed to inhibit 90% of bacterial growth (leucine incorporation), and very much lower than concentrations used in other studies (1000 to 2000 $\mu g.g^{-1}$ soil, Bailey et al., 2003; Boyle et al., 2008). At 78 $\mu g.g^{-1}$ soil, bronopol reduced respiration of the Avignon soil, the Theix soil, the Montpellier soil and the Lusignan soil by 42%, 11%, 40% and 18% respectively. The "FungiResp" protocol was therefore performed with a concentration of 78 $\mu g.g^{-1}$ soil of the bactericide bronopol applied as a volume of 25 µl in each deep-well microplate.

The correlations between ELFAMEs and FungiResp measurements confirm the interest of "FungiResp" method to assess soil fungal and microbial biomasses. Whereas, the slopes of the correlations for microbial and fungal biomasses are different. Total microbial ELFAMEs values seem to be till positive even with no more SIR of the soil samples. This may be explain by a possible conservation of FAMEs on dead or non active microbial cells one day after the end of the disturbance (Bérard et al., 2011).

### 4.2. Soils of different origins

Microbial biomass and activities (especially cellulolytic fungi) are known to be enhanced in soils with incorporated crop residues (Cookson et al., 1998). Avignon soil was a bare soil with no incorporated crop residues in the previous two years. By contrast, the grassland sites of Montpellier, Lusignan and Theix presented high volumes of rhizosphere soil and regularly received grass residues on their surfaces. This would explain their higher

microbial biomasses and activities (particularly fungal biomass and activities, Frey et al., 1999; Millard and Singh, 2010), especially in Theix soil, which presented the most total C and highest C/N ratio (Bailey et al., 2002; Six et al., 2006). Also, the soil pH can influence the relative numbers of fungi and bacteria (Rousk et al., 2009a), as fungal biomass and SIR fungal/microbial (%) were closely inversely correlated with pH (Pearson correlation, $p = 0.040$, $R^2 = 0.92$ and $p = 0.041$; $R^2 = 0.92$ respectively). As shown by the partial RDA, the pH structured the catabolism of microbial and fungal communities. Finally, the historic tillage of Avignon soil may have lowered the microbial activities and biomasses and especially the fungal ones (Bailey et al., 2002; Hossain and Sugiyama, 2011). The higher basal respiration of Lusignan soil compared with the other grassland soils may be due to a "Birch effect" (Bérard et al., 2011) induced by rewetting of this dried soil (Table 1) during the 7-day period of pre-incubation.

Microbial communities of Avignon soil were characterized by a higher mineralization of the easily mineralized carbohydrates glucose and sucrose, while grassland soils were characterized by a higher mineralization of amino acids (glycine and L-alanine), more recalcitrant carbohydrates (dextrin, D+cellobiose, D+mannose) and fungi-derived carbohydrates (trehalose), suggesting a broader diversity of available organic matter promoting the development of these substrate-adapted microbial communities and a higher proportion of fungal activities in these microbial communities (Degens and Harris, 1997). This is consistent with partial RDA results showing a structuring of C and C/N on CLPP measurements. The structuration of CLPP measurements by the historic tillage (partial RDA results) and the fact that microbial and fungal Theix soil communities were characterized by a higher mineralization of malic acid and a lower mineralization of glycine, L-alanine, sucrose and glucose, are consistent with the results of Cookson et al. (2008), who noted that no-till soils had greater utilization of carboxylic acids and lower utilization of amino acids and carbohydrates than conventional-till ones. These CLPP patterns compared between grassland and agricultural soils confirm the importance of fungi in microbial communities for C sequestration and mineralization of more diversified and recalcitrant organic matter (Strickland and Rousk, 2010).

### 4.3. Avignon Soil subjected to drought and heat disturbances

The low impact of the 7-day drought treatment on microbial and fungal biomasses may be a consequence of an adaptation of this Mediterranean soil, which was frequently

exposed to water shortage *in situ* (during year 2010, annual precipitation and temperature amounted to 54 mm and 15.3 °C respectively and monthly soil moisture contents varied from 8.5 to 23.9 %). Heated soils (H and DH) were highly impacted and did not recover in either biomass or catabolic structure 28 days after the end of the 7-day 50°C disturbances. The direct killing of microbial biomass associated with some thermal denaturing of microbial enzymes (Joergensen et al., 1990) by heat disturbance may suffice to cause shifts in the allocation and fate of carbon in the entire microbial community (Bérard et al., 2011). The DH soil was less impacted than the H soil. In our experimental design we first dried soils for 12 h at a temperature of 25°C and then heated the soils in an incubator at 50°C (Bérard et al., 2011). Desiccation may then have partly "protected" microbial communities from subsequent heat (Bérard et al., 2012) by increasing the proportion of inactivated or tolerant microorganisms by starvation (Lundquist et al., 1999) and/or by inducing an adaptation of microorganisms at the colony scale (Or et al., 2007).

Heat disturbance induced killing of microbial and fungal biomasses, but the SIR fungal/microbial percentage remained high during the recovery time till the end of experiment, suggesting no shift induced by heating in recolonizing microbial biomasses from fungi towards bacteria as observed by Barcela-Moreno and Bååth (2009). However, in terms of functional activity, the catabolic structure of fungi in the microbial community seems to be heavily impacted by the heat disturbances with less resilience as measured with fungi CLPPs.

## 5. Conclusion

The objective of this study was to propose a simple, low cost, miniaturized method applicable to whole soil to characterize the fungal biomass and catabolic profiles in the soil microbial and fungal communities: the FungiResp method. We applied this method to compare different soils and perturbations (drought and temperature). The FungiResp method gave us further data on the fungal part of the microbial communities in these different contexts. In addition, the catabolic structure of microbial and fungal communities measured as patterns of substrate utilization (CLPPs) enabled us to contrast the functional contributions of the decomposer groups in the different soils studied (agricultural or grassland soils) and highlight the functional impacts of the different perturbations applied to these soils.

These first applications show that the FungiResp technique offers a convenient, rapid and sensitive method for assessing environmental factors affecting microbial and fungal

communities in soil ecosystems. Therefore, the FungiResp method seems to respond to the criteria for indicators of soil quality suggested by Doran and Zeiss (2000). To increase the relevance of this method, the inclusion of a range of more complex and recalcitrant substrates in the FungiResp assay could be tested (Oren and Steinberger, 2008), but to take advantage of this simple method, these substrates have to be soluble in water. Also, this method has to be further refined to cope with artefacts derived from $CaCO_3$–$CO_2$–$H_2O$ equilibria that occur in substrate-induced respiration (SIR) measurements, closely linked to variations in the soil solution pH, particularly in calcareous soils (Oren and Steinberger, 2008). Finally, we have to keep in mind that the selective inhibition (SI) procedure we use in the FungiResp method means that we apply, in a short-term bioassay, a chemical perturbation to the microbial communities, inducing reactions that could be variable among soils, because of variability in inhibitor bioavailability and variability in the sensitivity of microbial populations to this toxicant (Anderson and Domsch, 1975; Bailey et al., 2003; Bérard et al., 2004). Moreover, bronopol may impact to a degree the fungal community depending of its concentration. For these reasons inhibitor concentration must thus be checked for the new soils studied.

There is no one universal indicator that can be used to characterize soil ecosystems and their perturbations (Strickland and Rousk, 2010). The FungiResp method assessing fungal contribution to microbial activity and catabolic structure should be used as one of a range of diversified indicators for diversified functions and communities to address specific research questions in ecosystem functioning and environmental impacts on these ecosystems.

**Acknowledgements**

This study was supported by the "Soil-Aquifer Flux Experimental Facility" at INRA-Avignon and the sampling sites of the VALIDATE ANR project. The authors thank the UE-EA (INRA-Avignon) for technical assistance in the agricultural management of the crop field, D. Renard, B. Bes, G. Sévenier and C. Ginies for their support with soil sampling, suction table manipulations and biochemical analysis, and Odile Berge and Christelle Leyronas for scientific and technical advice on dilution plate cultures. The project received funding from the INRA Division Environment and Agronomy (Projets Innovants). The work was supported by the French Agency for Environment and Energy Management (ADEME) and INRA Division Environment and Agronomy. The text was language-edited by ATT, Clermont-Ferrand.

## References


Anderson, J.P., Domsch, K.H., 1973. Quantification of Bacterial and Fungal Contributions to Soil Respiration. Arch. Mikrobiol. 93, 113–127.

Anderson, J.P.E., Domsch, K.H., 1975. Measurements of bacterial and fungal contribution to respiration of selected agricultural and forest soils. Can. J. Microbiol. 21, 314–322.

Anderson, J.P.E., Domsch, K.H., 1978. A physiological method for the quantitative measurement of microbial biomass in soil. Soil Biol. Biochem. 10, 215–221.

Anderson, T.-H., Domsch, K.H., 1985. Determination of ecophysiological maintenance carbon requirements of soil microorganisms in a dormant state. Biol. Fert. Soils 1, 81–89.

Bailey, V.L., Smith, J.L, Bolton, H., 2003. Novel antibiotics as inhibitors for the selective respiratory inhibition method of measuring fungal: bacterial ratios in soil. Biol. Fertil. Soils 38, 154–160.

Bailey, V.L., Smith, J.L., Bolton, H., 2002. Fungal to bacterial ratios in soil investigated for enhanced C sequestration. Soil Biol. Biochem. 34, 997–1007.

Barcela-Moreno, G., Bååth, E., 2009. Bacterial and fungal growth in soil heated at different temperatures to simulate a range of fire intensities. Soil Biol. Biochem. 41, 2517–2526.

Bardgett, R.D., Hobbs, P.J., Frostegard, A ., 1996. Changes in soil fungal: bacterial biomass ratios following reductions in the intensity of management of an upland grassland. Biol. Fertil. Soils 22, 261–264.

Bell, T., Newman, J.A., Silverman, B.W., Turner, S.L., Lilley, A.K., 2005. The contribution of species richness and composition to bacterial services. Nature 436,1157–1160.

Bérard, A., Ben Sassi, M., Renault, P., Gros, R., 2012. Severe drought-induced community tolerance to heat wave. An experimental study on soil microbial processes. J. Soils S. DOI 10.1007/s11368-012-0469-1.

Bérard, A., Bouchet, T., Sévenier, G., Pablo, A.L., Gros, R., 2011. Resilience of soil microbial communities impacted by severe drought and high temperature in the context of Mediterranean heat-waves. Eur. J. Soil Biol. 47, 333–342.

Bérard, A., Rimet, F., Capowiez, Y., Leboulanger, C., 2004. Procedures for determining the pesticide sensitivity of indigenous soil algae - a possible bioindicator of soil contamination? Arch. Environ. Contam. Toxicol 46, 24–31.

Boyle, S.A., Yarwood, R.R., Bottomley, P.J., Myrold, D.D., 2008. Bacterial and fungal contributions to soil nitrogen cycling under Douglas fir and red alder at two sites in Oregon. Soil Biol. Biochem. 40, 443–451.

Branson E., 2002. Efficacy of bronopol against infection of rainbow trout (Oncorhynchus mykiss) with the fungus Saprolegnia species. Veterinary Record 2002;151:539-541.

Buyer, J.S., Roberts, D.P., Millner, P., Russek-Cohen, E., 2001. Analysis of fungal communities by sole carbon source utilization profiles. J. Microbiol. Meth. 45, 53–60.

Calbrix, R., Barray, S., Laval, K., 2005. Analysis of potential functional diversity of soil bacterial community using sole-carbon-source utilization profiles: a reproducible procedure. Eur. J. Soil Biol. 41, 11–20.

Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S., Potts, J.M., 2003. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate

amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Appl. Environm. Microbiol. 69, 3593–3599.

Campbell, C.D., Graystona, S.J., Hirst, D.J., 1997. Use of rhizosphere carbon sources in sole carbon source tests to discriminate soil microbial communities. J. Microbiol. Meth. 30, 33–41.

Chapman, S.J., Campbell, C.D., Artz, R.R.E., 2007. Assessing CLPPs using MicroResp$^{TM}$. A comparison with biolog and multi-SIR. J. Soil Sediment 7, 406–410.

Commission of the European Communities, 2002. Towards a Thematic Strategy for Soil Protection. 179 final. 35pp.

Cookson, W.R., Beare, M.H., Wilson, P.E., 1998. Effects of prior crop residue management on microbial properties and crop residue decomposition. Appl. Soil Ecol. 7, 179–188.

Cookson, W.R., Murphy, D.V., Roper, M.M., 2008. Characterizing the relationships between soil organic matter components and microbial function and composition along a tillage disturbance gradient. Soil Biol. Biochem. 40, 763–777.

Degens, B.P., Harris, J.A., 1997. Development of a physiological approach to measuring the catabolic diversity of soil microbial communities. Soil Biol. Biochem. 29, 1309-1320.

Dobranic, J.K., Zak, J.C., 1999. A microtiter plate procedure for evaluating fungal functional diversity. Mycologia 91, 756–765.

Doran, J.W., Zeiss, M.R., 2000. Soil health and sustainability: managing the biotic component of soil quality. Appl. Soil Ecol. 15, 3–11.

Frey, S.D., Elliott, E.T., Paustian, K., 1999. Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. Soil Biol. Biochem. 31, 573-585.

Gómez, B.M., Lores M., Domínguez J., 2010. A new combination of extraction and derivatization methods that reduces the complexity and preparation time in determining phospholipid fatty acids in solid environmental samples. Bioresource Technol. 101, 1348-1354.

Hardman, J.G., Limbird, L.E., Gilman, A.G., 2001. Goodman and Gilman's. The Pharmacological Basis of Therapeutics. 10th ed. New York, NY: McGraw-Hill, p. 2045.

Hossain, Z., Sugiyama, S., 2011. Geographical structure of soil microbial communities in northern Japan: effects of distance, land use type and soil properties. Eur. J. Soil Biol. 47, 88–94.

Joergensen, R.G., Brookes, P.C., Jenkinson, D.S., 1990. Survival of the soil microbial biomass at elevated temperatures. Soil Biol. Biochem. 22, 1129–1136

Klamer, M., Bååth, E., 1998. Microbial community dynamics during composting of straw material studied using phospholipid fatty acid analysis. FEMS Microbiol. Ecol. 27, 9–20.

Lin, Q., Brookes, P.C., 1999[a]. An evaluation of the substrate-induced respiration method. Soil Biol. Biochem. 31, 1969–1983.

Lin, Q., Brookes, P.C., 1999[b]. Comparison of substrate induced respiration, selective inhibition and biovolume measurments of microbial biomass and its community structure in unamended, ryegrass-amended, fumigated and pesticide-treated soils. Soil Biol. Biochem. 31, 1999-2014.

Lundquist, E.J., Jackson, L.E., Scow, K.M., 1999. Wet-dry cycles affect dissolved organic carbon in two California agricultural soils. Soil Biol. Biochem. 31, 1031–1038.

Millard, P., Singh, B.K., 2010. Does grassland vegetation drive soil microbial diversity? Nutr. Cycl. Agroecosyst. 88, 147–158.

Millennium Ecosystem Assessment, 2005. Ecosystems and Human Well-being: Synthesis. Island Press, Washington, DC.

Nakamoto, T., Wakahara, S., 2004. Development of Substrate Induced Respiration (SIR) Method Combined with selective Inhibition for Estimating Fungal and Bacterial Biomass in Humic Andosols.. Plant Prod. Sci. 7, 70–76

Or D., Smets, B.F., Wraith, J.M., Deschesne, A., Friedman, S.P., 2007. Physical constraints affecting bacterial habitats and activity in unsaturated porous media – a review. Adv. Water Ress. 30, 1505–1527.

Oren, A., Steinberger Y., 2008. Catabolic profiles of soil fungal communities along a geographic climatic gradient in Israel. Soil Biol. Biochem. 40, 2578–2587.

Piotrowska-Seget, Z., Engel, R., Nowak, E., Kozdro, J., 2008. Successive soil treatment with captan or oxytetracycline affects non-target microorganisms. World J. Microbiol. Biotechnol. 24, 2843–2848.

Rajapaksha, R.M.C.P., Tobor-Kapłon, M.A., Bååth, E., 2004. Metal Toxicity Affects Fungal and Bacterial Activities in Soil Differently. Appl. Environ. Microbiol. 70, 2966–2973.

Reynolds, H.L., Packer, A., Bever, J.D., Clay, K., 2003. Grassroots ecology: Plant-microbe-soil interactions as drivers of plant community structure and dynamics. Ecology 84, 2281–2291.

Ritz, K., Black, H.I.J., Campbell, C.D., Harris, J.A., Wood, C., 2009. Selecting biological indicators for monitoring soils: a framework for balancing scientific and technical opinion to assist policy development. Ecol. Indic. 9, 1212–1221.

Romaniuk, R., Giuffre, L., Costantini, A., Nannipieri, P., 2011. Assessment of soil microbial diversity measurements as indicators of soil functioning in organic and conventional horticulture systems. Ecol. Indic. 11, 1345–1353.

Rousk, J., Brookes, P.C., Bååth, E., 2009a. Contrasting soil pH effects on fungal and bacterial growth suggests functional redundancy in carbon mineralisation. Appl. Environ. Microbiol. 75, 1589–1596.

Rousk, J., Brookes, P.C., Bååth, E., 2010. Investigating the mechanisms for the opposing pH relationships of fungal and bacterial growth in soil. Soil Biol. Biochem. 42, 926–934.

Rousk, J., Demoling, L.A., Bååth, E., 2009b. Contrasting Short-Term Antibiotic Effects on Respiration and Bacterial Growth Compromises the Validity of the Selective Respiratory Inhibition Technique to Distinguish Fungi and Bacteria. Microb. Ecol. 58, 75–85.

Saul-Tcherkas, V., Steinberger, Y., 2009. Substrate utilization patterns of desert soil microbial communities in response to xeric and mesic conditions. Soil Biol. Biochem. 41, 1882–1893.

Schutter, M.E., Dick, R.P., 2000. Comparison of fatty acid methyl ester (FAME) methods for characterizing microbial communities. Soil Sci. Soc. Am. J. 64, 1659-1668.

Shepherd, J.A., Roger, D., Waigh, R.D., Gilbert, P., 1988. Antibacterial Action of 2-Bromo-2-Nitropropane-1,3-Diol (Bronopol). Antimicrob. Agents Chemother. 32, 1693–1698.

Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Sci. Soc. Am. J. 70, 555–569.

Strickland, M.S., Rousk, J., 2010. Considering fungal:bacterial dominance in soils - Methods, controls, and ecosystem implications. Soil Biol. Biochem. 42, 1385–1395.

Ter Braak, C.J.F., Smilauer, P., 1998. CANOCO Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination (Version 4). Microcomputer Power, Ithaca, New York, 352 pp.

Thiele-Bruhn, S., Beck, I-C., 2005. Effects of sulfonamide and tetracycline antibiotics on soil microbial activity and microbial biomass. Chemosphere 59, 457–465.

Tlili, A., Marechal, M., Montuelle, B., Volat, B., Dorigo, U., Bérard, A., 2011. Use of the Microresp™ method to assess Pollution-Induced Community Tolerance for lotic biofilms Environ. Pol. 159, 18–24.

Uhlirova, E., Elhottova, J., Triska J., Santruckova, H., 2005. Physiology and microbial community structure in soil at extreme water content. Folia Microbiol. 50, 161–166.

Welp, G., Brummer, G.W., 1997. Microbial Toxicity of Cd and Hg in Different Soils Related to Total and Water-Soluble Contents. Ecotox. Environm. Safe. 38, 200–20.

# Chapitre III

# RESULTATS ET DISCUSSION

Ce chapitre est constitué de deux parties. La première partie décrit les conséquences d'un évènement de type canicule et/ou sécheresse sur les communautés microbiennes d'un sol Méditerranéen. Elle inclut deux articles en préparation intitulés respectivement: "Heat waves and soil Mediterranean microbial communities: Differential influence of drought, high-temperature and perturbation duration" (Article 4) et "Functional and taxonomic stability of soil Mediterranean microbial communities subjected to drought and/or high temperature" (Article 5). La deuxième partie est consacrée à l'étude de l'influence de l'épandage de composts sur la réponse des communautés microbiennes à une perturbation de type canicule-sécheresse. Elle inclut un article en préparation intitulé: "Effects of compost amendment on functional and taxonomic stability of soil Mediterranean microbial communities subjected to drought and high temperature" (Article 6).

# Partie III.1

# Quelles sont les conséquences d'un événement de type canicule et/ou sécheresse sur les communautés microbiennes d'un sol Méditerranéen ?

Cette partie est constituée de deux sections rédigées sous forme d'articles en préparation. La première section aborde les effets à court-terme de perturbations de type canicule et/ou sécheresse appliquées aux communautés microbiennes (structures et fonctions) d'un sol Méditerranéen agricole ; influence du type et de la durée de la perturbation (Article 4). La deuxième section aborde les effets à long-terme de perturbations de type canicule et/ou sécheresse appliquées aux communautés microbiennes (structures et fonctions) d'un sol Méditerranéen agricole (Article 5).

# Section III.1.1

# Effets à court-terme de perturbations de type canicule et/ou sécheresse appliquées aux communautés microbiennes (structures et fonctions) d'un sol Méditerranéen agricole; influence du type et de la durée de la perturbation (Article 4)

Cette section est présentée sous forme d'un article en préparation intitulé : **"Heat waves and soil Mediterranean microbial communities: Differential influence of drought, high-temperature and perturbation duration"**.

# Heat waves and soil Mediterranean microbial communities: Differential influence of drought, high-temperature and perturbation duration


## Abstract

Shifts in the frequency and intensity of drought events and heat-waves are predicted within the current Mediterranean context. These extreme events may affect the physic-chemical soil characteristics', the microbial growth, the microbial activity and thereby the biodiversity and the function of soil. Therefore we performed an experiment in microcosms mimicking severe drought and/or high temperature perturbation with different durations (2, 7, 14, 21 and 28 days). We then studied the resistance of microbial Mediterranean soil structure (functional and taxonomic) using FungiResp, ELFAMEs, Bacterial ARISA and qPCR techniques. In this Mediterranean context, the effects of heat treatments (heat: H and dry-heat: DH) seemed to be more important than the effects of the drought perturbation (D). Whether heat perturbation (H) has altered the catabolic and the microbial taxonomic structure from 2 day perturbation, the drought-heat perturbation had stronger effects after long-perturbation duration. The 21day drought-heat perturbation induced the strongest soil physico-chemical changes' was considered a critical threshold duration from which the physiology and the structure of soil microbial communities may have undergone the strongest shift.



**Keywords:** Drought, High-temperature, Duration of perturbation, Mediterranean microbial communities, Resistance.

## 1. Introduction

Soil is a complex and dynamic biological system, it governs plant productivity of terrestrial ecosystems and it maintains biogeochemical cycles (Nannipieri et al., 2003). However, soils are one of the great unknown realms on earth, despite decades of extensive research (Coleman, 2011). Most of the processes in soil are reactions mediated by microbes. Therefore, soil microorganisms can degrade a wide range of organic compounds and contribute to nutrient cycling, decomposition and nutrient mineralization and immobilization (Swift et al., 1998; Saetre and Stark, 2005). Soil bacteria and fungi contribute also to plant nutrition by enhancing nutrient uptake and translocation and soil structure (Hinsinger et al., 2009).

Global Circulation Models predict longer and more severe drought periods over the next three or four decades and an increase of global surface temperatures (IPCC, 2007), with increasing frequency and intensity of extreme events such as droughts and heat waves (Goebel et al., 2011).This overall trend towards warming and drought is especially expected for Mediterranean ecosystems (Gibelin and Déqué, 2003; IPCC, 2012). It will decrease soil water-availability and create stressful conditions for microorganisms (Schimel et al., 2007), as temperature and water are key factors in the regulation of many terrestrial biogeochemical processes (Asensio et al., 2007; Wu et al., 2011), including the activities and the composition of soil microbial community (Pettersson and Bååth, 2003; Clark et al., 2009).

Numerous field and laboratory experiments have been performed on different soils to study the effects of a single climate change factor on microbial community (Jensen et al., 2003; Pietikaïnen et al., 2005; Clark et al., 2009; Sanaullah et al., 2011). Studies have shown that high temperatures generally increase soil respiration and may lead to a rapid decrease of the soil microbial biomass (Pietikaïnen et al., 2005, Hamdi et al., 2011). Drought acts as an obstacle to microbial activity (Sardans et al., 2006) and causes a decrease in total microbial biomass (Griffiths et al., 2003). Both disturbances, high temperatures and drought, lead to shifts between microbial groups (Pietikaïnen et al., 2005; Schimel et al., 2007). However, less is known about the combined impacts of high temperatures and drought (Yuste et al., 2007; Andresen et al., 2009; Bérard et al., 2011) and the responses remain unclear (Wu et al., 2011). The level and the duration of the environmental perturbation are the main factors that affect soil microbial responses (Schimel et al., 1999; Certini, 2005). Long-duration of stress could have stronger effects than shorter one and alter communities: for example, two weeks drought

in birch litter reduced more strongly microbial biomass and activity than did shorter droughts (Schimel et al., 1999). The disturbance duration may also increase shifts in microbial taxonomic structure (Bérard et al., 2011). Moreover, high stress duration can have comparable impacts on microbial communities to a high stress level. This has been shown in ecotoxicological studies on fluvial biofilm communities where Zn-tolerance values indicated that exposure to high levels of metals has similar effects to a longer exposure to lower levels (Tili et al., 2011). The researchers hypothesized that microbial communities are impacted by the stresses along a temporal gradient.

A better understanding of the stability responses of microbial diversity and activities to high temperature and/or severe drought is required to protect soil ecosystem under climate change. The aim of this study was to assess the soil microbial composition, structure and catabolic functions and the resistance (i.e. the magnitude of change after a system is disturbed; Pimm, 1984) of these microbial parameters to heat and/or drought of different durations, which were conducted in microcosms. We hypothesized that i) these perturbations have direct effects on soil microorganisms, ii) depending on the nature of the perturbation indirect effects *via* physicochemical soil parameters changes' (particularly Mn mobility and DOC) could in turn affect soil microorganisms, iii) the experimental duration of the perturbation has an influence on the resistance of the microbial community, iv) there is a threshold duration that causes shifts in the microbial community composition and catabolic functions, v) although drought and heat perturbations occurred in the Mediterranean context, moisture perturbation which is the most common environmental stress that soil microorganisms experience may be less important than effects of heat perturbations on soil catabolic function and microbial structure.

## 2. Material and methods

### 2.1. Soil sampling

The soil used in this study was collected on June 2010 from the 0-10 cm layer of a bare field located in the South Eastern of France at INRA-Avignon farm . This soil is an alkaline soil (pH 8.51) with a fine textured silty clay loam (323 g $kg^{-1}$ clay, 259 g $kg^{-1}$ silt and 41 g $kg^{-1}$ sand). It had an organic carbon content of 13.2 g $kg^{-1}$ and a large amount of carbonate (347 g $kg^{-1}$ CaCO3). This soil was air-dried and sieved into size fraction of between 2 and 3 mm.

### 2.2. Incubation experiments

Four treatments were conducted: (1) Control soil incubated at constant conditions ("C", 25% of dry basis gravimetric water content ≈ -0.01 MPa, 25°C), (2) Soil subjected to high-temperature 50°C ("H", 25% of gravimetric water content ≈ -0.01 MPa, 50°C), (3) Soil subjected to drying and incubated at 25°C ("D", 5% of gravimetric water content ≈ -10 MPa), (4) Soil subjected to drying and incubated at 50°C ("DH", of gravimetric water content ≈ -10 MPa). For each treatment different durations of disturbance were applied (2, 7, 14, 21 and 28 days).

After sampling, drying and sieving, the soil was slowly moistened and maintained at -0.01 MPa on a suction table during 13 days at ambient temperature (25 °C +/- 2) and in the dark. It was then distributed in Petri dishes (40 $g.dish^{-1}$, 3 replicates per treatment for each incubation time). All treatments were conducted in darkness and at a constant temperature by putting each microcosm within desiccators put within temperature-controlled incubators. For the treatments "D" and "DH", the soils in the microcosms were air-dried at a temperature of 25°C to adjust the water content close to the target water potential of -10 MPa. Then "D" and "DH" microcosms were kept in desiccators (with a beaker containing saturated aqueous solution of potassium nitrate salt; Bérard et al., 2011) put in incubators maintained at 25°C and 50°C respectively. For the treatments "C" and "H", microcosms were directly kept in desiccators (with a beaker containing free water to maintain high humidity) put in incubators maintained at 25°C and 50°C respectively. Soil water contents were regularly checked by gravimetric measurements throughout the course of the experiment.

At the end of each duration treatment, soils were rewetted by adding ultra-pure water and adjusted to achieve an average water gravimetric content of 25% (approximately -0.01 MPa). The microcosms were then placed in desiccators (with a beaker containing free water) within an incubator placed at 25°C +/- 2. For each duration disturbance, soil analyses were done 24 h after the restoration of environmental soil conditions (rewetting and lowering of temperature). Three soil sub-samples were collected from each treatment scenario and pooled to perform community-level physiological profiling (CLPP) analyses immediately after sampling. However, DNA extraction and ARISA analysis were performed subsequently on triplicate deep-frozen (-80°C) soil samples.

### 2.3. Physico-chemical analyses

*Soil dissolved organic C (DOC)*

Seven gram of soil were mixed with 35 mL of water and shaken for 1 h. The mixture was centrifuged at 7000 rpm and 5°C for 10 min in a SIGMA 6K15 centrifuge. Five milliliters of the supernatant solution was filtered through 0.2 µm filters and injected into Venoject tubes. Soil dissolved organic C (DOC) contents in soil filtered extracts was added with 100 µL of HCl (0.2 M) and analyzed for C (Shimadzu TOC-5050A total organic C analyser).

*Manganese (Mn)*

Seven gram of soil were mixed with 35 mL of 1 mol $L^{-1}$ KCl solution and shaken for 1 h. The mixture was centrifuged at 7000 rpm and 5°C for 10 min in a SIGMA 6K15 centrifuge. Ten milliliters of the supernatant solution were filtered through 0.2 µm filters and injected into Venoject tubes. This solution was added with 50 µL of HCl (38%). Total concentration of Mn was obtained by a SpectrAA 220 atomic absorption spectrometer (Varian, Austria).

### 2.4. Soil microbial measurements

*Metabolic activity*

To assess the catabolic fingerprint of the microbial and fungal communities, the FungiResp method using the MicroResp™ technique combined with a selective inhibition (SI) was applied (Campbell et al., 2003; Ben Sassi et al., 2012; Renault et al., unpublished, Chapter II PhD). The respiratory response to 10 dissolved carbon sources was tested: seven carbohydrates (glucose, sucrose, trehalose, mannose, dextrin, glucosamine and D+cellulobiose); two amino acids (glycine, alanine) and one carboxylic acid (malic acid). The carbon substrates were selected for ecological relevance: relevant to soil agroecosystems (i.e. plant residue, root exudates etc), involved in desiccation tolerance (osmolytes like sucrose, trehalose and glycine). Water was also added to assess basal respiration. A stock solution was made from 25 µl of each substrate that was added to the 96-deep-well plate to deliver 42 mg per mL of soil water to each one. To obtain the catabolic fingerprint of the fungal communities, we added 25 µL of the bactericide bronopol with a concentration of 78 $\mu g.g^{-1}$

soil in each deep-well. However, to obtain the catabolic fingerprint of the whole microbial communities we added 25 µL of water. Soil (≈ 40% of WHC) was then added using a volumetric dispenser system in each deep well of the microplate, following Campbell et al. (2003). After 1 h pre-incubation with the bacterial inhibitor (to let the toxicant penetrate the cells and induce maximal inhibition), each deep-well microplate was sealed to the microplate containing a gel with an indicator dye that responds to the gel pH and, indirectly, to $CO_2$, *via* a silicone seal (MicroResp$^{TM}$, UK) and incubated in the dark at 23°C (±2) for 6 h. Gel absorbance was measured at 570 nm (Bioteck L-800 spectrophotometer) immediately before sealing to the soil deep-well plate, and after 6 h incubation.

When using MicroResp$^{TM}$ technique as described by Campbell et al. (2003), absorbance values were converted to $CO_2$ concentration after the construction of a calibration curve of absorbance *versus* headspace equilibrium $CO_2$. Doing so, researchers assume generally that the microbially produced $CO_2$ accumulates in the gaseous headspace and that this $CO_2$ is of microbial origin exclusively. However, $CO_2$ is also trapped within the Agar gel and the soil solution. Moreover, the soil we used in our study is a calcareous soil with pH>7 exacerbating the risk of $CO_2$ retention in soil solution and the risk of abiotic $CO_2$ emission with acidic substrates. Thus, absorbance values were converted to $CO_2$ concentration after correction using a new geochemical model that assesses the biases in microbial respiration measurements resulting from the $CO_2$ solubilisation and dissociation in the soil solution as in the Agar gel and the effect of substrate supply on $CO_2$ physicochemical equilibriums (Renault et al., unpublished, Chapter II PhD). We unfortunately lost the 28 day perturbation samples for microrespirometric measurements.

The metabolic quotient $qCO_2$ is the ratio of microbial basal respiration (BR) to microbial biomass (Anderson and Domsch, 1985). Microbial and fungal biomasses can be estimated by the glucose-induced respiration (Anderson and Domsch, 1978, Ben Sassi et al., 2012). We divided microbial BR by the glucose-induced respiration to obtain an index directly correlated to $qCO_2$ but without units. This quotient indicates how efficiently the microbial biomass is utilizing available carbon for biosynthesis, and is considered as an ecophysiological indicator of environmental conditions-induced stress in soil (Anderson and Domsch, 1993).

*Microbial community structure (EL-FAME analysis)*

In order to extract microbial fatty acids, we performed the method proposed by Gómez et al. (2010). Total fatty acids were extracted from 5 g of each sample with 20 mL of chloroform-methanol, 2:1 (v/v) shaken vigorously. The phases allowed to separate overnight. The supernatant was then reduced by evaporation. Lipids were redissolved in 500 µL of methyl *tert*-butyl ether. One hundred microliters of this solution were placed in a vial and added with 10 µL of the internal standard methyl nonadecanoate (19:0, 230 µg $mL^{-1}$) and 50µL of the derivatization agent. This solution was then vortex-mixed for 30 s and allowed to react for 30 min. The fatty acid methyl esters (FAMEs) were separated on an Elite-5MS capillary column (30 m, 0.25 mm, 0.25 µm) in gas chromatograph equipped with a mass spectrometer (GCMS-QP 2010, Shimadzu). The injector temperature was 250°C.

The FAMEs peaks were identified by comparing retention times with peaks of standard mixture of BAME (Bacterial Acid Methyl Ester Mix, 47080-U, SUPELCO Analytical, USA and Grain FAME Mix (47801, SUPELCO Analytical, USA) and specific fatty acid markers. The relative abundance of FAMEs is expressed in µg $g^{-1}$ soil and specific lipid indicators are used to assess the abundance of fungal and bacterial structural groups. The specific lipid indicators used to assess the abundance of fungi were 18:2ω6,9 and 18:1ω9 (Klamer and Bååth, 1998). Gram-negative bacteria were characterized by 16:1ω7, 16:1ω5, 17:1ω8, 18:1ω7, cyclo17:0 and cyclo19:0 (Kaur et al., 2005). However, Gram-positive bacteria were characterized by i14:0, 14:0, i15:0, a15:0, 15:0, i16:0, i17:0 and a17:0 (Klamer and Bååth, 1998; Feng and Simpson, 2009) and gram positive thermophile bacteria were characterized by i16:0, a17:0 and i17:0 (Klamer and Bååth, 1998). Actinomycetes were characterized by 10Me16:0 and 10Me18:0 (Williams and Rice, 2007).

*DNA extraction, amplification, PCR quantification and Automated Ribosomal Intergenic Spacer (ARISA)*

The ISO-11063 procedure was used to extract DNA from the soil samples. Briefly, 250 mg of soil containing 0.5 g of 106 µm diameter glass beads and 2 glass beads of 1 mm diameter were homogenized in 1 mL of extraction buffer (100 mM Tris (pH 8.0), 100 mM EDTA, 100 mM NaCl, 1% (w/v), polyvinylpyrrolidone and 2% (w/v), sodium dodecyl sulfate) for 30 s at 1600 rpm in a mini-bead beater cell disrupter (Mikro-Dismembrator S, B. Braun Biotech International, Germany). After the removal of centrifuged soil and cell debris,

the proteins were eliminated using sodium acetate precipitation. Then, nucleic acids were precipitated with cold isopropanol and washed with 70% ethanol. They were purified using a polyvinylpolypyrrolidone and Sepharose 4B spin columns. The quality and the size of the soil DNAs were checked by electrophoresis on 1% agarose gels.

The abundance of the total bacterial and of the *Actinobacteria, Acidobacteria, Bacteriodetes, Firmicutes, Gemmatimonadetes, Verrucomicrobia, α-Proteobacteria, β-Proteobacteria, γ-Proteobacteria, Planctomycetes and Crenarchaeota* was determined by quantitative real-time PCR using taxa-specific 16S rRNA primers previously described (Muyzer et al. 1993; Ochsenreiter et al. 2003; Fierer et al. 2005; Mûhling et al. 2008; Philippot et al. 2009). The presence of PCR inhibitors in DNA extracted from soil was estimated for all samples by mixing a known amount of the pGEM-T plasmid (Promega) with water or with the soil DNA extracts. PCR reactions were then performed in an ABI7900HT thermal cycler (Applied Biosystems) with the T7 and SP6 primers. The measured quantity of plasmid for the different DNA extracts and the controls with water were in some soil DNA extracts significantly different indicating that inhibition occurred. We then diluted all samples until we detected no inhibition and perform PCR reactions. Quantification was based on the increasing fluorescence intensity of the SYBR Green dye during amplification in a total volume of 20 µl using Absolute qPCR SYBR Green Rox (ABgene), 10 µM or 20 µM of each primer (the concentration depended on the primer used), soil DNA and T4 Gp32. The standard curves were carried out using serial dilutions of linearized plasmids containing environmental clones of each taxon.

To analyze the bacterial community structure, ARISA was applied on DNA extracted from soils. The bacterial intergenic spacer region between the small (16S) and large (23S) subunit ribosomal DNA genes was amplified from each soil replicate with the universal primers $1552_f$ (5′-TCG GGC TGG ATC ACC TCC TT-3′) and $132_r$ (5'-GCC GGT TTC CCC ATT CGG-3') (Ranjard et al., 2001). ARISA fragments were resolved on 3.7 % acrylamide gels and run for 15 h at 2500 V/ 80 Won a Li-Cor 4300 DNA analysis system. RISA profiles were analyzed with One-D-Scan program which allows the matrices production (size and relative density of each band).

### 2.5. Data analysis

The values of microbial and fungal chemical group SIR for the different treated soils were normalized to the control values % change from control (Chaer et al., 2009). The

significance of differences between the data obtained with the different perturbation was tested using XLSTAT (Addinsoft, Paris, France). As our data were not following a normal distribution, we applied non-parametric statistical procedures. Kruskal–Wallis tests ($p < 0.05$) were performed on SIRs (4 replicates), ELFAMEs biomasses and bacterial taxa abundances (3 replicates) to assess the difference between perturbations. Mann-Withney tests were performed to assess the differences on SIR glucose data between the control and perturbed soils (4 replicates). Community level physiological profiles (corresponding to substrate-induced respiration measurements normalized to the sum of all SIR measurements; 4 replicates), EL-FAMEs profiles (3 replicates) and bacterial taxa abundances (3 replicates) were carried out with principal component analyses (PCA). Non-parametric partial redundancy analysis (RDA), followed by a Monte Carlo permutation test were then used on CLPPs and ELFAMEs data to test the statistical significance of each explanatory variable (factor): perturbance types (humidity and temperature) and duration. RISA fingerprints were analysed using PrepRISA and ADE-4. The data from the 1D-Scan were converted into a matrix summarizing the bands' presence (i.e. peaks) and intensity (i.e. peak heights) using PrepRISA. Then, using ADE-4, principal component analysis (PCA) was performed (3 replicates).

## 3. Results

### 3.1. Fungal and microbial respirations and soil carbon and manganese content

Heated soils had the highest DOC concentrations with a maximum value of 107.27 µg C $g^{-1}$ soil after 21 day heat perturbation duration (H21) (Fig. 1A, Table 1). However H soils presented the lowest microbial basal respiration (BR) and the lowest SIR glucose whatever the duration of the perturbation (Fig. 2A-B). These biological parameters were consistently lower in H than in control microcosms and the differences were highly significant ($p< 0.0001$) when all sample durations were considered together. Mn concentrations in H soils were comparable to those of Controls.

**Table 1:** Mean values of microbial and fungal SIR-glucose and $qCO_2$ after 2 and 21 day of perturbation (standard deviation in brakets; n=4) and DOC and Mn content. D=Dried, H= Heated soils, DH= Dried-heated soils, and C= control soils.

| Parameters | Perturbation duration (days) | C | D | H | DH |
|---|---|---|---|---|---|
| Microbial SIR glucose (µg C-$CO_2$ $g^{-1}$ soil $h^{-1}$) | 2 | 3.36 (0.75) | 2.44 (0.32) | 0.25 (0.01) | 0.67 (0.25) |
| | 21 | 2.52 (0.65) | 3.09 (0.38) | 0.22 (0.01) | 1.40 (0.05) |
| Fungal SIR glucose (µg C-$CO_2$ $g^{-1}$ soil $h^{-1}$) | 2 | 1.05 (0.04) | 0.39 (0.05) | 0.16 (0.01) | 0.26 (0.00) |
| | 21 | 1.16 (0.17) | 1.21 (0.19) | 0.17 (0.01) | 0.30 (0.01) |
| $qCO_2$ | 2 | 0.08 (0.02) | 0.08 (0.01) | 0.40 (0.02) | 0.35 (0.11) |
| | 21 | 0.12 (0.03) | 0.11 (0.01) | 0.69 (0.54) | 0.32 (0.09) |
| DOC (µg C- $g^{-1}$ soil) | 2 | 47.13 | 56.31 | 63.44 | 46.00 |
| | 21 | 37.44 | 36.13 | 107.25 | 88.63 |
| Mn (µg C- $g^{-1}$ soil) | 2 | 4.13 | 0 | 5.84 | 6.88 |
| | 21 | 4.58 | 2.11 | 0 | 42.24 |

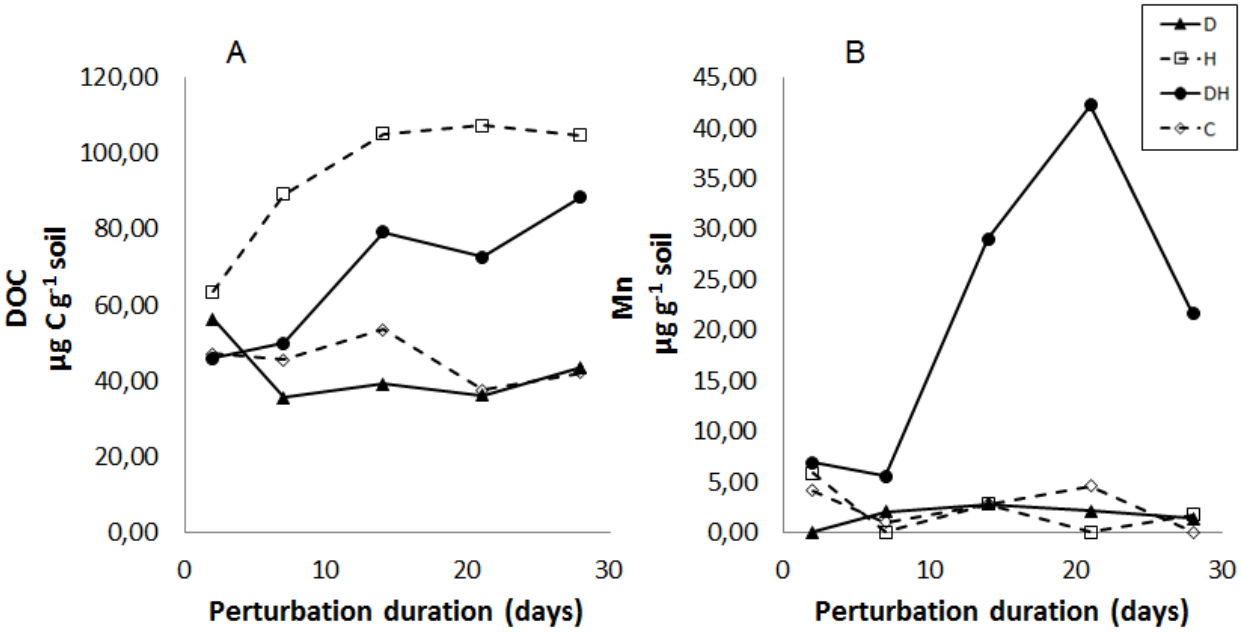


**Fig.1.** DOC (A) and Mn (B) concentration after the different perturbation duration. D=Dried soils, H= Heated soils, DH= Dried-heated soils, and C= control soils.

Except for the 2 days disturbance duration, dried-heated soils (DH) presented the highest microbial BR one day after the end of the perturbation (Fig. 2A). These samples presented also the highest Mn concentration with a maximum value for the 21 day perturbation duration (DH21) (Fig. 1B; Table 1). The DH perturbation durations (2, 7, 14, 21 days) had significant effects on microbial BR, microbial SIR glucose and fungal SIR glucose (Kruskal-Wallis tests; $p<0.05$; $n=4$). Generally, the longer the DH perturbation duration, the higher are the Mn and DOC concentrations in soils, the microbial BR, the microbial SIR glucose (Fig. 1.A-B, Fig. 2.A-B) and the fungal SIR glucose (Table 1).

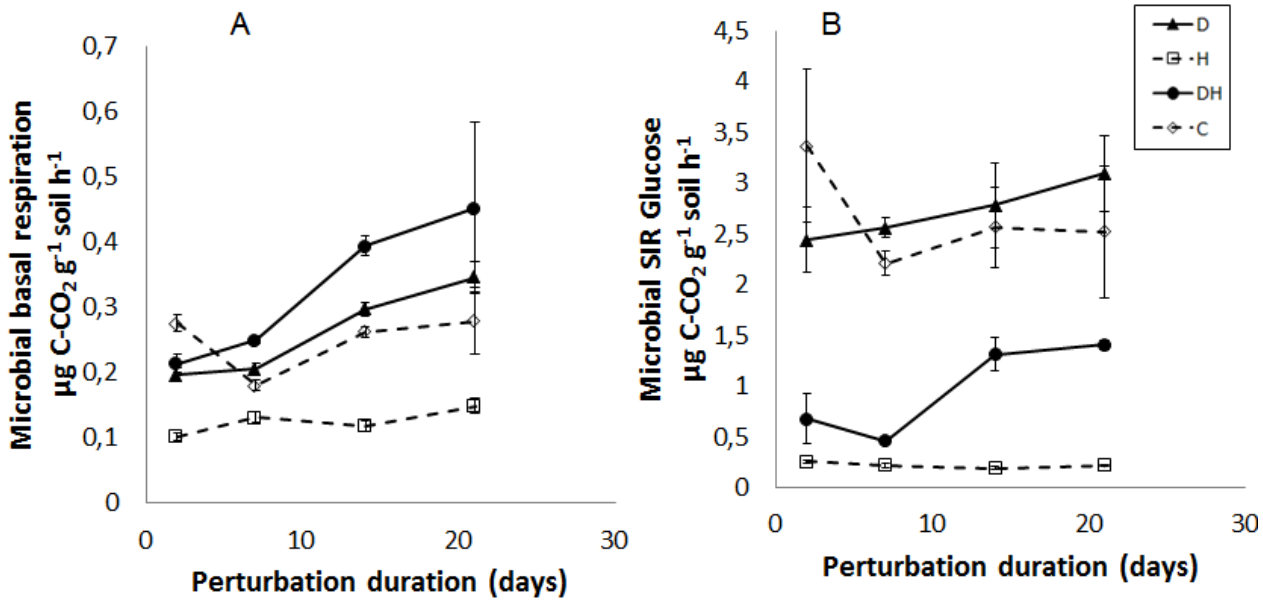


**Fig.2.** Microbial basal respiration (A) and SIR-glucose (B) after the different perturbation duration. D=Dried, H= Heated soils, DH= Dried-heated soils, soils and C= control soils. Error bars represent standard deviation of a mean value (n=3).

Soil microcosms subjected to drought (D) showed an increase in microbial BR and microbial and fungal SIR glucose with maximum values after 21 days of perturbation (Fig. 2.A-B, Table 1). However, except after 2 days of drought (D) where microbial BR and microbial and fungal SIR glucose were lower than in the control, these values were not significantly higher in dried soils (D) than in the control (Mann-Whitney tests, $p>0.05$; Fig. 2.A-B, Table 1).

The metabolic quotient $qCO_2$ was higher in the heat-treated soils (H and DH), while it was lower and stable in the dried soils (D) and the control (C) (Table 1).

### 3.2. Microbial and fungal community level physiological profiles (CLLPs)

The first two axes of the PCA performed on microbial CLLP accounted for more than 81% of the variability (Fig. 3A). There was a high degree of similarity of the ordination of the dried (D) and control (C) soils within PC1 accounting for more than 63% of the overall variation. The PCA1 axis separated the heated soils (H) from all the other soils. This axis seemed to separate also the dried-heated soils from the control (C). However, with increasing the duration of perturbation, the microbial dried-heat perturbation (DH) CLPP came closer to control ones. Particularly, a 21 day dried-heated soil (DH21) presented a microbial CLPP closely similar to C21 soil along PC1. Heated soils (H) were characterized by the mineralization of alanine and glycine. Dried-heated soils (DH) were characterized by the mineralization of dextrin whereas dried soils (D) and control (C) were characterized by the mineralization of sucrose, glucose, D+mannose and D+cellobiose. Partial redundancy analysis (RDA) performed on microbial CLPPs, and followed by the permutation test, showed that drought, heat and the duration of perturbation had highly significant effects ($F=2.46$; 11.86; 1.84 and $p<0,0001$ respectively) on the catabolic functions of the soil microbial communities.

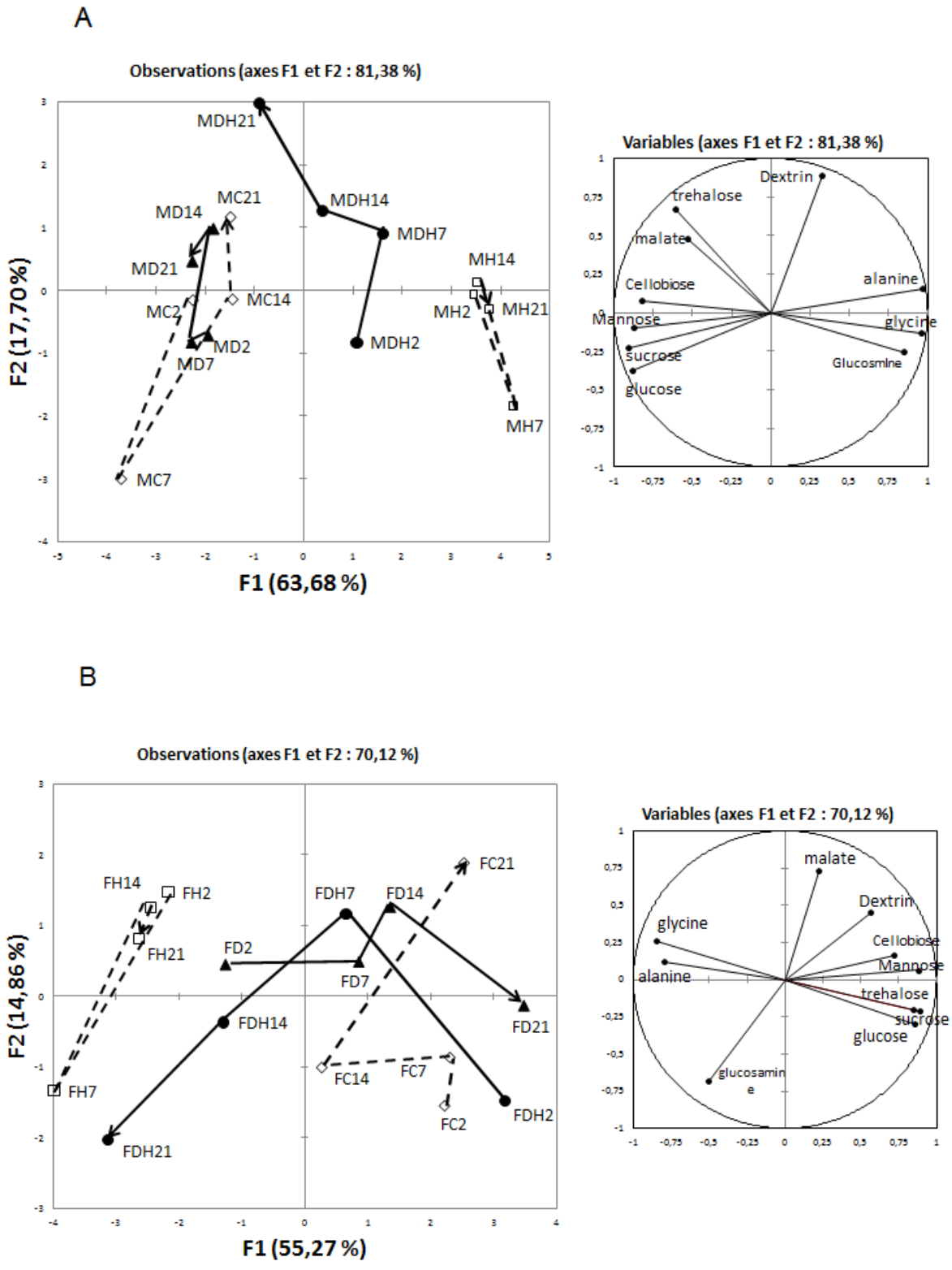


**Fig.3.** Principal component analysis of the microbial (A) and the fungal (B) CLPP measurements after the different perturbation durations (2: 2 days, 7: 7 days, 14: 14 days, 21: 21 days and 28: 28 days). D=Dried soils, H= Heated soils, DH= Dried-heated soils, and C= control soils.

The principal component analysis performed on fungal CLPP measurements showed a total of variability of 70.12% for the first two PCs axes (Fig. 3B). The PC 1 accounting for more than 55% of the variability confirmed the difference between the heated soils (H) and the other treated soils and the control. A 2 day dried-heated soil (DH2) presented a fungal CLPP closely similar to C2. However, with increasing the duration of perturbation, the fungal dried-heat perturbation (DH) CLPP came closer to the fungal heat perturbation (H) CLPP. Particularly, at 21 day perturbation duration, fungal dried-heated soil (DH21) CLLPP was markedly separated from control one (C21) on both PC1 and PC2 whereas the latter CLLP (DH21) was closely similar to fungal heat soil CLLP on PC1. As microbial CLLP, heated soils (H) were characterized for fungal CLPP by the mineralization of alanine and glycine and dried soils (D) and control (C) were characterized by the mineralization of sucrose, glucose, trehalose, D+mannose and D+cellobiose. At 21 day perturbation, dried-heated soil (DH21) was characterized by the mineralization of D+glucosamine. The partial redundancy analysis (RDA) performed on fungal CLPPs, and followed by the permutation test, confirmed the highly significant effect of heat but not of drought ($F=3.35$, $p<0.0001$ and $F=0.61$, $p=0.41$ respectively) on the catabolic functions of the soil fungal communities.

**Table 2:** Mean percentage of change relative to control for microbial and fungal chemical group SIR after 21 day of perturbation (standard deviation in brakets; n=4). D=Dried, H= Heated soils and DH= Dried-heated soils.

| Parameters | | D | H | DH |
|---|---|---|---|---|
| Microbial SIR | Carbohydrates | 10.30 (3.68) | -87.45 (1.00) | -25.23 (2.70) |
| | Amino acids | -7.52 (3.06) | -76.19 (1.91) | -29.66 (9.90) |
| | Carboxylic acid | -23.44 (9.23) | -92.28 (0.17) | -52.24 (5.66) |
| Fungal SIR | Carbohydrates | -3.38 (2.62) | -79.03 (0.46) | -60.19 (2.89) |
| | Amino acids | -14.58 (2.17) | -68.37 (0.28) | -53.68 (3.77) |
| | Carboxylic acid | -30.57 (11.66) | -83.90 (0.59) | -75.43 (0.74) |

For all treatments and all perturbation duration, the percentages of change from control for both microbial and fungal SIR groups (carbohydrates, amino acids, carboxylic acids) were calculated. The results showed that all the microbial and fungal SIR groups studied are stronger negatively impacted by the heat treatments after 21 day of perturbation (H and DH; Table 2). Moreover, fungal SIR groups seemed to be more impacted after 21 day of perturbation than microbial ones particularly in dried-heated soils (Table 2).

### 3.3. Microbial community structure

About 63% of the total variability in the EL-FAMEs profiles was represented by a PCA plot (35.90% along axis 1, and 27.11% along axis 2; Fig. 4). Except for the 2 days perturbation duration, the first axis PC1 separated dried-heated soils (DH) from dried (D) and control soils. However, except for the 2 days perturbation duration, the second axis PC2 separated heated soils (H) from all the other soils. Heated soils (H) were characterized by the dominance of G+ biomarkers (a17, i17 and i16). Dried (D) and control soils were characterized by the dominance of fungal biomarkers (18:2ω6,9 and 18:1ω9) and G negative bacteria biomarkers (16:1ω7, 17:1ω8 and 18:1ω7). A 21 day dried-heated soil (DH21) presented EL-FAMEs profiles closely similar to DH28 soil's ones and both were not characterized by the dominance of fungal biomarkers (18:2ω6,9 and 18:1ω9), while DH2 soils were characterized by fungal dominance. The partial redundancy analysis (RDA) performed on EL-FAMEs profiles, and followed by the permutation test, showed that drought, heat and duration of the perturbation had highly significant effects (F=3.11; 6.22; 2.97 and p<0.0001 respectively) on the soil microbial structure.

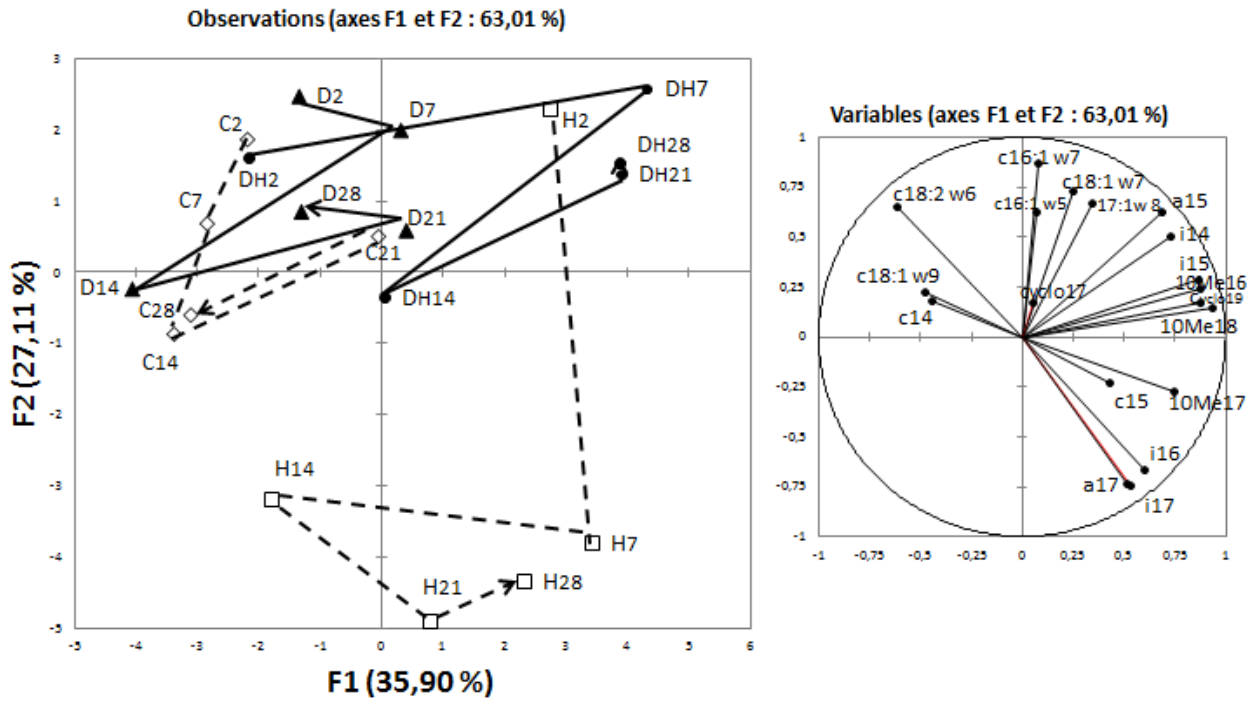


**Fig.4.** Principal component analysis of the ELFAMEs profiles after the different perturbation durations (2: 2 days, 7: 7 days, 14: 14 days, 21: 21 days and 28: 28 days). D=Dried soils, H= Heated soils, DH= Dried-heated soils, and C= control soils.

### 3.4. Bacterial community structure

To evaluate changes that could occur after the different perturbation duration PCA was performed on B-ARISA profiles. The first principal axis (PC1) represented 21.93 % of the variability while the second principal axis (PC2) represented 16.87 %. The heated perturbation (H) was strongly separated from the other perturbations (Fig. 5). However, dried soils (D) were close to the control (C) on PC1 and PC2. For microcosms subjected to drying heating (DH) perturbation, there was a shift of the soil bacterial structure from 21 day of perturbation. Despite the low total variability in B-ARISA profiles, the PCA performed on the abundances of the different bacterial taxa targeted accounted for more than 78% of the total abundance variability (61.17% along axis 1, and 17.72% along axis 2; Fig. 6). The PC1 separated dried soils (D) and the control from heat treated soils (H and DH). For microcosms subjected to drying heating (DH) perturbation, there was a shift of the soil bacterial structure from 21 day of perturbation. Three groups discriminated between the perturbations. The first group consisted of the *Acidobacteria, Gemmatimonadetes, Verrucomicrobia, γ-Proteobacteria, Planctomycetes* and *Crenarchaeota* and characterized dried soils (D) and the control (C). The second group consisted of *Actinobacteria* and *α-Proteobacteria* and characterized heated soils (H) and dried-heated ones (DH) except for DH after 21 and 28 days which were characterized by *Firmicutes* and *β-Proteobacteria* (Fig. 6).

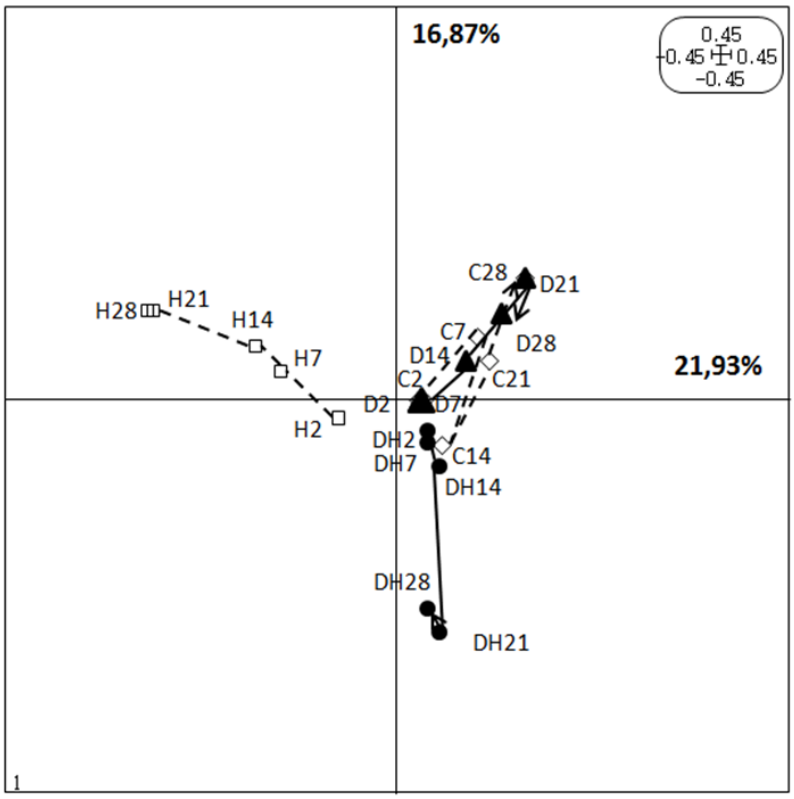


**Fig.5.** Principal component analysis performed from RISA fingerprints obtained from DNA extracted from soils after the different perturbation durations (2: 2 days, 7: 7 days, 14: 14 days, 21: 21 days and 28: 28 days). D=Dried soils, H= Heated soils, DH= Dried-heated soils, and C= control soils. Each circle, rectangle, triangle and diamond is the barycentre coordinate from 3 replicates of each perturbation duration.

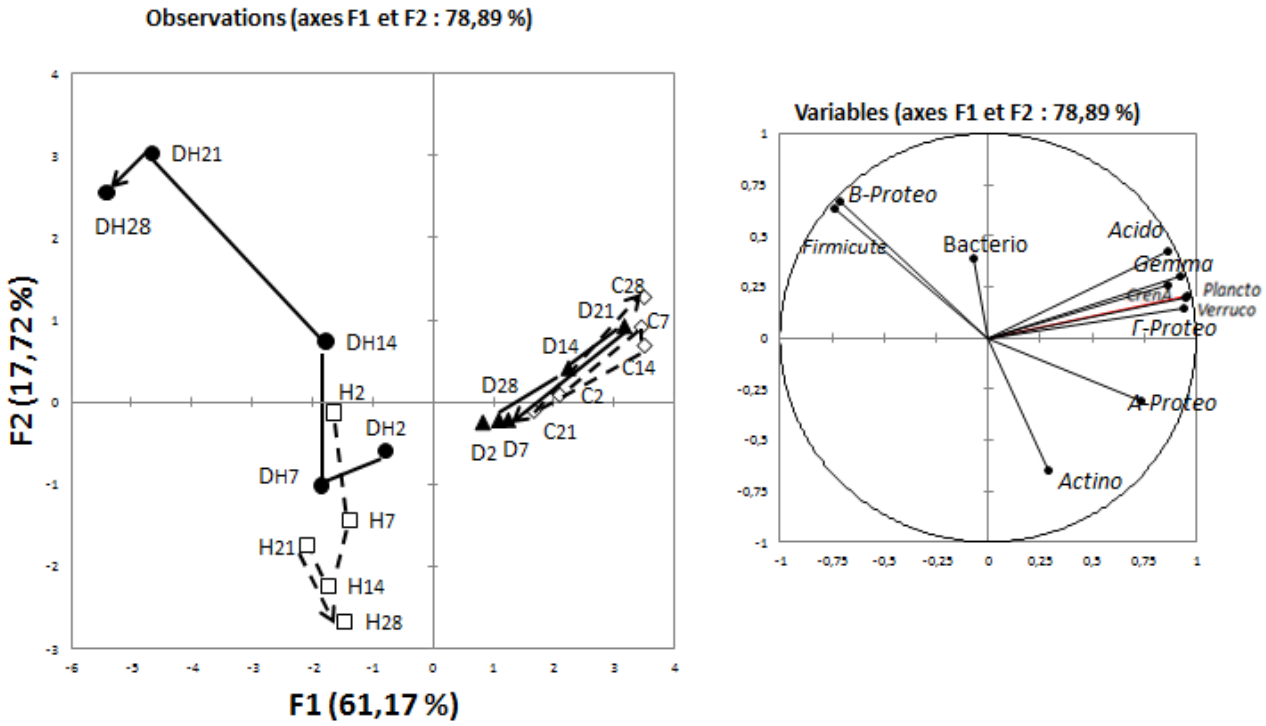


**Fig.6.** Principal component analysis performed from qPCR bacterial taxa abundances (*Acido=Acidobacteria, Gemma=Gemmatimonadetes, Verruco=Verrucomicrobia, γ-Proteo=γ-Proteobacteria, Plancto=Planctomycetes, CrenA=Crenarchaeota*, Actino=*Actinobacteria, α-Proteo=α-Proteobacteria, Firmicute=Firmicutes* and *β-Proteo=β-Proteobacteria*) after the different perturbation durations (2: 2 days, 7: 7 days, 14: 14 days, 21: 21 days and 28: 28 days). D=Dried soils, H= Heated soils, DH= Dried-heated soils, and C= control soils.

### 3.5. Microbial soil functions and structure after 21 days perturbations duration

To highlight the effects of the 21 days perturbation duration, we focused on the following PCA performed: microbial CLLPs, ELFAMEs profiles and bacterial taxa abundances and then selected microbial and fungal substrates-induced respiration (SIR), as well as EL-FAMEs biomarkers and bacterial taxa, which discriminate between the different perturbations (Fig. 3A, 4, 6). We then compared values between the different perturbations (H, DH and D), the control (C) after 21 days perturbation and before perturbation (t0) (Fig. 7, 8, 9). Whatever the parameter selected (microbial and fungal substrates-induced respiration (SIR), EL-FAMEs biomarkers and bacterial taxa) there are differences between t0 and the other treatments.

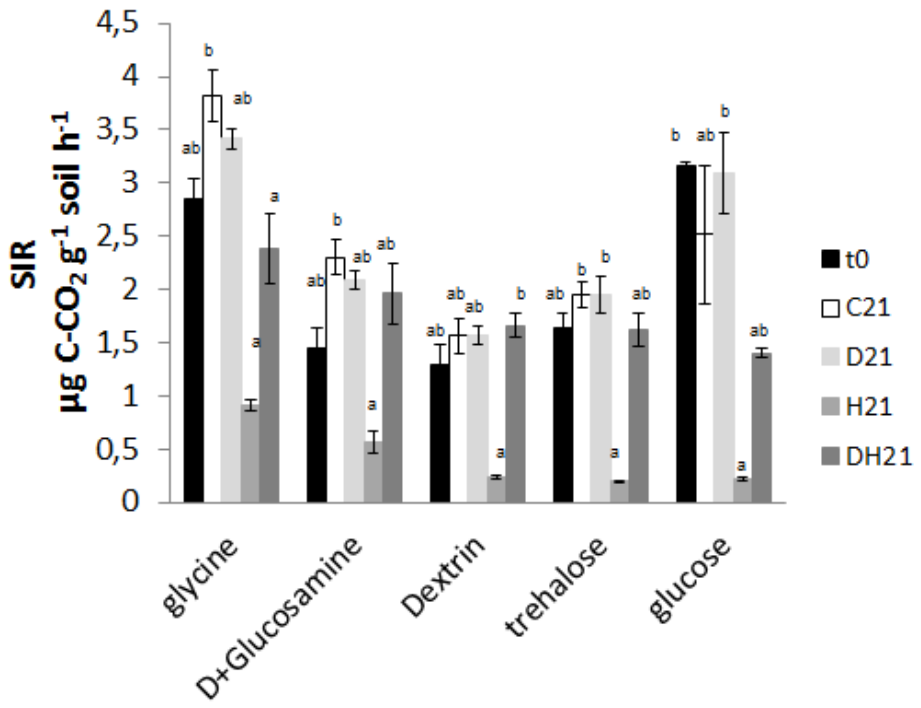


**Fig.7.** Substrate induced respiration of glycine, D+glucosamine, dextrin, trehalose and glucose at t0 and after 21 days of perturbation. D=Dried, H= Heated soils, DH= Dried-heated soils, soils and C= control soils.

Except t0, whatever the treatment (D, DH, H and C) glycine was the most mineralized substrate with a maximum value of 3.82 µg C-$CO_2$ $g^{-1}$ soil $h^{-1}$ for control and a minimum value of 0.91 µg C-$CO_2$ $g^{-1}$ soil $h^{-1}$ for microcosms subjected to heat perturbation (H). For all selected substrates, the lowest values of induced respiration were observed in heated soils (H). Significantly differences between the control and H perturbation were observed for SIR glycine, SIR D+Glucosamine and SIR trehalose (Fig. 7). SIR glycine and SIR glucose were highly lower in dried-heated soils (DH) than in the control whereas all the other SIR substrates were not significantly lower than the control. For dried soils (D), SIRs of all the substrates studied were significantly closely similar to the control values (Fig. 7).

After the end of the 21 perturbation, total EL-FAMEs ranged from 15.11 µg $g^{-1}$ soil to 16.81 µg $g^{-1}$ soil and bacteria EL-FAMEs biomarkers ranged from 8.98 µg $g^{-1}$ soil to 10.01 µg $g^{-1}$ soil and both were higher in soils subjected to DH21 and D21 soils as compared to the 21

days heat perturbation and the control. Fungi EL-FAMEs biomarkers were not significantly higher in DH21 and D21. Gram negative ELFAMEs biomarkers were lower in H21, while gram positive ELFAMEs biomarkers and thermophile ones were higher (Fig. 8). However, *Actinobacteria* biomarkers were lower in H21. EL-FAMEs showed no significant differences between the perturbations whatever the biomarker studied.

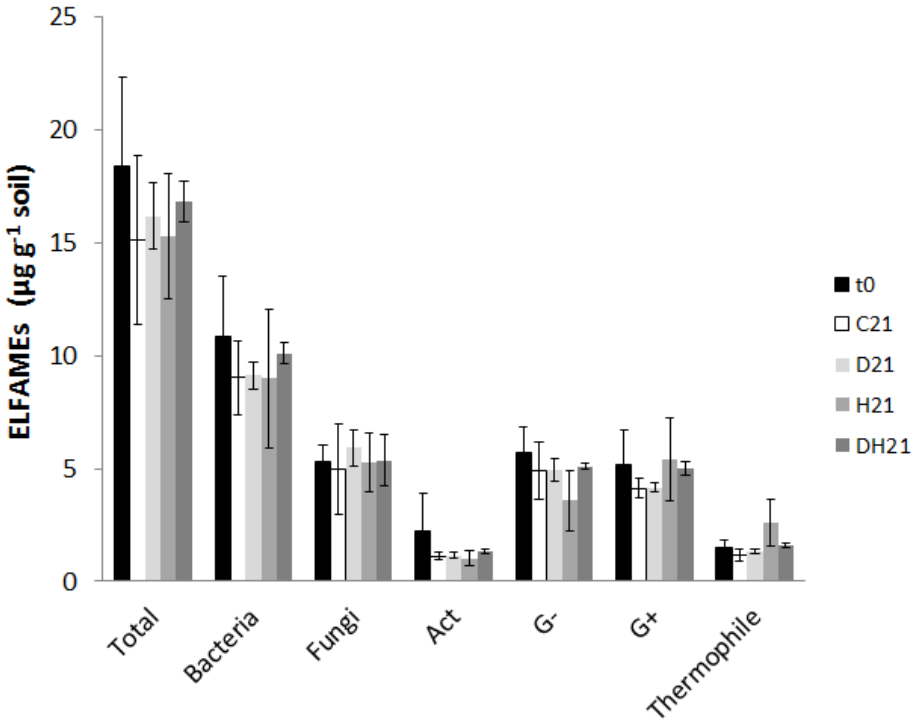


**Fig.8.** Total, bacterial, fungal, actinobacteria, gram-negative bacteria, gram-positive bacteria and thermophile ELFAMEs at t0 and after 21 days of perturbation. D=Dried soils, H= Heated soils, DH= Dried-heated soils, and C= control soils.

The copy numbers of the total 16S rRNA genes ranged from $5.95 \times 10^9$ 16S rRNA gene copies $g^{-1}$ soil to $7.48 \times 10^9$ 16S rRNA gene copies $g^{-1}$ soil with a higher abundance of the total bacteria in the DH21 soils as compared to the other 21 day treatments (Fig. 9). Even there were no significantly differences of the abundances of the total bacteria between the different treatments; there were significant ones within taxa. More precisely, a significantly higher abundance of *Firmicutes* was found in the dried-heated soil DH21 compared to the control. However, a significantly lower abundance of *Verrucomicrobia* was found in heated

soils (DH21 and H21) compared to the dried soil (D21) and the control. Higher significant abundances of *Acidobacteria* and *α-Proteobacteria* were observed in the D21 soil compared to H21 and DH21 respectively. For heated soils (DH21 and H21), differences were significant only for the *Actinobacteria* which abundance was higher in H21 (Fig. 9).

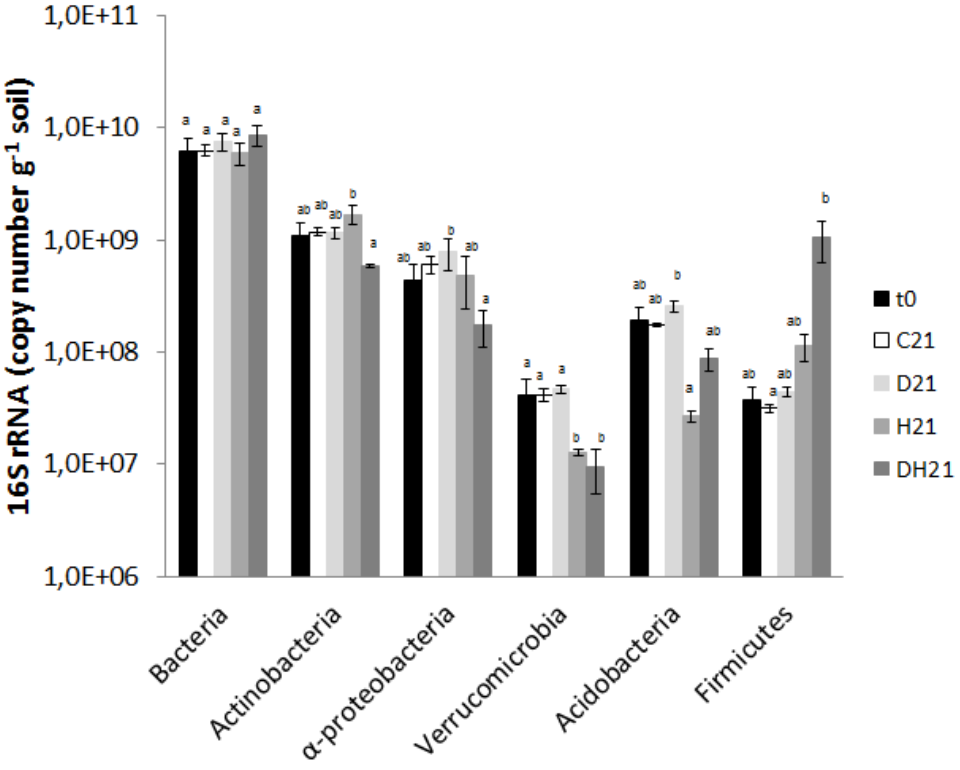


**Fig.9.** 16S rRNA sequences copy number of total bacteria, *Actinobacteria, Alphaproteobacteria, Verrucomicrobia, Acidobacteria* and *Firmicutes* at t0 and after 21 days of perturbation. D=Dried soils, H= Heated soils, DH= Dried-heated soils, and C= control soils.

## 4. Discussion

### 4.1. Consequences of drought, high temperature and duration on soil biological parameters

Heated soils (H) were highly negatively impacted in their BR and SIR glucose suggesting a lower microbial biomass concomitant with an increase in DOC amounts. Since the microbial biomass (SIR-glucose) was the most killed in these soils, the $qCO_2$ was the

highest. Dead and lysed microbial cells in the heated soils may mainly release dissolved carbon increasing the DOC concentration (Hamdi et al., 2011). However, in the dried-heated soils (DH) BR and SIR glucose increased with the duration of the perturbation. In these DH soils, DOC concentration was higher. This is partly consistent with studies describing that rewetting of dry soils caused a short-term increase in microbial activity named the "Birch-effect" process (Birch, 1958; Bloem et al., 1992), inducing a flush of carbon and nitrogen mineralization. This stimulation of respiration, after rewetting of dried-heated soils, could be explained by lysis of live microbial cells, release of compatible solutes and exposure of previously protected organic matter (Fierer and Schimel, 2003; Schimel et al., 2007). For the dried-heated soils not only the DOC concentration was higher but also the Mn concentration. As diverse microorganisms (bacteria and fungi) oxidize soluble Mn (II) (Miyata et al., 2007) and adequate supply of the reduced Mn (II) co-factor is required for a large number of cellular functions (Tebo et al., 2005; Krishnan et al., 2007), added manganese resulted in significantly higher cell densities (Watanabe et al., 2012). Thus, the respiration pulse observed in DH soils suggested that Carbon substrate availability and Mn are limiting factors for both microbial activities and growth. Although DOC and Mn concentrations were lower in dried soils (D), the respiration pulse was also observed in these soils. However, several studies have shown that drying increased Mn solubilility and exchangeability both at laboratory and field scales (Bartlett and James, 1980; Makino et al., 2000; Ross et al., 2001). We could hypothethise that microbial dried soils (D) communities were lower impacted during drying. However, DH perturbation is a combination of two different abiotic perturbations: drought and heat. Thus we could suggest that even drought increased Mn concentration, heat factor mainly destroyed microbial biomass. Therefore, while higher microbial biomass could be reactivated within the first hours of restored conditions after dried perturbation (D), lower one were after dried-heat perturbation (DH) inducing a lower oxidation of Mn during the first 24 h after restored conditions. After remoistening of these dried-heated soils (DH), Mn could be a stimulus for the revival soil microbial communities rendered inactive during the perturbation.

The catabolic structure of the soil microbial and fungal communities was highly impacted by heat treatments (H and DH). However, moist-heat perturbation (H) had stronger effects on the catabolic structure than dry-heat perturbation. Moreover, microbial community composition differed between heat perturbation (H) and the other perturbations and there were neither microbial nor fungal functions resistance whatever the duration of the perturbation. This is consistent with several studies describing killing of microbial biomass at high

temperatures (Hamdi et al., 2011) with a modification of the composition and the function of microbial communities (Bárcenas-Moreno et al., 2009). Despite the same high temperature, dry-heat perturbation (DH) had an intermediate effect on the catabolic structure of the microbial and fungal community and on the microbial community composition between moist-heated soils (H) on one hand and dried soils (D) and the control on the other hand. Thus, dried-heated soil (DH) was less impacted than moist-heated soil (H). Indeed, even we have done our experiments at laboratory scale (microcosms) the lower decline of microbial and fungal biomasses observed in DH soils could be related to the dried condition that transmits heat slower than in the moist conditions of H soils (Certini, 2005). Moreover, in our study we dried soil and then incubated it at 50°C. This previous drying may have partly "protected" microbial communities from subsequent heat (Bérard et al., 2011). For the dried-heated soils (DH) there were neither microbial nor fungal functions resistance and the fungal functions seemed to be more impacted by the combined effect of dried-heat than the total microbial community. Moreover, while a 2 day dried heated soil (DH2) presented a fungal CLLP closely to the control; it seemed that more longer the dried-heat perturbation duration more closer were dried-heat (DH) and heat (H) CLLPs'. As fungi are stated to be adapted to changes in drought (Beare et al., 1992; Jensen et al., 2003) and are sensitive to high temperatures (Klamer and Bååth, 1998), we could suggest that heat factor predominates with increasing the duration of drought-heat perturbation (DH). Nevertheless, wathever the duration, the dought-heat (DH) perturbation killed fungal biomass (fungal SIR glucose) and could induce fungal microbial community change having high level of functional redundancy only at shorter duration. Whereas it seemed that fungal biomass remained stable (a slight increase) with increasing DH perturbation duration. Thus heat could select for heat tolerant fungal species (Bollen, 1969) and this selection may be time dependent.

Heat treatments (H and DH) led to higher changes in the El-FAMEs profiles. This is consistent with Pettersson and Bååth, 2003 studies who showed that higher temperatures led to higher rates of change on microbial community composition. For heat perturbation (H) the changes found from the 7 day perturbation were drastic changes from gram negative bacteria towards gram positive thermophilic community (i16:0, a17:0 and i17:0). This is consistent with and Bååth (1998) studies, which observed shift from mesophilic to a completely different compost thermophilic community at high temperatures. The EL-FAMEs community structure of the shorter dried-heated perturbation (DH 2 days) seemed to be similar to the control one (C2), while from 7 days perturbation duration the increase of the duration of DH perturbation

induced ELFAMEs community structure highly different from the control one. Moreover, DH21 presented EL-FAME profiles closely similar to the DH28. It seemed that change in ELFAMEs community structure appearing sooner (in term of perturbation duration) could be related to changes of membrane structure. Indeed, bearing in mind that membrane structure is modified phenotypically in response to changes in temperature (Petersen and Klug, 1994), our results suggested that changes due to phenotypic acclimation occur sooner than changes in species composition (Pettersson and Bååth, 2003). As we haven't performed the fungal ARISA analyses, this hypothesis could be partially confirmed by Bacterial ARISA profiles. Indeed, changes in DH soils ELFAMEs profiles appeared sooner (in term of perturbation duration) than changes in Bacterial ARISA profiles. There was a strong shift of the soil bacterial structure from 21 day of perturbation. In addition, as shown with ELFAMEs profiles, there is similitude between DH21 and DH28 bacterial ARISA profiles which was emphazed by the qPCR bacterial taxa abundance analysis.

In this Mediterranean context, the effects of heat treatments (H and DH) seemed to be more important than the effects of the drought perturbation (D) on the resistance of soil microbial and fungal biomasses as well as on catabolic functions and taxonomic structure. Indeed, the moisture regimen to which this soil is naturally exposed *in situ* may be responsible for the resistance of microbial communities to drying and rewetting stress (Zelles et al., 1991; Schimel et al., 1999). This historic of habitats by inducing soil microbial communities tolerance to drought events, could also explain the lower impact of drought-heat (DH) perturbation (Bérard et al. 2012 Annexe 2) compared to heat one (H).

For drought-heat perturbation it seemed that short-term perturbation had less effect than longer one on soil physicochemical characteristics (e.g. Mn mobilization) as well as on functional and taxonomic microbial structure. Indeed, longer disturbance duration induced greater degree of change than did shorter one (Schimel et al., 1999). In our case, as observed in Bérard et al. (2011) study, it is suggested that from 21 day of drought-heat perturbation, considered as critical threshold duration, soil microbial communities may have undergone the strongest shift in fungal CLLPs and microbial community structure particularly taxonomic bacteria structure.

## 4.2. Consequences of the 21 day perturbation duration on microbial communities

There are differences between t0 and C21 substrates mineralization values, ELFAMEs and qPCR results. These differences could probably correspond not only to successional stages of microbial communities, but also to a possible carbon source starvation and confinement taking place in the microcosms when isolating soils from the field (Bérard et al., 2012 Annexe 2).

To highlight the effects of the 21 day perturbation duration, we selected substrates which discriminated between the different perturbations. We observed a high microbial mineralization of dextrin in drought-heat soil. Therefore, knowing that dextrin is a polymeric substrate and the organic material produced at higher temperatures is more recalcitrant than at lower temperatures (Dalias et al., 2001); this high mineralization may suggest that drought-heated microbial communities are adapted to degrade complex substrates. However, even high temperature we observed a low mineralization of dextrin in heat perturbation (H). These lowest values of respiration are probably a consequence of a low microbial biomass (SIR glucose) and a drastic change in community structure induced by the heat perturbation. This is consistent with Banning and Murphy 2008 studies, who suggested that there was a threshold value below which the microbial biomass is not able to respond as rapidly to the added substrate. The microbial and fungal mineralization of trehalose in dried soils (D) were not significantly higher than the control, while in dried-heated soils (DH) the microbial mineralization of trehalose was lower and fungal one seemed to be inhibited (data not shown). Moreover, dried-heat perturbation (DH) highly reduced fungal and microbial biomasses (fungal and microbial SIR glucose). Nevertheless, like Bérard et al., 2011 studies restricted only to microbial mineralization we couldn't show for dried soils (D and DH) if the mineralization of the osmolyte substrate (trehalose) would be related to the total abundance of microorganisms and/or to microbial communities adapted to mineralizing such substrates; indeed the prokaryotes and fungi accumulate compatible solutes to reduce water cellular potential and prevent damages to membranes and proteins during soil drying (Kempf and Bremer 1998; Billi and Potts, 2002).

It seemed that heat perturbation (H) enhanced gram positive bacteria, more specifically thermophile ones while it decreases gram negative group (ELFAMEs biomass) and fungal community (fungal SIR glucose). However, drought perturbation (D) enhanced the

fungal dominance (fungal SIR glucose and fungal ELFAMEs biomass). Indeed, as mentioned above it is often stated that fungi are more adapted to low soil moisture conditions than bacteria (Beare et al., 1992; Jensen et al., 2003) and are sensitive to high temperatures (Klamer and Bååth, 1998). We observed that heat perturbation (H) decreased *Actinobacteria* (ELFAMEs) which is consistent with Klamer and Bååth (1998) studies using phospholipid fatty acid analysis who reported that G+ Actinomycetes group may be sensitive to high temperatures. Conversely our qPCR results showed that heat perturbation (H) increased *Actinobacteria*. This result is consistent with Xiao et al., 2011 studies on *Actinobacteria* communities during continuous thermophilic composting revealed by qPCR. Indeed, the genome is identical in each cell of a particular organism and group-specific primers were designed to specifically amplify 16S rRNA gene fragments of the taxa. However, gene expression is differentiated in time (i.e. growth state), in space (i.e. cell type) and/or characteristic of a given situation (i.e. stress, stimuli) and enzymes catalyze lipids production. Therefore, we could consider that like proteins and in the context of our perturbation experiment, ELFAMEs may be indicators of the physiological state of the microbial communities, explaining these differences in *Actinobacteria* biomass estimations. Despite that each method presents some limitations, we could consider that qPCR is more efficient than ELFAMEs to estimate group abundance. Moreover, as individual species contain a significant number of the fatty acids exhibited by a whole community, ELFAMEs pattern is an indicator of microbial community structure but not of diversity (Griffiths et al., 2000).

RISA results showed that after 21 days perturbation DH21 and D21 led to the establishment of bacterial communities differing in their structure. These findings were in accordance with changes observed in the taxonomic composition of the bacterial community studied by quantifying the abundances of different bacterial taxa. The qPCR results showed that *Acidobacteria, Verrucomicrobia* and *Alphaproteobacteria* dominated in the previously dried soils (D). It seemed that *Acidobacteria* tend to be favoured in oligotrophic soils with lower carbon availability (Fierer et al., 2007) and are predominant taxa within the dry treatment soils (Castro et al., 2010). Whereas, Gram-negative bacteria like *Proteobacteria* are r-strategists (or copiotrophic, Fierer et al., 2007) and have the ability to grow fast. In addition, Placella et al., 2012 showed that wet-up events resuscitate and preserve the capacity of *Verrucomicrobia* to synthesize proteins rapidly (within 1 h). These different biological traits may favour *Acidobacteria, Verrucomicrobia* and *Alphaproteobacteria* in the context of rewetting after a severe drought.

*Firmicutes*, however dominated in the dried-heated soil (DH). Indeed, *Firmicutes* are Gram-positive bacteria and are well known for their ability to produce a highly resistant endospore (Placella et al., 2012). Moreover, as perturbation could made easier the isolation of rare bacterial group (Rokikto et al., 2003), *Firmicutes* which were minor taxa before perturbation became dominant after dried-heated perturbation (DH). *Firmicutes* may have then subsisted at low levels or perhaps as spores in our soil before perturbation "awaiting" favourable conditions for growth and dried-heated perturbation selected for them as suggested Norris et al., 2002 in their studies after geothermal activity in Yellowstone National Park which selected for thermotolerant species. In addition, in our study we studied the resistance 24 h after remoistening of soils. This time would have been sufficient for spore outgrowth as Placella et al., 2012 classified *Firmicutes* as intermediate responders to wet-up events and found increased activity of Bacilli from 3 to 24 h after wet-up. This enhancement of activity especially protein production (e.g. hydrolytic enzymes) could also be related to Mn oxidation and would explain the higher degradation of dextrin in dried-heated soil mentioned above.

## 5. Conclusion

Our laboratory-scale drought and/or heat experiment perturbation with different durations revealed that the effects of heat treatments (H and DH) seemed to be more important than the effects of the drought perturbation (D). We have also noted that heat perturbation (H) had stronger effects on the resistance of the catabolic and the microbial taxonomic structure from shorter perturbation duration, whereas the drought-heat perturbation had stronger effects after long-perturbation duration. As our Mediterranean soil is frequently exposed *in situ* to moisture disturbances, our results underlined that resistance of microbial communities could depend on the historic of habitats. Moreover, in the context of our experiment we have emphasized that drought-heat perturbation induced the strongest physico-chemical soil parameters changes and that this change is time dependent. Particularly Mn concentration was highest in drought-heat perturbation and reached its maximum concentration after 21 day of perturbation. This enhancement in co-factor and substrate availability increased microbial activity. Despite this activity enhancement we confirm that the 21 day perturbation duration is a critical threshold duration from which the structure of soil microbial communities of this Mediterranean soil may have undergone a strong shift suggested in Bérard et al. (2011) study. Thus microbial community resistance depends not only on the nature and the length of the perturbation, but also on the soil characteristics and

the nutrient availability. Our experimental conditions could induce a confinement and did not take into account the light and sun energy daily variations. Our study was also limited to the resistance of the microbial communities after perturbation. Since the stability of an ecosystem is defined by its resistance and resilience (Pimm, 1984), further research is required to assess whether the soil microbial communities recover after threshold duration of a perturbation (Bérard et al. 2011) and if so, the time required for this recovery.

***Contexte experimental et analytique: contributions**

Les prélèvements d'échantillons sur la placette et la mise en place de l'expérimentation au laboratoire ont été réalisés avec l'aide de D. Renard, B. Bes, M. Debroux, P. Renault et A. Bérard (UMR EMMAH, Avignon).

Les analyses moléculaires ont été réalisées àl'UMR MSE, (INRA Dijon) encadrées et assistées par F. Martin-Laurent et J. Béguet.

Les analyses biochimiques (ELFAMEs) ont été réalisées à l'UMR SQPOV, (INRA Avignon) encadrées et assistées par C. Giniès et G. Sévenier.

**References**

Anderson, J.P.E., and Domsch, K.H., 1978. A physiological method for the quantitative measurement of microbial biomass in soil. Soil Biology and Biochemistry 10, 215–221.

Anderson, T.H., and Domsch, K.H., 1985. Determination of ecophysiological maintenance carbon requirements of soil microorganisms in a dormant state. Biology and Fertility of Soils 1, 81–89.

Anderson, T.H., and Domsch, K.H., 1993. The metabolic quotient for CO2 (qC02) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. Soil Biology and Biochemistry 25, 393-395.

Andresen, L.C., Michelsen, A., Jonasson, S., Beier, C., Ambus, P., 2009. Glycine uptake in heath plants and soil microbes responds to elevated temperature, CO2 and drought. Acta Oecologica 35, 786–796.

Asensio, D., Penuelas, J., Ogaya, R., Llusia, J., 2007. Seasonal soil and leaf CO2 exchange rates in a Mediterranean holm oak forest and their responses to drought conditions. Atmospheric Environment 41, 2447–2455.

Banning, N.C., and Murphy, D.V., 2008. Effect of heat-induced disturbance on microbial biomass and activity in forest soil and the relationship between disturbance effects and microbial community structure. Applied Soil Ecology 40, 109-119.

Bárcenas-Moreno, G., Gómez-Brandón, M., Rousk, J., Bååth, E., 2009. Adaptation of soil microbial communities to temperature: comparison of fungi and bacteria in a laboratory experiment. Global Change Biology 15, 2950–2957.

Bartlett, R., and James, B., 1980. Studying dried, stored soil samples-Some pitfalls. Soil Science Society of America Journal 44, 721-724.

Beare, M.H., Parmalee, R.W., Hendrix, P.F., Cheng, W., Coleman, D.C., Crossley, D.A., 1992. Microbial and faunal interactions and effects on litter nitrogen and decomposition in agroecosystems. Ecological Monographs 62, 569–591.

Ben Sassi, M., Dollinger, J., Renault, P., Tlili, A., Bérard, A., 2012. The FungiResp method: An application of the MicroRespTM method to assess fungi in microbial communities as soil biological indicators. Ecological Indicators 23, 482-490.

Bérard, A., Ben Sassi, M., Renault, P., Gros, R., 2012. Severe drought-induced community tolerance to heat wave. An experimental study on soil microbial processes. Journal of Soils and Sediments 12, 513-518.

Bérard, A., Bouchet, T., Sévenier, G., Pablo, A.L., Gros, R., 2011. Resilience of soil microbial communities impacted by severe drought and high temperature in the context of Mediterranean heat waves. European Journal of Soil Biology 47, 333-342.

Billi, D., and Potts, M., 2002. Life and death of dried prokaryotes. Research in Microbiology 153, 7–12.

Birch, H.F., 1958. The effect of soil drying on humus decomposition and nitrogen availability. Plant and Soil 10, 9-31.

Bloem, J., De Ruiter, P.C., Koopman, G.J., Lebbink, G., Brussaard, L., 1992. Microbial numbers and activity in dried and rewetted arable soil under integrated and conventional management. Soil Biology and Biochemistry 24, 655-665.

Bollen, G.J., 1969. The selective effect of heat treatment on the microflora of a greenhouse soil. Netherlands Journal of Plant Pathology 75, 157-163.

Campbell, C. D., Chapman, S. J., Cameron, C. M., Davidson, M. S., Potts, J. M., 2003. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Applied and Environmental Microbiology 69, 3593–3599.

Castro, H.F., Classen, A.T., Austin, E.E., Norby, R.J., Schadt, C.W., 2010. Soil microbial community responses to multiple experimental climate change drivers. Applied and Environmental Microbiology 76, 999-1007.

Certini, G., 2005. Effects of fire on properties of forest soils: a review. Oecologia 143, 1–10.

Chaer, G., Fernandes, M., Myrold, D., Bottomley, P., 2009. Comparative resistance and resilience of soil microbial communities and enzyme activities in adjacent native forest and agricultural soils. Microbial Ecology. 58, 414-424.

Clark, J.S., Campbell, J.H., Grizzle, H., Acosta-Martìnez, V., Zak, J.C., 2009. Soil Microbial Community Response to Drought and Precipitation Variability in the Chihuahuan Desert. Microbiol Ecology 57, 248–260.

Coleman, D.C., 2011. Understanding soil processes: one of the last frontiers in biological and ecological research. Australian Plant Pathology 40, 207-214.

Dalias, P., Anderson, J.M., Bottner, P., Coûteaux, M., 2001. Temperature responses of carbon mineralization in conifer forest soils from different regional climates incubated under standard laboratory conditions. Global Change Biology 6, 181-192.

Feng, X., and Simpson, M.J., 2009. Temperature and substrate controls on microbial phospholipid fatty acid composition during incubation of grassland soils contrasting in organic matter quality. Soil Biology and Biochemistry 41, 804-812.

Fierer, N., and Schimel, J.P., 2003. A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. Soil Science Society of America Journal 67, 798-805.

Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology 88, 1354-1364.

Fierer, N., Jackson, J.A., Vilgalys, R., Jackson, R.B., 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Applied and Environmental Microbiology 71, 4117–4120.

Gibelin, A. L., Déqué, M., 2003. Anthropogenic climate change over the Mediterranean region simulated by a global variable resolution model. Climate Dynamics 20, 327–339.

Goebel, M.O., Bachmann, J., Reichstein, M., Janssens, I.A., Guggenberger, G., 2011. Soil water repellency and its implications for organic matter decomposition – is there a link to extreme climatic events? Global Change Biology 17, 2640–2656.

Gómez, B.M., Lores M., Domínguez J., 2010. A new combination of extraction and derivatization methods that reduces the complexity and preparation time in determining phospholipid fatty acids in solid environmental samples. Bioresource Technology 101, 1348-1354.

Griffiths, B.S., Ritz, K., Bardgett, R.D., Cook, R., Christensen, S., Ekelund, F., Sørensen, S.J., Bååth, E., Bloem, J., de Ruiter, P.C., Dolfing, J., Nicolardot, B., 2000. Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity–ecosystem function relationship. Oikos 90, 279–294.

Griffiths, I.R., Whiteley, A.S., O'Donnell, A.G., Bailey, M.J., 2003. Physiological and Community Responses of Established Grassland Bacterial Populations to Water Stress. Applied and Environmental Microbiology 69, 6961–6968.

Hamdi, S., Chevallier, T., Ben Aïssa N., Ben Hammouda, M., Gallali, T., Chotte, J.L., Bernoux, M. 2011. Short-term temperature dependence of heterotrophic soil respiration after one-month of pre-incubation at different temperatures. Soil Biology and Biochemistry 43, 1752–1758.

Hinsinger, P., Bengough, A.G., Vetterlein, D., Young, I.M., 2009. Rhizosphere: biophysics, biogeochemistry and ecological relevance. Plant Soil 321, 117–152.

IPCC, 2007. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller (eds.). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 996 pp.

IPCC, 2012. Summary for Policymakers, in *Managing the risks of extreme events and disasters to advance climate change adaptation*, Cambridge University Press., C. B. Field et al. , éd. Cambridge, UK, and New York, NY, USA, 19 p.

ISO-11063, 2010. Soil quality—Method to directly extract DNA from soil samples. International organization for Standardization (ISO).

Jensen, K. D., Beier, C., Michelsen, A., Emmett, B., 2003. Effects of experimental drought on microbial processes in two temperate heathlands at contrasting water conditions. Applied Soil Ecology 24, 165–176.

Kaur, A., Chaudhary, A., Kaur, A., Choudhary, R., Kaushik, R., 2005. Phospholipid fatty acid- A bioindicator of environment monitoring and assessment in soil ecosystem. Current Science 89, 1103-1112.

Kempf, B., and Bremer, E., 1998. Uptake and synthesis of compatible solutes as microbial stress responses to high osmolality environments. Archives of Microbiol 170, 319–330.

Klamer, M., and Bååth, E., 1998. Microbial community dynamics during composting of straw material studied using phospholipid fatty acid analysis. FEMS Microbiology Ecology 27, 9-20.

Krishnan, K.P., Fernandes, S.O., Chandan, G.S., Loka Bharathi, P.A., 2007. Bacterial contribution to mitigation of iron and manganese in mangrove sediments. Marine Pollution Bulletin 54, 1427–1433.

Makino, T., Hasegawa, S., Sakurai, Y., Ohno, S., Utagawa, H., Maejima, Y., Momohara, K., 2000. Influence of Soil-Drying under Field Conditions on Exchangeable Manganese, Cobalt, and Copper Contents. Soil Science and Plant Nutrition 46, 581-590.

Mühling, M., Woolven-Allen, J., Colin Murrell, J., Joint, I., 2008. Improved group-specific PCR primers for denaturing gradient gel electrophoresis analysis of the genetic diversity of complex microbial communities. International Society for Microbial Ecology 2, 379–392.

Muyzer, G.A., de Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial population by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes coding for 16S rRNA. Applied and Environmental Microbiology 59, 695-700.

Nannipieri, P., Ascher, J., Ceccherini, M. T., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. European Journal of Soil Science 54, 655–670.

Norris, T.B., Wraith, J.M., Castenholz, R.W., McDermott, T.R., 2002. Soil microbial community structure across a thermal gradient following a geothermal heating event. Applied and Environmental Microbiology 68, 6300–6309.

Ochsenreiter, T., Selezi, D., Quaiser, A., Bonch-Osmolovskaya, L., Schleper, C., 2003. Diversity and abundance of Crenarchaeota in terrestrial habitats studied by 16S RNA surveys and real time PCR. Environmental Microbiology 5, 787-797.

Petersen, S.O., and Klug, M.J., 1994. Effects of Sieving, Storage, and Incubation Temperature on the Phospholipid Fatty Acid Profile of a Soil Microbial Community. Applied and Environmental Microbiology 60, 2421-2430.

Pettersson, M., and Bååth, E., 2003. Temperature-dependent changes in the soil bacterial community in limed and unlimed soil. FEMS Microbiology Ecology 45, 13-21.

Philippot, L., Bru, D., Saby, N.P.A., Cuhel, J., Arrouays, D., Simek, M., Hallin, S., 2009. Spatial patterns of bacterial taxa in nature reflect ecological traits of deep branches of the 16S rRNA bacterial tree. Environmental Microbiology 11, 3096-3104.

Pietikaïnen, J., Pettersson, M., Bååth, E., 2005. Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. FEMS Microbiology Ecology 52, 49–58.

Pimm, S. L., 1984. The complexity and stability of ecosystems. Nature 307, 321–326.

Placella, S.A., Brodie, E.L., Firestone, M.K., 2012. Rainfall-induced carbon dioxide pulses result from sequential resuscitation of phylogenetically clustered microbial groups. Proceedings of the National Academy of Sciences 27, 10931–10936.

Ranjard, L.F.P., Lata, J.C., Muogel, C., Thioulouse, J., Nazaret, S., 2001. Characterization of bacterial and fungal soil communities by automated ribosomal intergenic spacer analysis fingerprints: biological and methodological variability. Applied and Environmental Microbiology 67, 4479–4487.

Rokitko, P.V., Romanovskaya, V.A., Malashenko, Y.R., Chernaya, N.A., Gushcha, N.I., Mikheev, A.N., 2003. Soil drying as a model for the action of stress factors on natural bacterial populations. Microbiology 72, 756-761.

Ross, D.S., Hales, H.C., Shea-McCarthy, G.C., Lanzirotti, A., 2001. Sensitivity of Soil Manganese Oxides: Drying and Storage Cause Reduction. Soil Science Society of America Journal 65, 736–743.

Saetre, P., and Stark, J.M., 2005. Microbial dynamics and carbon and nitrogen cycling following re-wetting of soils beneath two semi-arid plant species. Oecologia 142, 247–260.

Sanaullah, M., Blagodatskaya, E., Chabbi, A., Rumpel, C., Kuzyakov, Y., 2011. Drought effects on microbial biomass and enzyme activities in the rhizosphere of grasses depend on plant community composition. Applied Soil Ecology 48, 38–44.

Sardans, J., Penuelas, J., Estiarte, M., 2006. Warming and drought alter soil phosphatase activity and soil P availability in a Mediterranean shrubland. Plant and Soil 289, 227–238.

Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88, 1386–1394.

Schimel, J.P., Gulledge, J.M., Clein-Curley, J.S., Lindstrom, J.E., Braddock, J.F., 1999. Moisture effects on microbial activity and community structure in decomposing birch litter in the Alaskan taiga. Soil Biology and Biochemistry 31, 831–838.

Swift, M.J., Andren, O., Brussaard, L., Briones, M., Couteaux, M.,M., Ekschmitt, K., Kjoller, A., Loiseau, P., Smith, P., 1998. Global change, soil biodiversity, and nitrogen cycling in terrestrial ecosystems: three case studies. Global Change Biology 4, 729-743.

Tebo, B.M., Johnson H.A., McCarthy, J.M., Templeton, A.S., 2005. Geomicrobiology of manganese (II) oxidation. TRENDS in Microbiology 13, 421-428.

Tlili, A., Corcoll, N., Bonet, B., Morin, S., Montuelle, B., Volat B., Bérard A., Guasch, H., 2011. In situ spatio-temporal changes in pollution-induced community tolerance to zinc in autotrophic and heterotrophic biofilm communities. Ecotoxicology 20, 1823-1839.

Watanabe, M., van der Veen, S., Nakajima, H., Abee, T., 2012. Effect of respiration and manganese on oxidative stress resistance of Lactobacillus plantarum WCFS1. Microbiology 158, 293–300.

Williams, M.A., and Rice, C.W., 2007. Seven years of enhanced water availability influences the physiological, structural, and functional attributes of a soil microbial community. Applied Soil Ecology 35, 535-545.

Wu, Z., Dijkstra, P., Koch, G.W., Penuelas, J., Hungate, B.A., 2011. Responses of terrestrial ecosystems to temperatureand precipitation change: a meta-analysis of experimental manipulation. Global Change Biology 17, 927–942.

Xiao, Y., Zeng, G.M., Yang, Z.H., Ma, Y.H., Huang, C., Xu, Z.Y., Huang, J., Fan, C.Z., 2011. Changes in the actinomycetal communities during continuous thermophilic composting as revealed by denaturing gradient gel electrophoresis and quantitative PCR. Bioresource Technology 102, 1383–1388.

Yuste, J.C., Baldocchi, D.D., Gershenson, A., Goldstein, A., Misson, L., Wong, S., 2007. Microbial soil respiration and its dependency on carbon inputs, soil temperature and moisture. Global Change Biology 13, 2018-2035.

Zelles, L., Adrian, P., Bai, Q. Y., Stepper, K., Adrian, M. V., Fischer, K., Maier, A., Ziegler, A., 1991. Microbial activity measured in soils stored under different temperature and humidity conditions. Soil Biology and Biochemistry 23, 955–962.

# Section III.1.2

# Effets à long-terme de perturbations de type canicule et/ou sécheresse appliquées aux communautés microbiennes (structures et fonctions) d'un sol Méditerranéen agricole (Article 5)

Cette section est présentée sous forme d'un article en préparation intitulé : **"Functional and taxonomic stability of soil Mediterranean microbial communities subjected to drought and/or high temperature"**.

# Functional and taxonomic stability of soil Mediterranean microbial communities subjected to drought and/or high temperature


## Abstract

In the next decades, climate scenarios foresee many periods of prolonged drying combined with heat wave events within Mediterranean soils. The nature of these abiotic factors combined with the physicochemical soil characteristics changes', historical and environmental contexts could affect the microbial and fungal biomass, the microbial and fungal activity and thereby determine the stability of the soil microbial ecosystem. Therefore we performed an experiment in microcosms mimicking a 21 day severe drought and/or high temperature perturbation. We then studied the stability of microbial Mediterranean soil structure (functional and taxonomic) using FungiResp, ELFAMEs and qPCR techniques. In this Mediterranean context, the extreme events induced physico-chemical soil changes' depending on the nature of the perturbation combined with a restructuration of microbial communities during the recovery time. Nevertheless, while drought induced resistance of the biological parameters, heat treatments induced neither resistance nor resilience. We propose that there is not only a selection pressure during the perturbation but also an additional selection related to the recovery time after restored conditions.



**Key words:** Drought, high-temperature, Mediterranean microbial communities, Recovery.

## 1. Introduction

Future climate scenarios foresee more frequent and severe extreme events, such as rainfall, heat waves and drought (IPCC, 2007). This is especially true within the current Mediterranean context (Gibelin and Déqué, 2003) where longer drought periods and more extremely dry years are predicted (IPCC, 2007; Planton et al., 2008). These extreme climatic events may affect the biodiversity and the function of terrestrial ecosystems (Millennium, 2005). Indeed soils provide essential ecosystem services such as regulation of biogeochemical cycles which are particularly supported by soil microorganisms. Their activities, abundance and diversity are generally sensitive to disturbances. Authors have illustrated that abiotic factors such as temperature, moisture have direct effects on soil microbial community composition, diversity and activity (Ge et al., 2010; Darby et al., 2011; Prevost-Boure et al., 2011) and that extreme climatic events may be more important drivers of ecosystem function than mean conditions (Heisler and Weltzin, 2006). Thus study of soil ecosystem stability to extreme climatic events is of increasing interest, particularly in Mediterranean context as soils' may be more affected (Bérard et al., 2011).

Resistance and resilience of soil properties and processes are component of the ecosystem stability (Pimm, 1984) and are emerging as key components of soil quality (Griffiths et al, 2005). Resistance is defined as the ability of a system to withstand a disturbance, while resilience could be defined as engineering or ecological resilience (Griffiths and Philippot, 2012). In this study, we focused on resistance and engineering resilience. The latter indicated the capacity of a system to recover after disturbance (Pimm, 1984; Seybold et al., 1999). Soil microbial community recovery depends not only on the nature of the perturbation, but also on the soil characteristics and the nutrient availability (Kuan et al., 2006). Moreover, different microbial parameters may display various degrees of resistance and resilience (Seybold et al., 1999)**.** Although, many microorganisms have a variety of evolutionary adaptations and a high degree of physiological flexibility that allow them to survive and remain active in the face of disturbances (Schimel et al., 2007; Allison and Martiny, 2008), certain specific groups could remain depressed for long time (Pesaro et al., 2004). Despite loss of biodiversity, the functions of soil microbial community could be maintained (Griffiths et al., 2000; Griffiths et al., 2001). For example, following rewetting of dried intact soil monoliths over a 2-month period, Griffiths et al. (2003) observed through culture-based analyses and Biolog measurements a physiological recovery of the microbial

population. In accordance, Pesaro et al. (2004) observed that respiration rates could recover quickly following a drying-rewetting stress. Inversely, Chowdhury et al. (2011) reported that even after 54 days at optimal water content recovery of microbial respiration after drying of soil for 14 days, may not be complete. Bérard et al. (2012 Annexe 2) also observed that microbial catabolic activities did not recover to control values 63 days after the end of a severe drought of 75 days followed by a drought-heat perturbation of 21 days. Therefore, recovery and the length of time required for recovery vary. Moreover, the initial resistance of function to stress was not predictive of recovery of function over time (Kuan et al., 2006; Philippot et al., 2008). Bérard et al. (2011) reported a lasting effect on taxonomic and catabolic structure after a drought-heat perturbation and suggested that this lasting effect was the consequence of direct drastic impacts on microbial biomass inducing a shift in composition and a shift in physiological traits. Otherwise, we recently observed that different durations of drought-heat perturbations impacted differently physico-chemical soil properties, which probably induced varying changes in microbial communities on one day after restoration of environmental conditions (Ben Sassi et al., in prep; Chapter III PhD). Therefore, if physico-chemical changes observed just after the end of disturbance continue to vary during the recovery time, this could also contribute to affect in the long term microbial communities and their functions.

In a previous laboratory experiment studying the soil microbial community resistance to drought-heat perturbation with different durations, we observed that the 21 day perturbation was a threshold duration from which soil microbial communities had undergone a restructuring associated with a shift in physiological traits (Ben Sassi et al., in prep; Chapter III PhD). In the same way Bérard et al. (2011) suggested that duration perturbation influence recovery. Therefore we performed this second study to insight resilience of soil properties in term of soil microbial and fungal communities' functions and taxonomic structure directly and indirectly impacted by a 21 day drought and/or heat perturbation. We hypothesised that 1) resilience is governed by the nature of the perturbation, 2) recovery time governs the microbial community resilience, more precisely 3) recovery time governs the microbial community resilience *via* physico-chemical soil characteristics and 4) recovery time governs microbial community functions *via* their structure.

## 2. Material and methods

### 2.1. Soil sampling

The soil used in this study was collected on June 2010 from the 0-10 cm layer of a bare field located in the South Eastern of France at INRA-Avignon farm. This soil is an alkaline soil (pH 8.51) with a fine textured silty clay loam (323 g $kg^{-1}$ clay, 259 g $kg^{-1}$ silt and 41 g $kg^{-1}$ sand). It had an organic carbon content of 13.2 g $kg^{-1}$ and a large amount of carbonate (347 g $kg^{-1}$ CaCO3). This soil was air-dried and sieved into size fraction of between 2 and 3 mm.

### 2.2. Incubation experiments

Four treatments were conducted: (1) Control soil incubated at constant conditions ("C", 25% of gravimetric dry basis water content ≈ -0.01 MPa, 25°C), (2) Soil subjected to high-temperature 50°C ("H", 25% of gravimetric water content ≈ -0.01 MPa, 50°C), (3) Soil subjected to drying and incubated at 25°C ("D", 5% of gravimetric water content ≈ -10 MPa), (4) Soil subjected to drying and incubated at 50°C ("DH", of gravimetric water content ≈ -10 MPa). For each treatment a perturbation of 21 days was applied.

After sampling, drying and sieving, the soil was slowly moistened and maintained at -0.01 MPa on a suction table during 13 days at ambient temperature (25 °C +/- 2) and in the dark. It was then distributed in Petri dishes (40 $g.dish^{-1}$, 3 replicates per treatment for each measurement date). All treatments were conducted in darkness and at a constant temperature by putting each microcosm within desiccators put within temperature-controlled incubators. For the treatments "D" and "DH", the soils in the microcosms were air-dried at a temperature of 25°C to adjust the water content close to the target water potential of -10 MPa. Then "D" and "DH" microcosms were kept in desiccators (with a beaker containing saturated aqueous solution of potassium nitrate salt; Bérard et al., 2011) put in incubators maintained at 25°C and 50°C respectively. For the treatments "C" and "H", microcosms were directly kept in desiccators (with a beaker containing free water to maintain high humidity) put in incubators maintained at 25°C and 50°C respectively. Soil water contents were regularly checked by gravimetric measurements throughout the course of the experiment.

At the end of 21-day treatment, soils were rewetted by adding ultra-pure water and adjusted to achieve an average water gravimetric content of 25% (approximately -0.01 MPa).

The microcosms were then placed in desiccators (with a beaker containing free water) within an incubator placed at 25°C (±2). For each treatment, soil analyses were done 1 day, 7 days, 28 days and 42 days after the restoration of environmental soil conditions (rewetting and lowering of temperature). Throughout the course of the recovery time, soil water contents were regularly checked by gravimetric measurements. Three soil sub-samples were collected from each treatment scenario and pooled to perform community-level physiological profiling (CLPP) analyses immediately after sampling. However, ELFAMEs and DNA extractions were performed subsequently on triplicate deep-frozen (-20 and -80°C respectively) soil samples. Dissolved organic carbon (DOC) and Manganese (Mn) were performed on soil extracts stored at 4°C.

### 2.3. Physico-chemical analyses

*Soil dissolved organic C (DOC)*

Seven gram of soil were mixed with 35 mL of water and shaken for 1 h. The mixture was centrifuged at 7000 rpm and 5°C for 10 min in a SIGMA 6K15 centrifuge. Five milliliters of the supernatant solution was filtered through 0.2 μm filters and injected into Venoject tubes. Soil dissolved organic C (DOC) contents in soil filtered extracts was added with 100 μL of HCl (0.2 M) and analyzed for C (Shimadzu TOC-5050A total organic C analyser).

*Manganese (Mn)*

Seven gram of soil were mixed with 35 mL of 1 mol $L^{-1}$ KCl solution and shaken for 1 h. The mixture was centrifuged at 7000 rpm and 5°C for 10 min in a SIGMA 6K15 centrifuge. Ten milliliters of the supernatant solution were filtered through 0.2 μm filters and injected into Venoject tubes. This solution was added with 50 μL of HCl (38%). Total concentration of Mn was obtained by a SpectrAA 220 atomic absorption spectrometer (Varian, Austria).

## 2.4. Soil microbial measurements

*Metabolic activity*

To assess the catabolic fingerprint of the microbial and fungal communities, the FungiResp method using the MicroResp™ technique combined with a selective inhibition (SI) was applied (Campbell et al., 2003; Ben Sassi et al., 2012; Renault et al., unpublished, Chapter II PhD). The substrate induced respiration (SIR) of 10 dissolved carbon sources was tested: seven carbohydrates (glucose, sucrose, trehalose, mannose, dextrin, glucosamine and D+cellulobiose); two amino acids (glycine, alanine) and one carboxylic acid (malic acid). The carbon substrates were selected for ecological relevance: relevant to soil agroecosystems (i.e. plant residue, root exudates etc), involved in desiccation tolerance (osmolytes like sucrose, trehalose and glycine). Water was also added to assess basal respiration (BR). Microbial and fungal biomasses were estimated by the glucose-induced respiration (Anderson and Domsch, 1978; Ben Sassi et al., 2012).

A stock solution was made from 25 µl of each substrate that was added to the 96-deep-well plate to deliver 42 mg per mL of soil water to each one. To obtain the catabolic fingerprint of the fungal communities, we added 25 µL of the bactericide bronopol with a concentration of 78 $\mu g.g^{-1}$ soil in each deep-well. However, to obtain the catabolic fingerprint of the whole microbial communities we added 25 µL of water. Soil (≈ 40% of WHC) was then added using a volumetric dispenser system in each deep well of the microplate, following Campbell et al. (2003). After 1 h pre-incubation with the bacterial inhibitor (to let the toxicant penetrate the cells and induce maximal inhibition), each deep-well microplate was sealed to the microplate containing a gel with an indicator dye that responds to the gel pH and, indirectly, to $CO_2$, *via* a silicone seal (MicroResp™, UK) and incubated in the dark at 23°C (±2) for 6 h. Gel absorbance was measured at 570 nm (Bioteck L-800 spectrophotometer) immediately before sealing to the soil deep-well plate, and after 6 h incubation.

When using MicroResp™ technique as described by Campbell et al. (2003), absorbance values were converted to $CO_2$ concentration after the construction of a calibration curve of absorbance *versus* headspace equilibrium $CO_2$. Doing so, researchers assume generally that the microbially produced $CO_2$ accumulates in the gaseous headspace and that this $CO_2$ is of microbial origin exclusively. However, $CO_2$ is also trapped within the Agar gel and the soil solution. Moreover, the soil we used in our study is a calcareous soil with pH>7

exacerbating the risk of $CO_2$ retention in soil solution and the risk of abiotic $CO_2$ emission with acidic substrates. Thus, absorbance values were converted to $CO_2$ concentration after correction using a new geochemical model that assesses the biases in microbial respiration measurements resulting from the $CO_2$ solubilisation and dissociation in the soil solution as in the Agar gel and the effect of substrate supply on $CO_2$ physicochemical equilibriums (Renault et al., unpublished, Chapter II PhD).

*Microbial community structure (EL-FAME analysis)*

In order to extract microbial fatty acids, we performed the method proposed by Gómez et al. (2010). Total fatty acids were extracted from 5 g of each sample with 20 mL of chloroform-methanol, 2:1 (v/v) shaken vigorously. The phases allowed to separate overnight. The supernatant was then reduced by evaporation. Lipids were redissolved in 500 µL of methyl *tert*-butyl ether. One hundred microliters of this solution were placed in a vial and added with 10 µL of the internal standard methyl nonadecanoate (19:0, 230 µg $mL^{-1}$) and 50µL of the derivatization agent. This solution was then vortex-mixed for 30 s and allowed to react for 30 min. The fatty acid methyl esters (FAMEs) were separated on an Elite-5MS capillary column (30 m, 0.25 mm, 0.25 µm) in gas chromatograph equipped with a mass spectrometer (GCMS-QP 2010, Shimadzu). The injector temperature was 250°C.

The FAMEs peaks were identified by comparing retention times with peaks of standard mixture of BAME (Bacterial Acid Methyl Ester Mix, 47080-U, SUPELCO Analytical, USA and Grain FAME Mix (47801, SUPELCO Analytical, USA) and specific fatty acid markers. The relative abundance of FAMEs is expressed in µg $g^{-1}$ soil and specific lipid indicators are used to assess the abundance of fungal and bacterial structural groups. The specific lipid indicators used to assess the abundance of fungi were 18:2ω6,9 and 18:1ω9 (Klamer and Bååth, 1998). Gram-negative bacteria were characterized by 16:1ω7, 16:1ω5, 17:1ω8, 18:1ω7, cyclo17:0 and cyclo19:0 (Kaur et al., 2005). However, Gram-positive bacteria were characterized by i14:0, 14:0, i15:0, a15:0, 15:0, i16:0, i17:0 and a17:0 (Klamer and Bååth 1998; Feng and Simpson 2009) and gram positive thermophile bacteria were characterized by i16:0, a17:0 and i17:0 (Klamer and Bååth, 1998). Actinomycetes were characterized by 10Me16:0 and 10Me18:0 (Williams and Rice, 2007). The sum of all EL-FAMEs was used as an estimation of microbial biomass (Ben Sassi et al., 2012 Annexe 2).

*DNA extraction, amplification and PCR quantification*

The ISO-11063 procedure was used to extract DNA from the soil samples. Briefly, 250 mg of soil containing 0.5 g of 106 µm diameter glass beads and 2 glass beads of 1 mm diameter were homogenized in 1 mL of extraction buffer (100 mM Tris (pH 8.0), 100 mM EDTA, 100 mM NaCl, 1% (w/v), polyvinylpyrrolidone and 2% (w/v), sodium dodecyl sulfate) for 30 s at 1600 rpm in a mini-bead beater cell disrupter (Mikro-Dismembrator S, B. Braun Biotech International, Germany). After the removal of centrifuged soil and cell debris, the proteins were eliminated using sodium acetate precipitation. Then, nucleic acids were precipitated with cold isopropanol and washed with 70% ethanol. They were purified using a polyvinylpolypyrrolidone and Sepharose 4B spin columns. The quality and the size of the soil DNAs were checked by electrophoresis on 1% agarose gels.

The abundance of the total bacterial and of the *Actinobacteria, Acidobacteria, Bacteriodetes, Firmicutes, Gemmatimonadetes, Verrucomicrobia, Alphaproteobacteria, Betaproteobacteria, gammaproteobacteria, Planctomycetes and Crenarchaeota* was determined by quantitative real-time PCR using taxa-specific 16S rRNA primers previously described (Muyzer et al. 1993; Ochsenreiter et al. 2003; Fierer et al. 2005; Mûhling et al. 2008; Philippot et al. 2009). The presence of PCR inhibitors in DNA extracted from soil was estimated for all samples by mixing a known amount of the pGEM-T plasmid (Promega) with water or with the soil DNA extracts. PCR reactions were then performed in an ABI7900HT thermal cycler (Applied Biosystems) with the T7 and SP6 primers. The measured quantity of plasmid for the different DNA extracts and the controls with water were in some soil DNA extracts significantly different indicating that inhibition occurred. We then diluted all samples until we detected no inhibition and perform PCR reactions. Quantification was based on the increasing fluorescence intensity of the SYBR Green dye during amplification in a total volume of 20 µl using Absolute qPCR SYBR Green Rox (ABgene), 10 µM or 20 µM of each primer (the concentration depended on the primer used), soil DNA and T4 Gp32. The standard curves were carried out using serial dilutions of linearized plasmids containing environmental clones of each taxon.

### 2.5. Data analysis

The values of microbial and fungal chemical group SIR for the different treated soils were normalized to the control values % change from control (Chaer et al., 2009). The

significance of differences between the data obtained with the different perturbation was tested using XLSTAT (Addinsoft, Paris, France). As our data were not following a normal distribution, we applied non-parametric statistical procedures. Kruskal–Wallis tests ($p < 0.05$) were performed on BR and SIR-glucose (4 replicates), total ELFAMEs and bacterial taxa abundances (3 replicates) to assess the difference between perturbations. Mann-Withney tests were performed to assess the differences on SIR glucose data between the control and perturbed soils (4 replicates). Community level physiological profiles (corresponding to substrate-induced respiration measurements normalized to the sum of all SIR measurements; 4 replicates), EL-FAMEs profiles after normalization of each ELFAMEs biomarker to the sum of the total ELAMEs (3 replicates) and the relative bacterial taxa abundances (3 replicates) were carried out with principal component analyses (PCA). Non-parametric partial redundancy analysis (RDA), followed by a Monte Carlo permutation test (999 permutations) were then used on CLPPs and ELFAMEs data to test the statistical significance of each explanatory variable (factor): disturbance types (drought and high temperature) and recovery time.

## 3. Results

### 3.1. Microbial respiration and soil carbon and manganese content

The recovery time after DH perturbation (1, 7, 28 and 42 days) had significant effects on microbial BR and microbial and fungal SIR glucose (Kruskal-Wallis tests; $p<0.05$; $n=4$). The DOC, Mn concentrations and microbial BR of dried-heated soils (DH) were higher 1 day after restored initial conditions (remoistening and lowering temperature) and lower 42 days after the end of the perturbation (Fig. 1.A-B; Fig. 2.A). However, while a drastic decrease of Mn concentration was observed within 6 days (between the 1$^{st}$ and the 7$^{th}$ recovery day), the DOC concentration was slightly stable (Fig. 1.A-B). At the shorter recovery time studied (1 day after restoration of environmental conditions) the microbial and fungal DH SIR glucose were lower than control values (Fig. 2.B-C). At the longer recovery time studied (42 days after restoration of environmental conditions); the microbial DH SIR glucose was still lower than the control while fungal SIR glucose was closely to the control value (Fig. 2.B-C).

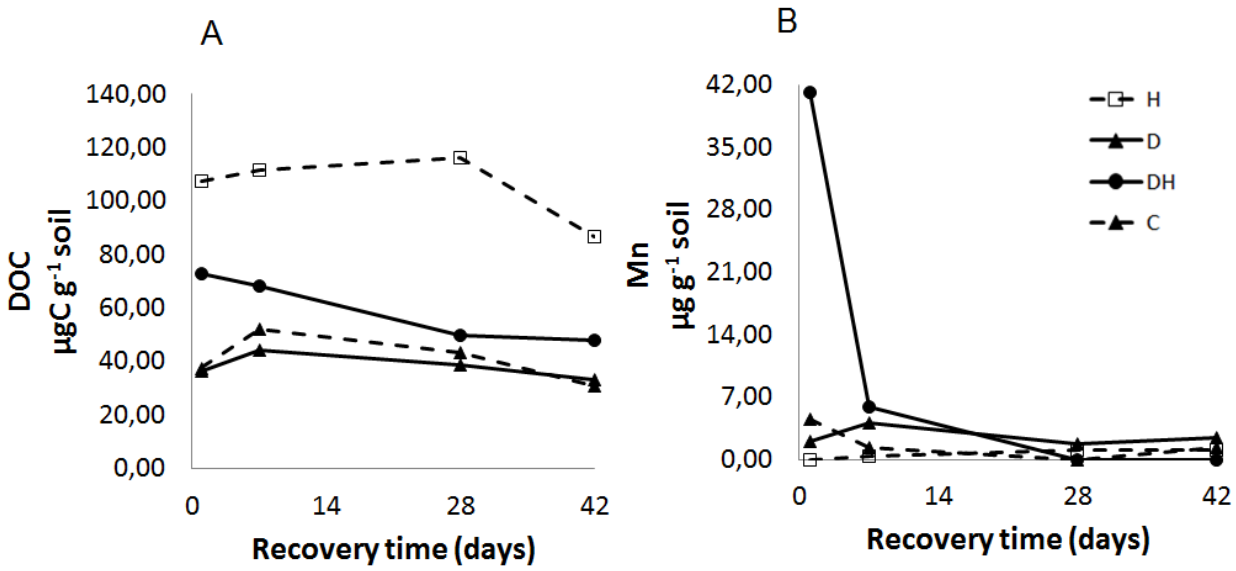


**Fig.1.** DOC (A) and Mn (B) concentration during the recovery time after perturbations of 21 days. D=Dried soils, H= Heated soils, DH= Dried-heated soils, and C= control soils.

Heated soils (H) had the highest DOC concentrations whatever the recovery time studied (Fig. 1.A). However, except for the 28 days recovery time studied, heated soils (H) had the lowest microbial and fungal SIR glucose (Fig. 2.B-C).

The DOC concentration of dried soils (D) was closely similar to the control (Fig. 1.A). The microbial basal respiration of these soils was higher than the control after 1 day restored environmental conditions (Fig. 2.A). In addition, microbial and fungal D SIR glucose were close to the control values at shorter recovery times, however at the longest recovery time studied (42 days) the microbial D SIR glucose was significantly lower than the control while the fungal D SIR glucose was significantly higher than the control (Mann-Withney tests; $p=0.029$; Fig. 2.B-C). At this recovery time, dried soils (D) presented the highest Mn concentration (Fig. 1.B).

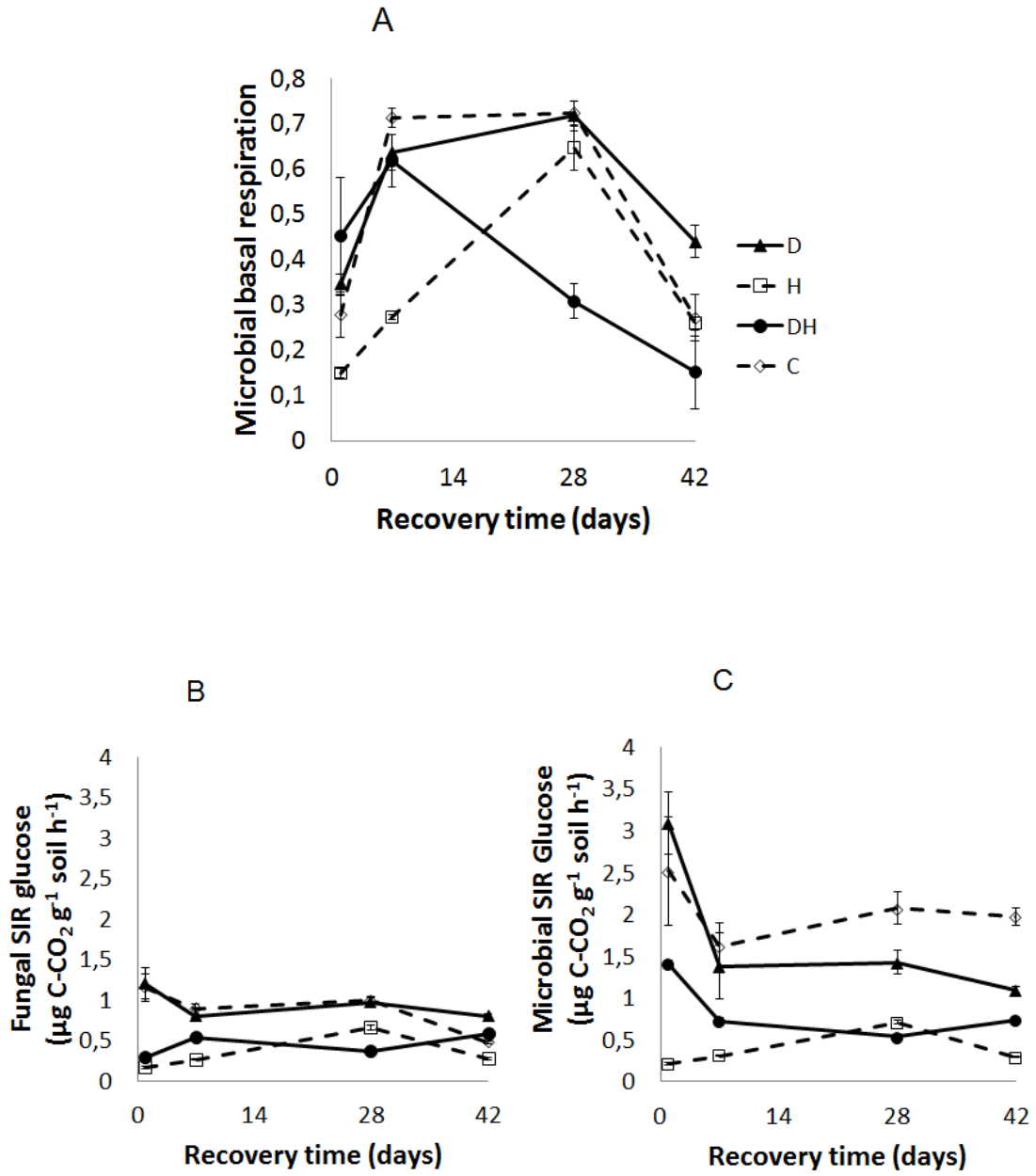


**Fig.2.** Microbial basal respiration (A), microbial SIR-glucose (B) and fungal SIR-glucose (C) during the recovery time after perturbations of 21 days. D=Dried, H= Heated soils, DH= Dried-heated soils, soils and C= control soils. Error bars represent standard deviation of a mean value (n=3).

At the end of recovery time studied (42 days after restored conditions) heated soils (H and DH) presented the highest fungal to microbial ratios (Table 1).

**Table 1:** Mean values of SIR fungal to microbial ratio, ELFAMEs biomass (µg $g^{-1}$ soil), fungal to bacterial, gram negative to gram positive and satured to unsaturated ELFAMEs biomarkers ratios 1 day and 42 days after restored conditions (standard deviation in brakets; n=3). D=Dried, H= Heated soils, DH= Dried-heated soils, and C= control soils.

| Parameters | Recovery time (days) | C | D | H | DH |
|---|---|---|---|---|---|
| SIR fungal to microbial | 1 | 0.49 (0.15) | 0.40 (0.07) | 0.80 (0.05) | 0.21 (0.01) |
| | 42 | 0.21 (0.01) | 0.73 (0.04) | 0.96 (0.06) | 0.81 (0.04) |
| Total ELFAMEs biomass | 1 | 15.11 (3.74) | 16.18 (1.49) | 15.29 (2.78) | 16.81 (0.90) |
| | 42 | 11.74 (0.52) | 12.40 (0.63) | 15.26 (2.74) | 8.29 (0.66) |
| Fungal to bacterial ELFAMEs biomarkers | 1 | 0.48 (0.12) | 0.58 (0.05) | 0.59 (0.34) | 0.47 (0.11) |
| | 42 | 0.72 (0.06) | 0.89 (0.14) | 0.48 (0.12) | 0.47 (0.18) |
| Gram negative to gram positive ELFAMEs biomarkers | 1 | 0.93 (0.17) | 0.93 (0.07) | 0.55 (0.01) | 0.81 (0.03) |
| | 42 | 1.01 (0.12) | 0.87 (0.12) | 0.44 (0.03) | 0.72 (0.09) |
| Satured to unsaturated ELFAMEs biomarkers | 1 | 0.56 (0.13) | 0.49 (0.02) | 0.72 (0.19) | 0.61 (0.08) |
| | 42 | 0.41 (0.06) | 0.40 (0.03) | 0.89 (0.12) | 0.66 (0.07) |

### 3.2. Microbial and fungal community level physiological profiles (CLLPs)

The first two axes of the PCA performed on both microbial and fungal CLLPs accounted for more than 84% of the variability (Fig. 3). Just after the end of perturbations (1 day after restored conditions), there was a high degree of similarity of the ordination of the fungal catabolic structure between heated soils (FH1 and FDH1) in one hand and between dried (FD1) and the control (FC1) soils on the other hand within PC1 accounting for more than 59% of the overall variation. However, during the recovery time studied there was similarity of the ordination of the fungal catabolic structure between dried soils (particularly FD7 and FDH7) and between moist soils and the control (particularly FH42 and FC42) soils on the other hand within PC1. The PCA2 axis separated the microbial catabolic structure of dried soils (MD) and the control (MC) from all the other soils. MC42 was predominantly

highly separated from all the other microbial catabolic structure on PCA2 axis. The PCA showed the same directional change over recovery time on both microbial and fungal catabolic structures of heat-moist soils (H) and the control on one hand and the drought soils (D and DH) on the other hand. These trajectories presented an opposite directional change over recovery time. Just after the end of perturbations, dried-heated soils (DH), dried soils (D) and the control (C) showed microbial catabolic structures highly different from fungal ones. However, the dried soils (D and DH) induced fungal catabolic structures more closely similar to microbial ones during the longer recovery time. Just after the end of perturbations, except for dried-heated soils (DH1), which were characterized by the fungal mineralization of D+Glucosamine, dried soils (D1) and heated soils (H1) were characterized by the mineralization of alanine and glycine. At the end of the recovery time studied (42 days after the end the perturbations), dried-heated soils (DH42 and D42) were characterized for both microbial and fungal CLPPs by the mineralization of dextrin, trehalose, sucrose, D+mannose and D+cellobiose. Control soils were characterized for microbial CLPPs by the mineralization of glucose, sucrose and malate particularly at the longest recovery time studied (42 days). Partial redundancy analysis (RDA) performed on microbial and fungal CLPPs showed that drought, heat and the recovery time had significant effects ($F=1.51$, $p<0.0001$; $F=0.62$, $p=0.023$ and $F=0.652$, $p=0.019$ respectively) on the catabolic functions of the soil microbial and fungal communities.

For all treatments and all recovery time studied, the percentages of change from control for both microbial and fungal SIR groups (carbohydrates, amino acids, carboxylic acids) were calculated. The results showed that all microbial SIR groups studied were strongly negatively impacted by heat treatments (H and DH; Table 2). Although the fluctuation observed in the changes from control for microbial SIR groups in dried soils (D), these soils were negatively impacted after 42 days of recovery. However, at this recovery time all fungal SIR groups were negatively impacted by the heat perturbation (H), while fungal SIR of carbohydrates and carboxylic acids were less or positively impacted by drought treatments (D and DH). The fungal SIR of amino acids in the latter soils (D and DH) as for H soils, were lower than control values (Table 2).

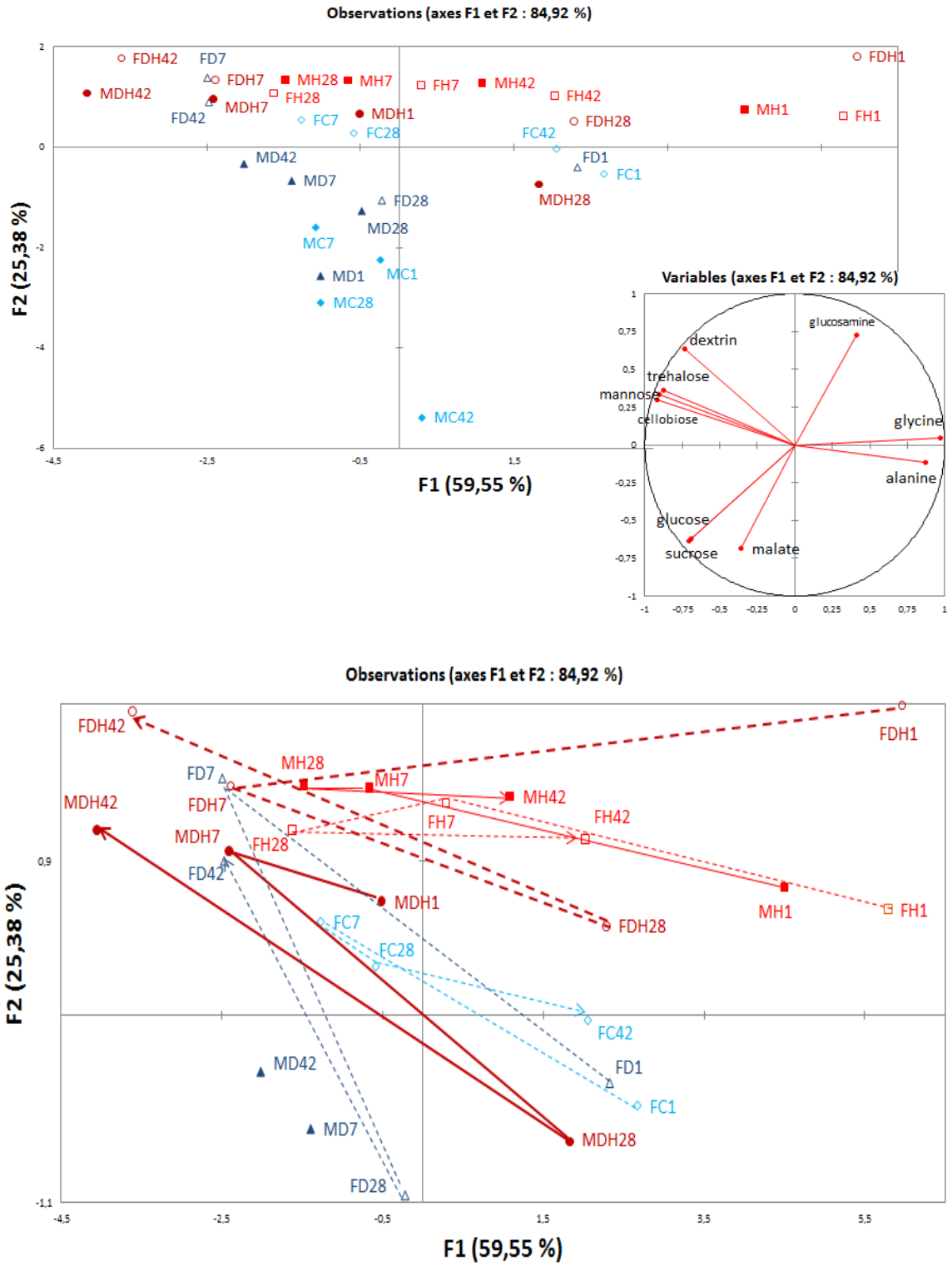


**Fig.3.** Principal component analysis of the microbial (M) and the fungal (F) CLPP measurements during the recovery time after perturbations of 21 days (1: 1 day, 7: 7 days, 28: 28 days and 42: 42 days). To make the figure clearer we focused on the upper and used colors. D=Dried soils, Dark blue; H= Heated soils, light red; DH= Dried-heated soils, dark red; and C= control soils, light blue.

**Table 2:** Mean percentage of change relative to control for microbial and fungal chemical group SIR 1 day and 42 days after restored conditions (standard deviation in brakets; n=4). D=Dried, H= Heated soils and DH= Dried-heated soils.

| Parameters | | Recovery time (days) | D | H | DH |
|---|---|---|---|---|---|
| Microbial SIR | Carbohydrates | 1 | 10.30 (3.68) | -87.45 (1.00) | -25.23 (2.71) |
| | | 42 | 12.69 (2.42) | -68.29 (0.99) | -41.36 (0.29) |
| | Amino acids | 1 | -7.52 (3.06) | -76.19 (1.11) | -29.66 (9.90) |
| | | 42 | -33.21 (8.04) | -62.35 (0.88) | -82.51 (4.24) |
| | Carboxylic acid | 1 | -23.44 (9.23) | -92.28 (0.17) | -52.24 (5.66) |
| | | 42 | -53.86 (6.88) | 81.56 (0.48) | -71.83 (2.33) |
| Fungal SIR | Carbohydrates | 1 | -3.38 (2.62) | -79.03 (0.46) | -60.19 (2.89) |
| | | 42 | 65.93 (3.64) | -27.29 (2.86) | 22.59 (8.29) |
| | Amino acids | 1 | -14.58 (2.17) | -68.37 (0.28) | -53.68 (3.77) |
| | | 42 | -37.94 (4.08) | -41.79 (3.11) | -78.40 (1.85) |
| | Carboxylic acid | 1 | -30.57(11.66) | -83.90 (0.59) | -75.43 (0.74) |
| | | 42 | 45.43 (11.31) | -29.55 (2.25) | 0.20 (9.16) |

### 3.3. Microbial community structure

About 67% of the total variability in the EL-FAMEs profiles was represented by a PCA plot (39.69% along axis 1, and 27.14% along axis 2; Fig .4). The first axis PC1 separated heated soils (H) from the other soils. However, the second axis PC2 separated dried-heated soils (DH) from all the other soils. During the recovery time, EL-FAMEs profiles of dried soils (D) were closely similar to control soils. Heated soils (H) were characterized by the dominance of G+ bacteria biomarkers (a17, i17 and i16). Dried (D) and control soils were characterized by the dominance of fungal biomarkers (18:2ω6,9 and 18:1ω9) and dried-heated soils (DH) were characterized by G+ bacteria biomarkers (a15 and

i14). The partial redundancy analysis (RDA) performed on EL-FAMEs profiles and followed by the permutation test, showed that drought, heat and recovery time after the perturbation had significant effects (F=7.25; 3.02 and p< 0.0001, and F=2.02 p=0.001 respectively) on the microbial structure of the soil.

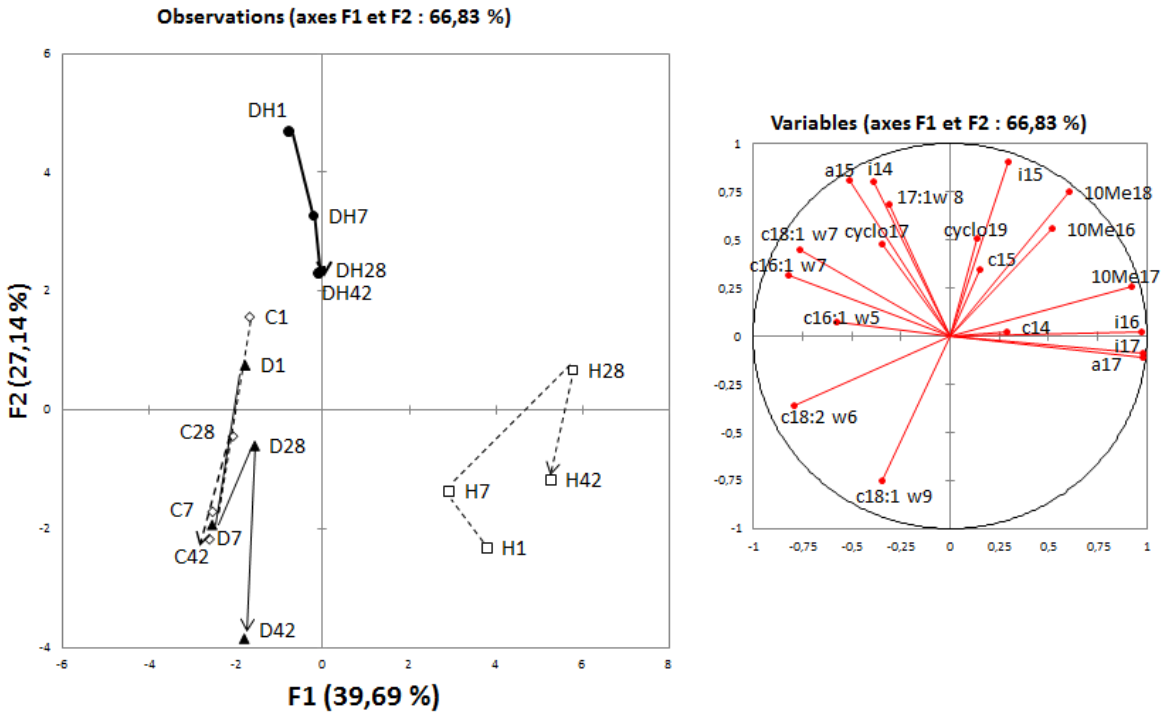


**Fig.4**. Principal component analysis of the ELFAMEs profiles during the recovery time after perturbations of 21 days (1: 1 day, 7: 7 days, 28: 28 days and 42: 42 days). D=Dried soils, H= Heated soils, DH= Dried-heated soils, and C= control soils.

For dried-heated soils (DH) total ELFAMEs significantly decreased with recovery time (Kruskall-Wallis, p=0.024) and differences between dried-heat perturbation and the control are more pronounced at the end of the recovery time studied than at the beginning (1 day and 42 days after perturbation; Table 2). Moreover while ELFAMEs biomass was lower in dried-heated soils (DH), ELFAMEs biomass was higher in heated soils (H) and closely similar to the control value in Dried soils (D) at the end of the recovery time studied.

Except for fungal to bacterial ratio after the end of perturbations, during the recovery time, fungal to bacterial and gram negative to gram positive ratios were lower in heated soils

(H and DH) compared to dried soils and control (Table 1). Differences of these ratios between the perturbations and control were not significant at the shortest recovery time (day 1; Kruskal-Wallis tests, p=0.6), while significant differences were observed at the longest one (day 42; Kruskal-Wallis tests, p=0.03 and p=0.02 respectively). However, saturated to unsaturated ratio was significantly higher in the heated soils (H and DH) (Kruskal-Wallis tests, $p<0.5$) whatever the recovery time studied. Values of all ratios were closely similar between dried soils (D) and the control.

### 3.4. Bacterial community structure

The relative abundances of the different bacterial taxa targeted were calculated and a principal component analysis was performed. The sum of the targeted groups represented 32-54% of the abundance of the total bacterial 16S rRNA gene copy numbers depending on the perturbation. The first principal axis (PC1) represented 50.72 % of the variability while the second principal axis (PC2) represented 22.34 %. At the shortest recovery time (1 day after the end of perturbations), the dried-heated soil (DH) was strongly separated from the other DH recovery times and all the other perturbations (Fig. 5). Dried soils (D) bacterial structure was closely similar to that of control (C) at this shortest recovery time. However, at the longest recovery time (42 days after the end of perturbations) PC1 separated dried soils (D) from the control. Heated soils (H and DH) were also separated from the control at this stage of the recovery time. *Acidobacteria, Alphaproteobacteria, Verrucomicrobia, gammaproteobacteria, Planctomycetes* and *Crenarchaeota* characterized dried soils (D) and the control (C). While, *Actinobacteria* and *Gemmatimonadetes* characterized heated soils (H) and dried-heated ones (DH) except for DH1 which were characterized by *Firmicutes* and *Betaproteobacteria.*

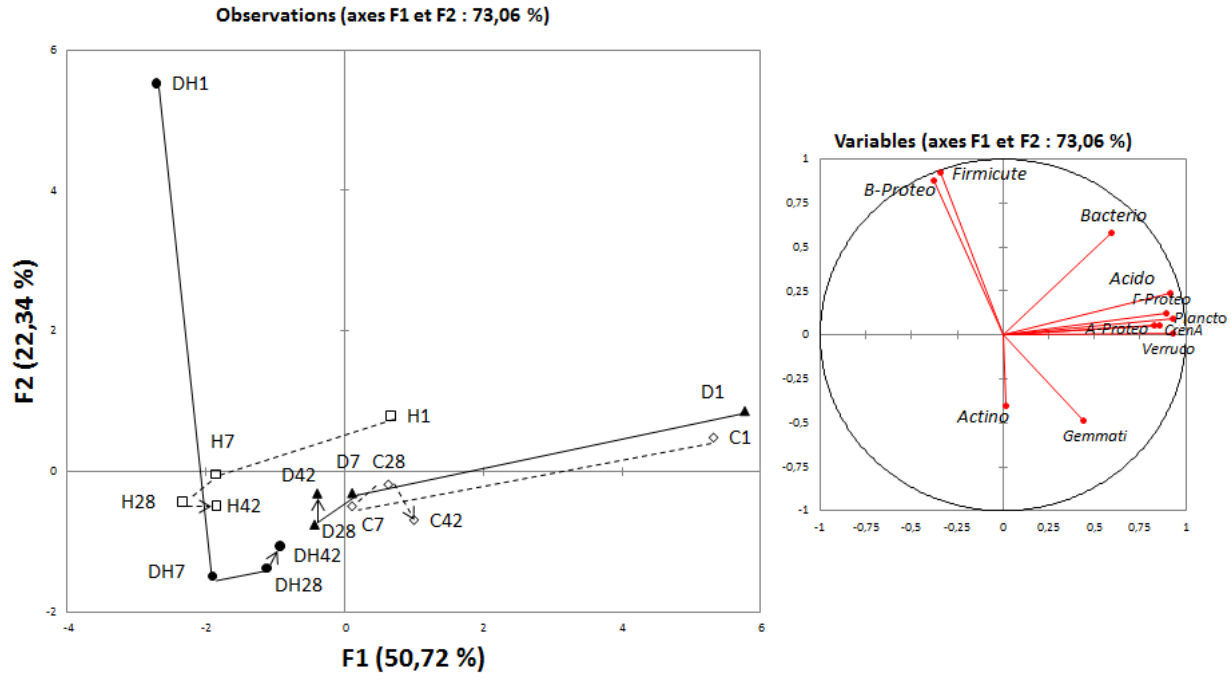


**Fig.5.** Principal component analysis performed from qPCR bacterial taxa abundances (*Acido=Acidobacteria, Gemma=Gemmatimonadetes, Verruco=Verrucomicrobia, γ-Proteo=γ-Proteobacteria, Plancto=Planctomycetes, CrenA=Crenarchaeota*, Actino=*Actinobacteria, α-Proteo=α-Proteobacteria, Firmicute=Firmicutes* and *β-Proteo=β-Proteobacteria*) during the recovery time after perturbations of 21 days (1: 1 day, 7: 7 days, 28: 28 days and 42: 42 days).

For further insights into the composition of the bacterial community, we calculated the relative abundances of the different phyla and classes within the total bacterial community (Fig. 6). The most dominant bacterial phylum after all perturbations was *Actinobacteria*. At the shortest recovery time studied (1 day after restored conditions); a significantly higher abundance of *Firmicutes* was found in the dried-heated soil DH1 compared to the control (Fig. 6.B). At the longest recovery time studied (42 days after restored conditions); a significantly higher abundance of *Gemmatimonadetes* and *Betaproteobacteria* but a significantly lower abundance of *Planctomycetes* and *Crenarchaeota* was found in the dried-heated soil DH42 compared to the control. However, lower significant abundances of *Acidobacteria* were observed in heated soils (H42) compared to the control. For dried soils (DH42 and D42), differences were significant only for the *Actinobacteria,* which abundance was higher in DH42. However, for heated soils (H42 and DH42), differences were significant only for the *Verrucomicrobia* which abundance was higher in DH42 (Fig. 6.C-D).

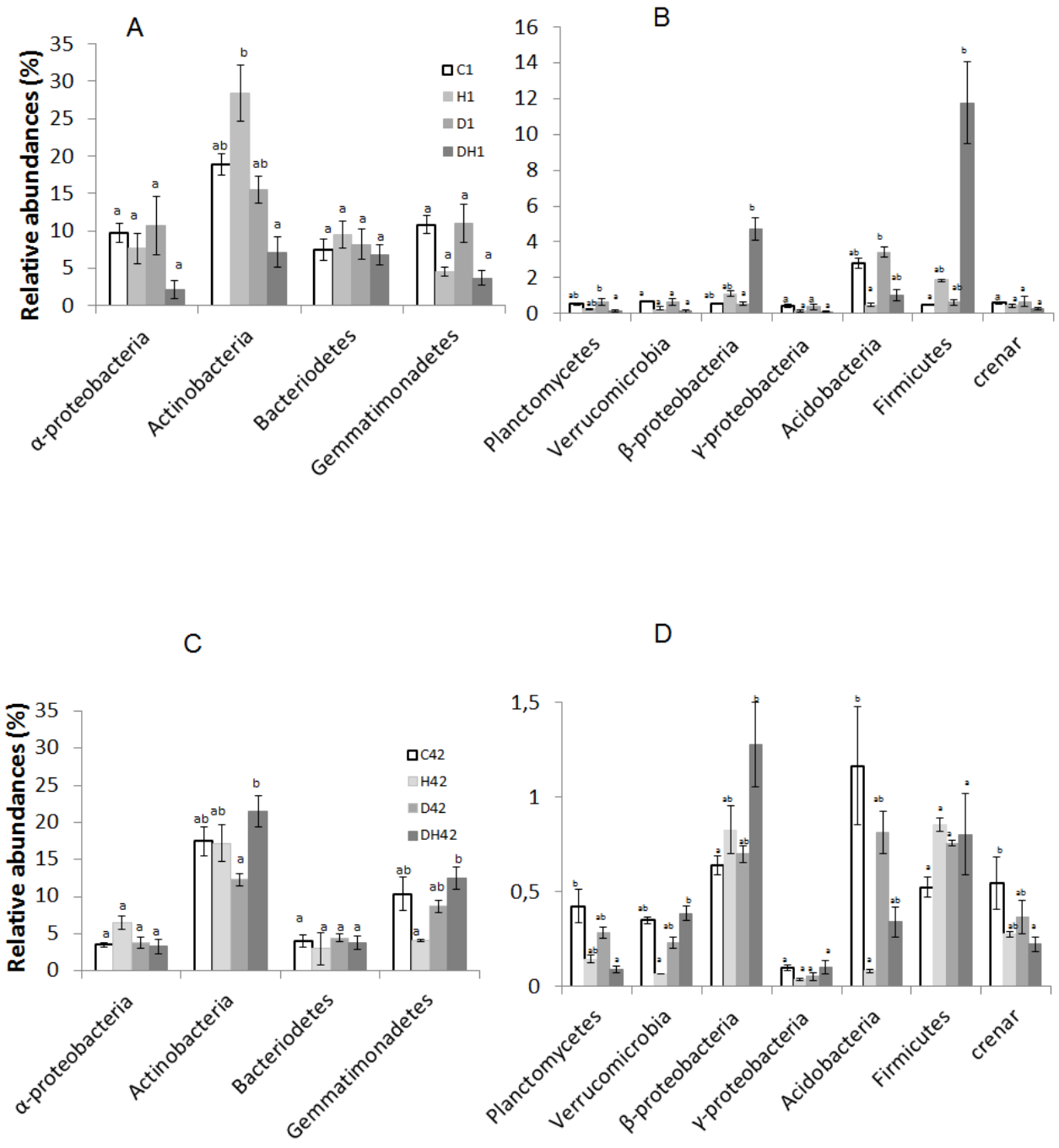


**Fig.6.** Relative abundances of *Alphaproteobacteria, Actinobacteria, Bacteriodetes and Gemmatimonadetes* (A-C), *Acidobacteria, Firmicutes, Verrucomicrobia, Betaproteobacteria, gammaproteobacteria, planctomycetes and Crenarchaeota* (B-D), 1 day (A-B) and 42 days (C-D) of restored conditions after perturbations of 21 days. D=Dried soils, H= Heated soils, DH= Dried-heated soils, and C= control soils.

## 4. Discussion

It seems that C substrate availability and Mn are limiting factors for both microbial activities and growth. Indeed, the stimulation of the BR one day after the end of the dried-heat perturbation (DH) was concomitant with an increase in DOC and Mn concentrations compared to the control soil. This is consistent with our previous study on different drought-heat perturbation durations, which described an increase of microbial activity with an enhancement of DOC and Mn concentrations (Ben Sassi et al. in prep, Chapter III PhD). Moreover, the increase of DOC concentration is consistent with studies describing that rewetting of dry soils caused a short-term increase in microbial activity named the "Birch-effect" process (Birch, 1958; Bloem et al., 1992), inducing a flush of carbon and nitrogen mineralization. Both microbial and non-microbial organic matter (OM) sources could contribute to this stimulation of respiration, after rewetting of dried-heated soil (Fierer and Schimel, 2003; Schimel et al., 2007). In addition, Mn concentration in soil increased under dried conditions (Bartlett and James, 1980; Makino et al., 2000; Ross et al., 2001) and adequate supply of the reduced Mn (II) co-factor is required for a large number of cellular functions (Tebo et al., 2005; Krishnan et al., 2007). This increase and supply suggest the contribution of Mn in microbial activities into an environment impacted by drought. Moreover, Mn concentrations, BR and microbial biomass (SIR glucose and total ELFAMEs) decreased during the recovery time after DH perturbation. Therefore, if after restoration of environmental conditions Mn may have been a stimulus for the revival soil microbial communities rendered inactive during the drought-heat perturbation, this microbial biomass by oxidizing soluble Mn (II) was then limited by low concentrations of the trace metal decreasing during the recovery time. We could thus suggest that resilience of microbial communities is partially governed by physico-chemical soil changes' depending on the nature of the perturbation (in our case the drought-heat impact) and on microbial limiting factors (especially in our case the Mn co-factor), which is recovery time dependent.

Resilience of microbial community structure is governed by the nature of perturbation and the length of the recovery time. Indeed, during the recovery time, while dried soils (D) seemed to have similar ELFAMEs community structure than the control (C), heat treatments (H and DH) led to higher changes in the El-FAMEs profiles. This is consistent with Pettersson and Bååth (2003) studies which showed that higher temperatures led to higher rates of change on microbial community composition. Despite the same high temperature, dried-

heated microbial community structure (DH) was less impacted than moist-heated one (H). Moreover, for this ELFAMEs community structure parameter resistance corroborate with resilience. Indeed, the previous drying of dried-heated soil may have partly "protected" microbial communities from subsequent heat (Bérard et al., 2011). Moreover, although drought and heat perturbations occurred in this Mediterranean context, moisture perturbation which is the most common environmental stress that soil microorganisms experience may led less important soil microbial structure changes than heat perturbations (H and DH) on soil microbial structure. Therefore, recovery of ELFAMEs community structure in dried soils (D) and its lack in heated soils (H and DH) suggests that stability is particular to the disturbance and the historic of habitats (Griffiths and Philippot, 2012).

The ratio of saturated to unsaturated fatty acids was higher in heated soils (H and DH) than in control soil during the recovery time. Indeed the degree of fatty acid unsaturation decreased during the heat perturbation (Klamer and Bååth, 1998). These changes in lipids may be partially attributed to rapid physiological adjustments by certain microorganisms (Petersen and Klug, 1994) and are necessary to maintain physicochemical properties such as a particular state of fluidity in the membrane bilayer (Russell, 2002). These physiological responses to perturbation have costs at the organism level and could contribute to alter the composition of the active microbial community (Schimel et al., 2007). Thus we observed that for heat treatments (H and DH) there were changes from fungal to bacterial community during the recovery time. Indeed, heat treatments of calcareous soil shifted the recolonisation of microbial community from fungi towards bacteria (Bárcenas-Moreno et al., 2011). Moreover within bacterial community, we observed that heat treatments (H and DH) induced changes from gram negative bacteria towards gram positive bacteria during the recovery time. Particularly thermophilic community (i16:0, a17:0 and i17:0) characterized heat perturbation (H). This is consistent with Klamer and Bååth (1998) studies, who observed shift from mesophilic to a completely different compost thermophilic community at high temperatures.

More insights in bacterial community composition during the recovery time based on the relative abundances of predominant taxa in soils showed also neither resistance nor resilience in heat-treated soils (H and DH). However, it seemed that this bacterial community composition is resistant but not resilient in the dried soil (D). Nevertheless, our results based on the sum of the targeted groups, represents only 32-54% of the abundance of the total microbial community depending on the perturbation. In other studies using the same qPCR assays the sum of the targeted groups was more than 79% (Pétric et al., 2011). As Wessén et

al. (2010) suggested, we could relate the low coverage of the bacterial community observed in our study to other bacterial groups, which we did not analyze.

Bacterial community composition stability depends on the nature of the perturbation and the initial resistance of some bacterial taxa was not predictive of recovery over time. Indeed, *Alphaproteobacteria, Planctomycetes* and *Acidobacteria* were resistant in dried soils (D). This is consistent with, Aanderud and Lennon (2011) studies, which showed relative resistance of *Alphaproteobacteria* following soil rewetting and Placella et al. (2012) studies, which showed that *Acidobacteria* displayed high relative activity within 1 h of wet-up after a drying stress. Moreover, it was showed that *Acidobacteria* tolerate desiccation stress (Ward et al., 2009). Whereas, even if *Acidobacteria* were higher in dried soils (D and DH) than in heated soils (H), they were not resilient. Their abundances decreased during the recovery time till the end of experiment (42 days of restored conditions). Accordingly, the recent work of Placella et al. (2012) reported declines in relative abundance of *Planctomycetes* and *Acidobacteria* with time after soil remoistening. Dried-heated soil (DH) bacteria community studied 1 day after the end of perturbation was characterized by *Firmicutes* and *Betaproteobacteria,* which were minor taxa before perturbation (data not shown). This is consistent with Rokikto et al. (2003) researchers, who observed that perturbation made easier the isolation of rare bacterial species. Moreover, *Firmicutes* are well known for their ability to produce a highly resistant endospore (Placella et al., 2012), thus rewetting of dried soils could be a stimulus for the revival of such soil microbial communities rendered inactive by low-water conditions (Saetre and Stark, 2005; Placella et al., 2012). However, despite their resistance and resilience (1 day and 42 days after restored conditions), a decrease of the abundance of *Firmicutes* and *Betaproteobacteria* were observed. Therefore, depending on the nature of the perturbation and on the bacterial taxa, our results showed different situations: 1) some bacterial taxa that were resistant were not resilient at the end of the recovery time studied; 2) some bacterial taxa were not resistant but resilient; 3) some bacterial taxa were resistant and resilient or 4) some bacterial taxa were not-resistant and not-resilient. These differences in "bacterial taxa trajectories" during recovery time may not only depend on the direct impacts of the different types of perturbation but could also depend on the resuscitation strategies (e.g. Placella et al., 2012) and on synergism and/or competition between taxa.

The fungal and the microbial catabolic structures of heated soils (H and DH) were neither resistant nor resilient. And in the case of Fungal catabolic structures results suggest that just after the end of perturbations, heat perturbation "governed" the fungal catabolic

structure (FDH1 catabolic structure was close to FH1). This could be explained by reduction of fungal and microbial biomass (as shown by fungal and microbial SIR glucose) concomitant with a modification of the composition of microbial communities (ELFAMEs profiles). This is consistent with Bárcenas et al. (2009) studies, which showed a modification of the composition and the function of microbial communities after heat perturbation. Whereas, while the catabolic structure of fungi seemed to be highly impacted, particularly by heat, within the microbial community just after the end of the dried-heat treatment (DH), it seemed that during the recovery time till the end of the experiment (from 7 to 42 days after the end of the perturbation) fungal catabolic structures impacted by DH perturbation were more and more similar to microbial ones. Despite that heat in drought-heat perturbation (DH) induced killing of fungal biomass (fungal SIR glucose), some fungal species may have developed highly resistant forms such as conidia (Dose et al., 2001) and some species inside fungal community are known to be heat tolerant (Bollen, 1969). Therefore, these resistant forms and tolerant species could have been stimulated after rewetting, increasing fungal to microbial active biomass during recovery time (as shown by microbial and fungal SIR glucose). Nevertheless, fungal growth could require longer time than some bacterial species, which may explain that it took 7 days of restored conditions to observe the dominance of fungi adapted to mineralize the studied carbon substrates within the microbial community. We suggest that changes in fungal biomasses during recovery time could explain changes in microbial community functions.

## 5. Conclusion

Biomass killing, the different resuscitation strategies, synergism and/or competition between taxa and thus shift in microbial community composition could explain the lasting effect observed on some soil microbial parameters. But the changes in availability of Mn and OM for microbial communities induced by perturbations could also explain changes in microbial parameters during recovery time. Two additional selection pressures must be taken into account when studying stability in term of resistance and resilience, the selection pressure directly induced by the nature of the perturbation and the selection pressure indirectly induced *via* the physico-chemical soil characteristics, themselves impacted by the nature of the perturbation and which changes are linked to the length of the recovery time. Therefore, the resistance to perturbation may be not predictive of recovery over time. In our study, while we

observed resistance of the biological parameters and a lasting effect on microbial biomass after drought perturbation, heat treatments induced neither resistance nor resilience.

However, we must take into account that our experimental conditions could induce a confinement and did not allow any recolonization strategies (Bérard et al., 2011). For example, Wertz et al. (2007) showed that recovery of the biotic capacity of the soil after perturbation can be reached by microorganisms' re-colonization from unperturbed soil through the active or passive cell movement and microbial growth. Therefore, further research is required to determine the link between the nature of the physical perturbation, the physico-chemical environmental contexts and bacterial and fungal re-colonization on taxonomic structure and microbial functions.

***Contexte experimental et analytique: contributions**

Les prélèvements d'échantillons sur la placette et la mise en place de l'expérimentation au laboratoire ont été réalisés avec l'aide de D. Renard, B. Bes, M. Debroux, P. Renault et A. Bérard (UMR EMMAH, Avignon).

Les analyses moléculaires ont été réalisées àl'UMR MSE, (INRA Dijon) encadrées et assistées par F. Martin-Laurent et J. Béguet.

Les analyses biochimiques (ELFAMEs) ont été réalisées à l'UMR SQPOV, (INRA Avignon) encadrées et assistées par C. Giniès et G. Sévenier.

**References**

Aanderud, Z.T., Lennon, J.T., 2011. Validation of heavy-water stable isotope probing for the characterization of rapidly responding soil bacteria. Applied and Environmental Microbiology 77, 4589–4596.

Allison, S.D. and Martiny, J.B.H., 2008. Resistance, resilience, and redundancy in microbial communities. Proceedings of the National Academy of Sciences 105, 11512-11519.

Anderson, J.P.E., and Domsch, K.H., 1978. A physiological method for the quantitative measurement of microbial biomass in soil. Soil Biology and Biochemistry 10, 215–221.

Bárcenas-Moreno, G., Gómez-Brandón, M., Rousk, J., Bååth, E., 2009. Adaptation of soil microbial communities to temperature: comparison of fungi and bacteria in a laboratory experiment. Global Change Biology 15, 2950–2957.

Bárcenas-Moreno, G., Rousk, J., Bååth, E., 2011. Fungal and bacterial recolonisation of acid and alkaline forest soils following artificial heat treatments Soil Biology and Biochemistry 43, 1023-1033

Bartlett, R., James, B., 1980. Studying dried, stored soil samples-Some pitfalls. Soil Science Society of America Journal 44, 721-724.

Ben Sassi, M., Dollinger, J., Renault, P., Tlili, A., Bérard, A., 2012. The FungiResp method: An application of the MicroRespTM method to assess fungi in microbial communities as soil biological indicators. Ecological Indicators 23, 482-490.

Bérard, A., Ben Sassi, M., Renault, P., Gros, R., 2012. Severe drought-induced community tolerance to heat wave. An experimental study on soil microbial processes. Journal of Soils and Sediments 12, 513-518.

Bérard, A., Bouchet, T., Sévenier, G., Pablo, A.L., Gros, R., 2011. Resilience of soil microbial communities impacted by severe drought and high temperature in the context of Mediterranean heat waves. European Journal of Soil Biology 47, 333-342.

Birch, H.F., 1958. The effect of soil drying on humus decomposition and nitrogen availability. Plant and Soil 10, 9-31.

Bloem, J., De Ruiter, P.C., Koopman, G.J., Lebbink, G., Brussaard, L., 1992. Microbial numbers and activity in dried and rewetted arable soil under integrated and conventional management. Soil Biology and Biochemistry 24, 655-665.

Bollen, G.J., 1969. The selective effect of heat treatment on the microflora of a greenhouse soil. Netherlands Journal of Plant Pathology 75, 157-163.

Campbell, C. D., Chapman, S. J., Cameron, C. M., Davidson, M. S., Potts, J. M., 2003. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Applied and Environmental Microbiology 69, 3593–3599.

Chaer, G., Fernandes, M., Myrold, D., Bottomley, P., 2009. Comparative resistance and resilience of soil microbial communities and enzyme activities in adjacent native forest and agricultural soils. Microbial Ecology 58, 414-424.

Chowdhury, N., Burns, R.G., Marschner, P. 2011. Recovery of soil respiration after drying. Plant and Soil 348, 269–279.

Darby, B.J., Neher, D.A, Housman, D.C., Belnap, J., 2011. Few apparent short-term effects of elevated soil temperature and increased frequency of summer precipitation on the abundance and taxonomic diversity of desert soil micro- and meso-fauna. Soil Biology and Biochemistry 43, 1474-1481.

Dose, K., Bieger-Dose, A., Ernst, B., Feister, U., Gomez-Silva, B., Klein, A., Risi, S., Stridde, C., 2001. Survival of microorganisms under the extreme conditions of the Atacama Desert. Origins of Life and Evolution of the Biosphere 31, 287-303.

Feng, X., Simpson, and M.J., 2009. Temperature and substrate controls on microbial phospholipid fatty acid composition during incubation of grassland soils contrasting in organic matter quality. Soil Biology and Biochemistry 41, 804-812.

Fierer, N., and Schimel, J.P., 2003. A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. Soil Science Society of America Journal 67, 798-805.

Fierer, N., Jackson, J.A., Vilgalys, R., Jackson, R.B., 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Applied and Environmental Microbiology 71, 4117–4120.

Ge, G.F., Li, Z.J., Fan, F.L., Chu, G.X., Hou, Z.A., Liang, Y.C., 2010. Soil biological activity and their seasonal variations in response to long-term application of organic and inorganic fertilizers Plant. Soil 326, 31–44.

Gibelin, A. L., and Déqué, M., 2003. Anthropogenic climate change over the Mediterranean region simulated by a global variable resolution model. Climate Dynamics 20, 327–339.

Griffiths, B.S., and Philippot, L., 2012. Insights into the resistance and resilience of the soil microbial community. FEMS Microbiology Reviews. 1–18.

Griffiths, B.S., Hallet, P.D., Kuan, H.L., Aitken, M.N., 2005. Biological and physical resilience of soil amended with heavymetal-contaminated sewage sludge. European Journal of Soil Science 56, 197–205.

Griffiths, B.S., Ritz, K., Bardgett, R.D., Cook, R., Christensen, S., Ekelund, F., Sørensen, S.J., Bååth, E., Bloem, J., de Ruiter, P.C., Dolfing, J., Nicolardot, B., 2000. Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity–ecosystem function relationship. Oikos 90, 279–294.

Griffiths, B.S., Ritz, K., Wheatley, R., Kuan, H.L., Boag, B., Christensen, S. et al. 2001. An examination of the biodiversity– ecosystem function relationship in arable soil microbial communities. Soil Biology and Biochemistry 33, 1713–1722.

Griffiths, I.R., Whiteley, A.S., O'Donnell, A.G., Bailey, M.J., 2003. Physiological and Community Responses of Established Grassland Bacterial Populations to Water Stress. Applied and Environmental Microbiology 69, 6961–6968.

Heisler, J.L., Weltzin, J.L., 2006. Variability matters: towards a perspective on the influence of precipitation on terrestrial ecosystems. New Phytologist 172, 189-192.

IPCC, 2007. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller (eds.). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 996 pp.

ISO-11063, 2010. Soil quality—Method to directly extract DNA from soil samples. International organization for Standardization (ISO).

Kaur, A., Chaudhary, A., Kaur, A., Choudhary, R., Kaushik, R., 2005. Phospholipid fatty acid- A bioindicator of environment monitoring and assessment in soil ecosystem. Current Science 89, 1103-1112.

Klamer, M., and Bååth, E., 1998. Microbial community dynamics during composting of straw material studied using phospholipid fatty acid analysis. FEMS Microbiology Ecology 27, 9-20.

Krishnan, K.P., Fernandes, S.O., Chandan, G.S., Loka Bharathi, P.A., 2007. Bacterial contribution to mitigation of iron and manganese in mangrove sediments. Marine Pollution Bulletin 54, 1427–1433.

Kuan, H.L., Fenwick, C., Glover, L.A., Griffiths, B.S., Ritz, K., 2006. Functional resilience of microbial communities from perturbed upland grassland soils to further persistent or transient stresses. Soil Biology and Biochemistry 38, 2300–2306.

Makino, T., Hasegawa, S., Sakurai, Y., Ohno, S., Utagawa, H., Maejima, Y., Momohara, K., 2000. Influence of Soil-Drying under Field Conditions on Exchangeable Manganese, Cobalt, and Copper Contents. Journal of Plant Nutrition and Soil Science 46, 581-590.

Millennium Ecosystem Assessment, 2005. Ecosystems and Human Well-being: Synthesis. Island Press, Washington, DC.

Mühling, M., Woolven-Allen, J., Colin Murrell, J., Joint, I., 2008. Improved group-specific PCR primers for denaturing gradient gel electrophoresis analysis of the genetic diversity of complex microbial communities. International Society for Microbial Ecology 2, 379–392.

Muyzer, G.A., de Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial population by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes coding for 16S rRNA. Applied and Environmental Microbiology 59, 695-700.

Ochsenreiter, T., Selezi, D., Quaiser, A., Bonch-Osmolovskaya, L., Schleper, C., 2003. Diversity and abundance of Crenarchaeota in terrestrial habitats studied by 16S RNA surveys and real time PCR. Environmental Microbiology. 5, 787-797.

Pesaro, M., Nicollier, G., Zeyer, J., Widmer, F., 2004. Impact of soil drying-rewetting stress microbial communities and activities and on degradation of two crop protection products. Applied and Environmental Microbiology 70, 2577–2587.

Petersen, S.O., and Klug, M.J., 1994. Effects of Sieving, Storage, and Incubation Temperature on the Phospholipid Fatty Acid Profile of a Soil Microbial Community. Applied and Environmental Microbiology 60, 2421-2430.

Petrić, I., Bru, D., Udiković-Kolić, N., Hršak, D., Philippot, L., Martin-Laurent, F., 2011. Evidence for shifts in the structure and abundance of the microbial community in a long-term PCB-contaminated soil under bioremediation. Journal of Hazardous Materials 195, 254– 260.

Pettersson, M. and Bååth, E., 2003. Temperature-dependent changes in the soil bacterial community in limed and unlimed soil. FEMS Microbiology Ecology 45, 13-21.

Philippot, L., Bru, D., Saby, N.P.A., Cuhel, J., Arrouays, D., Simek, M., Hallin, S., 2009. Spatial patterns of bacterial taxa in nature reflect ecological traits of deep branches of the 16S rRNA bacterial tree. Environmental Microbiology. 11, 3096-3104.

Philippot, L., Cregut, M., Cheneby, D., Bressan, M., Dequiet, S., Martin-Laurent, F., Ranjard, L., Lemanceau, P., 2008. Effect of primary mild stresses on resilience and resistance of the nitrate reducer community to a subsequent severe stress. FEMS Microbiology Letters 285, 51-57.

Pimm, S. L., 1984. The complexity and stability of ecosystems. Nature 307, 321–326.

Placella, S.A., Brodie, E.L., Firestone, M.K., 2012. Rainfall-induced carbon dioxide pulses result from sequential resuscitation of phylogenetically clustered microbial groups. Proceedings of the National Academy of Sciences 27, 10931–10936.

Planton, S., Déqué, M., Chauvin, F., Terray, L., 2008. Expected impacts of climate change on extreme climate events. Comptes Rendus Geoscience 340, 564-574.

Prevost-Boure, N.C., Maron, P.A., Ranjard, L., Nowak, V., Dufrene, E., Damesin, C., Soudani, K., Lata, J.C., 2011. Seasonal dynamics of the bacterial community in forest soils under different quantities of leaf litter. Applied Soil Ecology 4, 14–23.

Rokitko, P.V., Romanovskaya, V.A., Malashenko, Y.R., Chernaya, N.A., Gushcha, N.I., Mikheev, A.N., 2003. Soil drying as a model for the action of stress factors on natural bacterial populations. Microbiology 72, 756-761.

Ross, D.S., Hales, H.C., Shea-McCarthy, G.C., Lanzirotti, A., 2001. Sensitivity of Soil Manganese Oxides: Drying and Storage Cause Reduction. Soil Science Society of America Journal 65, 736–743.

Russell, N.J., 2002. Bacterial membranes: the effects of chill storage and food processing. An overview. International Journal of Food Microbiology 79, 27– 34.

Saetre, P., and Stark, J.M., 2005. Microbial dynamics and carbon and nitrogen cycling following re-wetting of soils beneath two semi-arid plant species. Oecologia 142, 247–260.

Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88, 1386–1394.

Seybold, C. A., Herrick, J. E., Brejda, J. J., 1999. Soil resilience: a fundamental component of soil quality. Soil Science 164, 224–234.

Tebo, B.M., Johnson H.A., McCarthy, J.M., Templeton, A.S., 2005. Geomicrobiology of manganese (II) oxidation. TRENDS in Microbiology 13, 421-428.

Ward, N.L., Challacombe, J.F., Janssen, P.H., Henrissat, B., Coutinho, P.M., et al., 2009.Three Genomes from the Phylum Acidobacteria Provide Insight into the Lifestyles of These Microorganisms in Soils. Applied and Environmental Microbiology 75, 2046–2056.

Wertz, S., Czarnes, S., Bartoli, F., Renault, P., Commeaux, C., Guillaumaud, N., Clays-Josserand, A., 2007. Early-stage bacterial colonization between a sterilized remoulded soil clod and natural soil aggregates of the same soil. Soil Biology and Biochemistry 39, 3127–3137.

Wessén, E., Hallin, S., Philippot, L., 2010. Differential responses of bacterial and archaeal groups at high taxonomical ranks to soil management. Soil Biology & Biochemistry 42, 1759-1765.

Williams, M.A., and Rice, C.W., 2007. Seven years of enhanced water availability influences the physiological, structural, and functional attributes of a soil microbial community. Applied Soil Ecology 35, 535-545.

# Partie III.2

# L'épandage de composts a-t-il une influence sur la réponse des communautés microbiennes à une perturbation de type canicule et sécheresse ?

Cette partie est constituée de deux sections. La première section présente le contexte de l'étude *in situ* en termes de suivi des biomasses microbiennes et de la structure catabolique après épandage de composts. La deuxième section, rédigée sous forme d'un article en préparation aborde les effets à long-terme d'une perturbation de type canicule et sécheresse appliquée aux communautés microbiennes (structures et fonctions) d'un sol Méditerranéen agricole après épandage de composts (Article 6).

# Section III.2.1

# Contexte de l'étude *in situ* ;

# suivi des biomasses microbiennes

# et de la structure catabolique

Cette partie rédigée sous forme de résultats et discussions, décrit l'évolution des communautés microbiennes des sols amendés par les composts (et du sol référence) au coursdu temps, correspondant à la durée de l'expérimentation en placette *in situ*.

Nous avons réalisé un suivi saisonnier sur 2 ans de 4 placettes amendées (NMa, NMb et M) ou non (R) avec des composts issus (cNMa et cNMb) ou non (cM) d'unité de méthanisation à des dates différentes. Les composts cNMa et cNMb non issus d'unité de méthanisation ont été épandus au mois d'Octobre 2009 et de Janvier 2011 respectivement. Le compost cM issu d'unité de méthanisation a été épandu au mois d'Octobre 2010 (voir les caractéristiques physico-chimiques des composts dans le Chapitre II, Tableau 4). La figure 1 représente les variations mensuelles moyennes de la température de l'air sous abri et des précipitations enregistrées au cours de ce suivi saisonnier (moyenne sur 30 jours avant chaque prélèvement de sol) ainsi que le taux d'humidité du sol Référence mesuré le jour même des prélèvements des échantillons sur les placettes. Les facteurs climatiques (température et précipitations) et le taux d'humidité varient au cours des saisons. En particulier, le taux d'humidité du sol référence est plus faible pendant les saisons chaudes, mais les valeurs mesurées supérieures à 14 % resteraient acceptables pour l'activité microbienne. La biomasse microbienne totale et la structure catabolique ont été déterminées selon la technique MicroResp$^{TM}$ (Chapitre II) à partir de sol (0-10cm) prélevé de ces placettes (3 prélèvements mélangés par placette et par date, 4 mesures de microrespirométrie par échantillon). La structure taxonomique et la biomasse microbienne des communautés ont été mesurées trois fois à l'aide la technique PLFA (Chapitre II) sur les échantillons de sol prélevés le jour de l'épandage de chacun des composts. La dynamique de la biomasse microbienne dans les sols est décrite au cours du temps: le jour de l'épandage ($j_0$), 1, 3, 6 et 9 mois pour les 4 placettes (R, NMa, M et NMb) ; 12 mois (R, NMa et M) et 24 mois (R et NMa). Des prélèvements supplémentaires ont été réalisés l'été 2011.

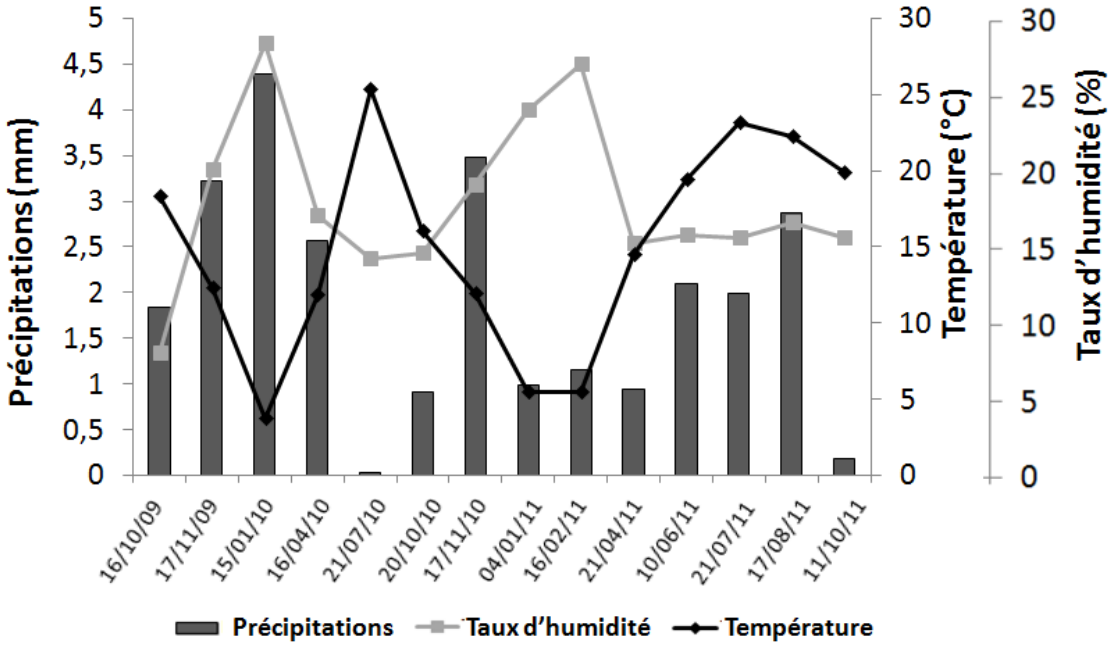


**Figure 1 :** Variations mensuelles moyennes de la température, des précipitations enregistrées et du taux d'humidité au cours du suivi saisonnier.

L'évolution de la biomasse microbienne active dans les différentes placettes estimée *via* la mesure de la respiration induite par le glucose (Anderson and Domsch, 1978 ; Chapitre II) est représentée dans la figure 2. Une différence significative a été enregistrée entre les biomasses microbiennes actives des différentes placettes quelle que soit la date de prélèvement (Mann-Withney : comparaison R et NMa du 16/10/2009 au 21/07/2010; $p<0,05$ et Kruskal-Wallis appliqué à partir du 20/10/2010 ; $p<0,05$ ; $n=4$).

Les mesures de SIR (glucose) réalisées sur les sols le jour de l'épandage nous donnent une indication sur la biomasse microbienne sous forme d'inoculum directement apportée par les différents composts. Les SIR glucose mesurées le jour de l'épandage ($j_0$), des sols amendés en compost sont plus élevées que celles du sol Référence mesurées aux 3 dates d'épandage (Tableau 1). Cependant, les biomasses microbiennes mesurées par analyse des acides gras phospholipidiques (PLFA) ne montrent pas toujours cette différence (Tableau 1). Il semblerait donc que la SIR glucose indique ici non seulement une biomasse microbienne active plus importante dans les sols amendés, mais aussi une activité accrue dès le jour de l'épandage (Saison et al., 2006). Les valeurs de respiration basale plus élevées dans les sols amendés que dans le sol référence, confirment cette hypothèse. Cette activité microbienne

accrue dans les sols amendés est probablement liée à l'apport du compost en nutriments (Albiach et al., 2001). Les analyses de COD réalisées le jour de l'épandage sur les sols M et NMb en comparaison avec le sol R confirment cette hypothèse (Tableau 1). Les analyses PLFA réalisées sur les sols le jour de l'épandage indiquent des proportions en biomarqueurs bactériens thermophiles (somme des biomarqueurs i16, i:17 et a:17 ; Klamer and Bååth, 1998) supérieures pour deux des sols amendés par rapport au sol référence (Tableau 1). Durant la phase thermophile du processus de compostage diverses espèces de bactéries gram-positives se développent et ont été identifiées par ces mêmes biomarqueurs d'acides gras membranaires (i16, i:17 et a:17 ; Klamer and Bååth, 1998). Nos résultats suggèrent donc que le compost peut-être source d'une communauté microbienne thermophile.

De plus, comme nous l'avons constaté (Figure 2) cette biomasse est significativement plus élevée dans les sols amendés que dans le sol référence non amendé sur le court, moyen et long-terme après l'épandage. En effet, la stabilité de la matière organique du compost est connue pour maintenir une disponibilité des nutriments dans le sol sur le long-terme (Guerrero et al., 2001). Cette disponibilité en nutriments associée à l'amélioration des propriétés physico-chimiques (Caravaca et al., 2002) induiraient des effets positifs sur la biomasse microbienne sur le long-terme (particulièrement visible pour le sol NMa après 2 ans d'épandage). Plusieurs études au champ ont rapporté une augmentation à court-terme de la biomasse microbienne (Ros et al., 2003 ; Calbrix et al., 2007). Cependant, les observations sont controversées sur le moyen et le long-terme. Calbrix et al. (2007) n'observent pas d'effet de l'apport de compost sur la biomasse au-delà de 6 mois après l'épandage. Tandis que Bastida et al. (2008) en comparant une parcelle contrôle avec des parcelles amendées avec du compost constatent encore une biomasse microbienne plus élevée 17 ans après l'épandage.

Plus précisément, nous constatons que les SIR glucose (et la respiration basale, le jour de l'épandage) mesurées le jour de l'épandage et durant le suivi de cette expérimentation sont plus élevées dans les sols amendés en compost non méthanisé (NMa et NMb) que dans le sol amendé avec du compost méthanisé (M) (Figure 2 ; Tableau 1). Il semblerait donc que la biomasse microbienne active soit plus importante dans les composts non méthanisés cNMa et cNMb que dans le compost méthanisé cM. Nous remarquons aussi que la teneur en carbone organique dissous[6] (COD), mesurée le jour de l'épandage, du sol NMb amendé en compost non méthanisé est plus élevée que celle du sol M amendé avec du compost méthanisé. En

[6] Nous n'avons pas de résultats d'analyses du COD dans le sol NMa le jour de l'épandage

effet, contrairement aux composts non méthanisés qui contiendraient une grande teneur de matières organiques facilement dégradables, les étapes du processus de méthanisation permettent d'obtenir un digestat contenant un faible pool de matières organiques facilement dégradables (Tambone et al., 2010). Ainsi, les composts non méthanisés (cNMa et cNMb) seraient le siège d'une activité microbienne plus intense et source plus importante de matières organiques facilement dégradables en comparaison avec le compost méthanisé (cM ; Gresse, 2011).

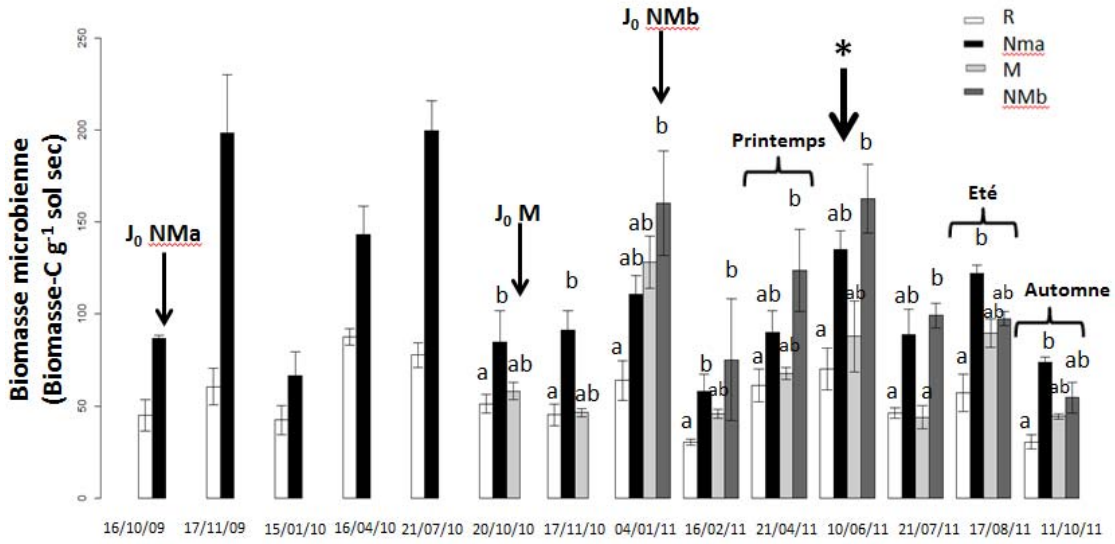


**Figure 2 :** Evolution de la biomasse microbienne dans les différents sols amendés (NMa, M et NMb) ou non (R) avec du compost. Les barres d'erreur correspondent aux écart-type (n=4) et les lettres différentes indiquent une différence significative entre les différentes placettes à une même date de prélèvement. ($J_0$ : jour de l'épandage des différents composts ; * : jour de prélèvement pour les manipulations en conditions contrôlées en microcosmes ; Printemps, Eté et Automne : jours de prélèvement pour la détermination de la structure catabolique et les bio-essais développés dans l'annexe 1).

**Tableau 1 :** Caractéristiques biologiques des sols le jour de leur épandage.

| Date de l'épandage | Paramètres | Sol R | Sol NMa | Sol M | Sol NMb |
|---|---|---|---|---|---|
| 16/10/2009 | Respiration basale (µgC-$CO_2$ $g^{-1}$ sol sec) | 0,42 (0,01) | 1,23 (0,11) | | |
| | Biomasse microbienne SIR glucose (µg biomasse-C $g^{-1}$ sol sec) | 44,89 (8,37) | 86,89 (1,44) | | |
| | Biomasse microbienne PLFA (µg $g^{-1}$ sol sec) | 8,85 (2,25) | 3,71 (1,08) | | |
| | Proportion des thermophiles (%) | 7,97 (2,80) | 15,81(0,41) | | |
| | COD (µg-C $g^{-1}$ sol sec) | non mesuré | non mesuré | | |
| 20/10/2010 | Respiration basale (µgC-$CO_2$ $g^{-1}$ sol sec) | 0,56 (0,02) | | 0,63 (0,02) | |
| | Biomasse microbienne SIR glucose (µg biomasse-C $g^{-1}$ sol sec) | 51,16 (5,03) | | 58,12(4,73) | |
| | Biomasse microbienne PLFA (µg $g^{-1}$ sol sec) | 6,63 (0,82) | | 21,43(2,96) | |
| | Proportion des thermophiles (%) | 11,02 (0,44) | | 13,69(1,12) | |
| | COD (µg-C $g^{-1}$ sol sec) | 69,73 | | 103,46 | |
| 04/01/2011 | Respiration basale (µgC-$CO_2$ $g^{-1}$ sol sec) | 0,84 (0,02) | | | 1,46 (0,21) |
| | Biomasse microbienne SIR glucose (µg biomasse-C $g^{-1}$ sol sec) | 63,93(10,76) | | | 160,49(28,43) |
| | Biomasse microbienne PLFA (µg $g^{-1}$ sol sec) | 11,14(10,69) | | | 27,38 (26,69) |
| | Proportion des thermophiles (%) | 12,13 (2,67) | | | 11,61 (1,01) |
| | COD (µg-C $g^{-1}$ sol sec) | 69,95 | | | 217,52 |

Des fluctuations de la biomasse microbienne sont observées au cours du temps indépendamment du type de sol (amendé ou non). Ces résultats sont en accord avec les travaux de Calbrix et al. (2007) qui ont montré que les conditions saisonnières ont une influence majeure sur la communauté bactérienne. De nombreuses controverses sur l'abondance des microorganismes en fonction des saisons ont été rapportées dans la littérature. Tandis que Bastida et al. (2008a) observent une diminution de la biomasse microbienne l'été (en comparaison avec le printemps et l'hiver), Grayston et al. (2001) rapportent une élévation de la biomasse bactérienne et fongique pendant la période estivale. Cependant, les variations saisonnières du climat sont accompagnées par des variations de biodisponibilités des ressources. Plus particulièrement, une augmentation de la biodisponibilité est observée au cours des saisons humides (Bastida et al., 2008a ; Oren et Steinberger, 2008). Alors qu'une diminution de la teneur en eau du sol restreint la disponibilité des nutriments (Jensen et al., 2003 ; Sardans et Penuelas, 2007) limitant donc leur diffusion aux micro-organismes et réduisant ainsi leur activité et leur croissance. D'après nos résultats il semble cependant que les biomasses soient plus élevées durant les saisons chaudes (printemps et été), que les saisons froides (hiver, automne) (test de Mann-Whitney significatif p=0,02 sur la comparaison des biomasses microbiennes du sol R classées en saison froide/ saison chaude). Dans le cadre de nos campagnes d'échantillonnage, la température a probablement été un facteur plus limitant que la sécheresse pour la croissance microbienne (Grayston et al., 2001).

L'ACP générée à partir des profils cataboliques des sols prélevés le printemps (le 21/04/2011), l'été (le 17/08/2011) et à la fin du suivi expérimental, soit l'Automne 2011 (le 11/10/2011) discrimine le sol référence non amendé (R) des sols amendés avec du compost quelle que soit la saison du prélèvement (Figure 3). En effet, l'apport de matière organique exogène associée à l'apport d'un inoculum de microorganismes pourrait se traduire par un remaniement de la communauté microbienne indigène du sol. Il en résulterait un changement de la biodiversité se traduisant par un changement de la structure fonctionnelle catabolique de la communauté microbienne (Gomez et al., 2006). En particulier, le sol Référence est caractérisé par une minéralisation du malate, de la glycine, l'alanine et la D+glucosamine alors que les sols amendés sont caractérisés par la minéralisation des carbohydrates (sucrose, glucose, tréhalose, dextrine, mannose, cellobiose) (Figure 3).

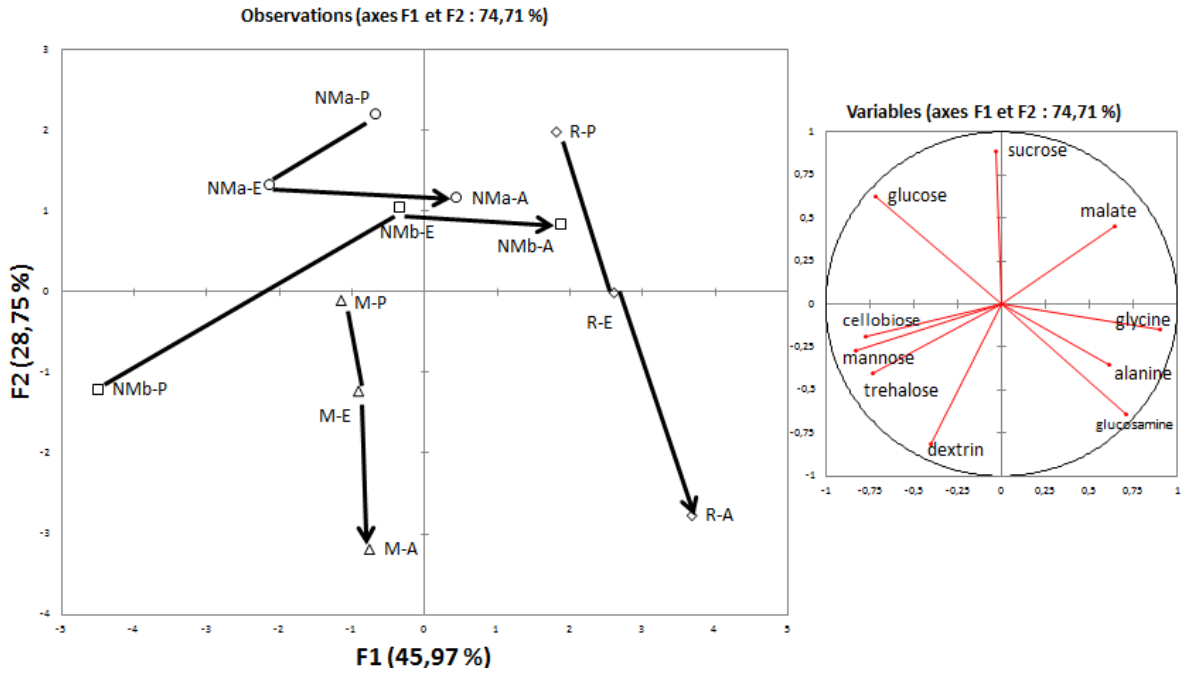


**Figure 3 :** Analyse en composantes principales de la structure catabolique des différents sols amendés (NMa, M et NMb) ou non (R) avec du compost en fonction des saisons (P : Printemps, E : Eté, A : Automne). Moyennes de 4 réplicas de mesures.

Il semblerait ici aussi que les variations saisonnières aient un impact sur les profils cataboliques des communautés microbiennes des sols amendés et non amendés avec du compost. En effet, la structure catabolique des différents sols évolue en fonction des prélèvements réalisés au cours de 3 saisons différentes. Comme nous l'avons suggéré précédemment, la biomasse microbienne, la composition taxonomique, mais aussi la biodisponibilité des ressources varient en fonction des variations climatiques de température et d'humidité affectant ainsi les activités microbiennes (Bell et al., 2009). Les résultats de l'ACP montrent aussi que les sols amendés avec du compost non méthanisé (NMa et NMb) présentent des trajectoires directionnelles de profils cataboliques similaires mais différentes du sol amendé avec le compost méthanisé (M) et du sol référence (R).

Nos résultats ne permettent pas de distinguer et de vérifier l'impact direct du à l'incorporation de microorganismes (*via* l'inoculum du compost) et leur sélection pouvant se produire au cours du temps, de l'impact indirect dû à la proportion de carbone minéralisable dans les produits organiques (apportés par le compost) et de leurs évolutions saisonnières dues aux facteurs environnementaux, qui pourraient induire simultanément ou

individuellement le changement ou la similitude de structure catabolique observés. Ces trois facteurs agissent probablement en interaction sur la dynamique des communautés microbiennes des sols amendés.

En résumé, l'amendement en compost s'est accompagné par une augmentation de la biomasse active microbienne et une modification de la structure catabolique sur le court et le long-terme des sols. Il semble que les variations saisonnières aient un impact majeur sur la biomasse microbienne et la dynamique structurale fonctionnelle de la communauté microbienne des sols, que ceux–ci soient amendés en compost ou non.

**Références**


Albiach. R., Canet. R., Pomares. F., Ingelmo. F., 2001. Organic matter components and aggregate stability after the application of different amendments to a horticultural soil. Bioresource Technology 76, 125-129.

Anderson, J.P.E., and Domsch, K.H., 1978. A physiological method for the quantitative measurement of microbial biomass in soil. Soil Biology and Biochemistry 10, 215–221.

Bastida, F., Barbera, G.G., Garcia, C., Hernandez, T., 2008a. Influence of orientation, vegetation and season on soil microbial and biochemical characteristics under semiarid conditions. Applied Soil Ecology 38, 62 – 70.

Bastida, F., Kandeler, E., Hernandez, T., Garcià, G., 2008. Long-term effect of municipal solid waste amendment on microbial abundance and humus-associated enzyme activities under semiarid conditions. Microbial Ecology 55, 651–661.

Bell, C.W., Acosta-Martinez, V., McIntyre, N.E., Cox, S., Tissue, D.T., Zak, J.C., 2009. Linking Microbial Community Structure and Function to Seasonal Differences in Soil Moisture and Temperature in a Chihuahuan Desert Grassland. Microbial Ecology 58, 827–842.

Calbrix, R., Barray, S., Chabrerie, O., Fourrie, R., Laval, K., 2007. Impact of organic amendments on the dynamics of soil microbial biomass and bacterial communities in cultivated land. Applied Soil Ecology 35, 511–522.

Caravaca, F., Barea, J.M. , Figueroa, D., Roldán, A. 2002. Assessing the effectiveness of mycorrhizal inoculation and soil compost addition for enhancing reafforestation with *Olea europaea* subsp. *sylvestris* through changes in soil biological and physical parameters. Applied Soil Ecology 20, 107–118.

Gomez, E., Ferreras, L., Toresani, S., 2006. Soil bacterial functional diversity as influenced by organic amendment application. Bioresource Technology 97, 1484–1489.

Grayston, S.J., Griffith, G.S., Mawdsley, J.L., Campbell, C.D., Bardgett, R.D., 2001. Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. Soil Biology and Biochemistry 33, 533-551.

Gresse, M., 2011. Apports de composts au sol, mineralization du carbone et respiration microbienne ; influence de l'aération et de la nature du compost. Rapport de stage 42pp.

Guerrero, C., Gomez, I., Moral, R., Mataix-Solera, J., Mataix-Beneyto, J., Hernandez, T., 2001. Reclamation of a burned forest soil with municipal waste compost: macronutrient dynamic and improved vegetation cover recovery. Bioresource Technology 76, 221–227.

Jensen, K. D., Beier, C., Michelsen, A., Emmett, B., 2003. Effects of experimental drought on microbial processes in two temperate heathlands at contrasting water conditions. Applied Soil Ecology 24, 165–176.

Klamer, M., and Bååth, E., 1998. Microbial community dynamics during composting of straw material studied using phospholipid fatty acid analysis. FEMS Microbiology Ecology 27, 9-20.

Oren, A., and Steinberger Y., 2008. Catabolic profiles of soil fungal communities along a geographic climatic gradient in Israel. Soil Biology and Biochemistry 40, 2578–2587.

Ros, M., Hernandez, M.T., Garcia, C., 2003. Soil microbial activity after restoration of a semiarid soil by organic amendments. Soil Biology and Biochemistry 35, 463-469.

Sardans, J., and Peñuelas, J., 2007. Drought changes phosphorus and potassium accumulation patterns in an evergreen Mediterranean forest. Functional Ecology 21, 191–201.

Tambone, F., Scaglia, B., D'Imporzano, G., Schievano, A., Orzi, V., Salati, S., Adani, F., 2010. Assessing amendment and fertilizing properties of digestates from anaerobic digestion through a comparative study with digested sludge and compost. Chemosphere 81, 577–584.

# Section III.2.2.

# Effets à long-terme d'une perturbation de type canicule et sécheresse appliquée aux communautés microbiennes (structures et fonctions) d'un sol Méditerranéen agricole après épandage de composts (Article 6)

Cette section est présentée sous forme d'un article en préparation intitulé : "**Effects of compost amendment on functional and taxonomic stability of soil Mediterranean microbial communities subjected to drought and high temperature**".

# Effects of compost amendment on functional and taxonomic stability of soil Mediterranean microbial communities subjected to drought and high temperature


## Abstract

Mediterranean soils are generally low in organic matter and suffer from water depletion. Moreover, heat-waves events and longer drought periods are predicted within the future Mediterranean context. Soils and their functions may be thus negatively impacted by these extreme events through changes in the biomass, composition and activities of the edaphic microbial communities. However, compost addition, due to the improvement of soil properties could be a key element to compensate the loss of soil microbial biodiversity and functioning. The aim of this study was to assess resilience of soil biological properties in term of soil microbial and fungal biomasses, soil microbial and fungal communities' taxonomic structure and functions impacted by a 21 day drought-heat perturbation after addition of 2 types of compost in the field at different periods. The amendments improved the physico-chemical soil properties and drought-heat perturbation induced microbial community structure changes, which were less pronounced in compost amended than unamended soils. These small short-term impacts may be due to the combined effects of physico-chemical soil structure, supplying organic matter and incorporation of microbial inoculum adapted to high temperatures. However, it seemed that the period duration between compost amendment of the field and drought-heat perturbation and seasonal environmental parameters experienced during that duration, rather than the type of compost influences the resilience of the soil microbial community structure and catabolic functions.



**Key words:** Drought and high-temperature, compost, Mediterranean microbial communities, stability, soil history.

## 1. Introduction

Mediterranean soils are generally low in organic matter (Ros et al., 2003) and suffer from water depletion. Moreover, prolonged soil dryness and heat-waves events are increasingly likely to occur in temperate regions, due to the foreseen climate (IPCC, 2007; Planton et al., 2008). Changes in soil temperature and water would deteriorate soil biology, which contributes significantly to soil quality and productivity. Decreasing the water content of soil alters nutrient availability (Jensen et al., 2003; Sardans and Penuelas, 2007) restricting therefore their diffusion to microorganisms and inducing a slower nutrient turn-over (Sardans and Penuelas, 2004). Increasing temperature affects also substrate availability and induces depletion of labile organic matter (Dalias et al., 2001). Studies have also shown that drought causes a decrease in total microbial biomass (Griffiths et al., 2003) and that high temperatures generally increase soil respiration and may lead to a rapid decrease of the soil microbial biomass (Pietikaïnen et al., 2005, Hamdi et al., 2011). Soils and their functions may be thus negatively impacted by these extreme events through changes in the biomass, composition and activities of the edaphic microbial communities (Bérard et al., 2011). These climatic changes would contribute to changes in farm management strategies and use of organic wastes for soil amendment (Hueso et al., 2011).

The production of urban and industrial organic waste materials such as composts, sewage sludges or municipal solid wastes is widespread and the main means to attain sustainable development is recycling such organic waste in agriculture. Several authors compared the addition of different organic waste amendments to soil and conclude that compost could be preferential organic material for enhancing soil properties (Albiach et al., 2000; Albiach et al., 2001; Ros et al., 2003). Thus compost amendment to soil is often viewed as a strategy to improve chemical, physical and biological properties. Compost may improve the stability of soil aggregates (Chenu et al., 2000; Albiach et al., 2001; Tejada and Gonzales, 2003) and may increase soil porosity and water holding capacity (Giusquiani et al., 1995). It has also been shown that compost amendments act as a source of nutrients and organic matter (Albiach et al., 2001 ; Caravaca et al., 2002; Ros et al., 2006a; Ros et al., 2006b; Bastida et al., 2008; Arthur et al., 2011) and may enhance microbial biomass (Garcia-Gil et al., 2000; Borken et al., 2002a,b; Caravaca et al., 2002; Ros et al., 2003; Saison et al., 2006; Bastida et al., 2008; Laudicina et al., 2011). Compost amendments to soil may also stimulate microbial activities and result in an increase in enzymatic activities (Garcia-Gil et al., 2000; Crecchio et

al., 2004; Bastida et al., 2008). Finally, compost amendments can modify the microbial community composition and enhance soil biodiversity (Bossio et al., 1998; Pérez-Piqueres et al., 2006; Bastida et al., 2008; Tabuchi et al., 2008).

Compost addition, due to the improvement of soil properties could be a key element to compensate the loss of soil microbial biodiversity and functioning. The use of compost is recommended to recover degraded soils in semiarid areas (Borken et al., 2002a,b; Ros et al., 2003). Moreover, the use of composts as amendments in burned Mediterranean ecosystems in order to promote soil restoration is increasing (Guerrero et al., 2001; Larchevêque et al., 2005; Cordovil et al., 2011; Cellier et al., 2012). However, organic amendments could be early applied to soils in order to prevent, minimize and avoid any damage which would be caused to the soil biological properties by the future extreme events (IPCC, 2007) and thus enhancing its stability. The stability of soil ecosystem could be defined by two components resistance and resilience (Pimm 1984). Resistance is defined as the ability of a system to withstand a disturbance, while resilience could be defined as the capacity of a system to recover after disturbance (Pimm, 1984; Seybold et al., 1999).

In a previous laboratory experiment studying the soil microbial community resilience to 21 day drought-heat perturbation, we observed that soil microbial and fungal communities' functions and taxonomic structure did not recover till 42 days after the end of the perturbation (Ben Sassi, et al. in prep, Chapter III PhD). Restoration experiments suggest that there could be benefits from adding organic amendments to soil before a perturbation like a drought-heat one. Whereas, in a field experiment, Calbrix et al. (2007) suggested that organic amendment type had fewer effects than seasonal variations on the microbial structure and function. We are then wondering, if these benefits induced by the amendment may be more or less pronounced relating to the period duration (i.e. seasons and number of seasons) between compost amendment of the field and the perturbation. The aim of this study was to assess resilience of soil biological properties in term of soil microbial and fungal biomasses, soil microbial and fungal communities' functions and taxonomic structure impacted by a 21 day drought-heat perturbation after addition of 2 types of compost in the field at different periods. Our questions are: 1) Do previous compost amendments have an influence on the resilience of biological soil properties after 21 days of drought-heat perturbation? 2) Is there resilience of biological soil properties related to the type of compost amended? and 3) Is there resilience of biological soil properties related to the period duration between compost amendment of the field soil and drought-heat perturbation (i.e. seasons and number of seasons)?

## 2. Material and methods

### 2.1. Soils and field organic amendments and microbial and fungal biomasses measurements

An amendment field experiment was conducted on a bare soil located in the South Eastern of France at INRA-Avignon farm. Before amendment, the soil properties, measured at the *Laboratoire d'Analyse des Sols* (LAS-INRA) in Arras (France), were as follows: 347 g $kg^{-1}$ $CaCO_3$ and after decarbonatation 323 g $kg^{-1}$ clay, 259 g $kg^{-1}$ silt; 41 g $kg^{-1}$ sand; 13.2 g $kg^{-1}$ organic carbon respectively. The field was divided into four plots (15 × 5 m). Two plots were amended with compost not derived from an urban waste methanization treatment plant (Launay-Lantic and Beaucaire composting units). The first one was amended on October 2009 (NMa) and the second on January 2011 (NMb). The third plot was amended with compost from an urban waste methanization treatment plant (Varenne-Jarcy composting unit), on October 2010 (M, Table 1). Composts were incorporated mechanically at 30 t/ha into the top 10 cm of the soil. A reference plot (R) also underwent mechanical treatment. Three soil samples were collected randomly at 0–10 cm depth (and pooled) from each of the fourth plot on June 2011 (i.e. 20 months after NMa amendment, 8 months after M amendment and 5 months after NMb amendment) and were subjected to the following laboratory-scale heat-waves experiment.

Microbial and fungal biomasses were measured in the field amendment experiment by the glucose-induced respiration (Anderson and Domsch, 1978; Ben Sassi et al., 2012, see Section III.2.1) at different sampling dates after amendments (after 3, 6, 9, 12, 18 or 24 months depending on the date of the amendment).

### 2.2. Laboratory-scale experiment

After sampling, drying and sieving into size fraction of between 2 and 3 mm, the different soils (R, NMa, M and NMb) were slowly moistened and maintained at -0.01 MPa on a suction table during 13 days at ambient temperature (25 °C +/- 2) and in the dark. Soils were then distributed in Petri dishes (40 $g.dish^{-1}$, 3 replicates per soil for each measurement date). For each soil (R, NMa, M and NMb), two treatments were conducted: (1) Control soils incubated at constant conditions (“C”, -0.01 MPa, 25°C), (2) Soils subjected to drying and incubated at 50°C (“DH”, -10 MPa). The DH perturbation lasted 21 days. All treatments were conducted in darkness and at a constant temperature by putting each microcosm within

desiccators put within temperature-controlled incubators. For the treatment "DH", the soils in the microcosms were air-dried at a temperature of 25°C to adjust the water content close to the target water potential of -10 MPa. Then microcosms were kept in desiccators (with a beaker containing saturated aqueous solution of potassium sulfate salt; Bérard et al., 2011) put in incubators maintained at 50°C respectively. For the control "C", microcosms were directly kept in desiccators (with a beaker containing free water to maintain high humidity) put in incubators maintained at 25°C.

At the end of 21-day treatment, "DH" soils were rewetted by adding ultra-pure water and adjusted to achieve an average water gravimetric content of 25% (approximately -0.01 MPa). The microcosms were then placed in desiccators (with a beaker containing free water) within an incubator placed at 25°C +/- 2. For each soil treatment, soil analyses were done 1 day, 7 days, 28 days and 42 days after the restoration of environmental soil conditions (rewetting and lowering of temperature). Three soil sub-samples were collected from each treatment scenario and pooled to perform community-level physiological profiling (CLPP), DOC and Mn analyses immediately after sampling. However, PLFA extraction was performed subsequently on triplicate deep-frozen (-20°C) soil samples.

### 2.3. Physico-chemical analyses

*Water holding capacity (WHC)*

Fifty gram of soil were placed in a funnel containing glass wool, added with 50 mL of water and leaved for 30 min to saturate soil. After these 30 min, water that drained from funnel was collected during an additional 30 min. Then, the amount of water that a soil holds against gravity was calculated.

*Soil dissolved organic C (DOC)*

Seven gram of soil were mixed with 35 mL of water and shaken for 1 h. The mixture was centrifuged at 7000 rpm and 5°C for 10 min in a SIGMA 6K15 centrifuge. Five milliliters of the supernatant solution was filtered through 0.2 µm filters and injected into Venoject tubes. Soil dissolved organic C (DOC) contents in soil filtered extracts was added with 100 µL of HCl (0.2 M) and analyzed for C (Shimadzu TOC-5050A total organic C analyser).

*Manganese (Mn)*

Seven gram of soil were mixed with 35 mL of 1 mol $L^{-1}$ KCl solution and shaken for 1 h. The mixture was centrifuged at 7000 rpm and 5°C for 10 min in a SIGMA 6K15 centrifuge. Ten milliliters of the supernatant solution were filtered through 0.2 µm filters and injected into Venoject tubes. This solution was added with 50 µL of HCl (38%). Total concentration of Mn was obtained by a SpectrAA 220 atomic absorption spectrometer (Varian, Austria).

### 2.4. Soil microbial measurements

*Metabolic activity*

To assess the catabolic fingerprint of the microbial and fungal communities, the FungiResp method using the MicroResp™ technique combined with a selective inhibition (SI) was applied (Ben Sassi et al., 2012; Renault et al., unpublished, Chapter II PhD). The respiratory response to 10 dissolved carbon sources was tested: seven carbohydrates (glucose, sucrose, trehalose, mannose, dextrin, glucosamine and D+cellulobiose); two amino acids (glycine, alanine) and one carboxylic acid (malic acid). The carbon substrates were selected for ecological relevance: relevant to soil agroecosystems (i.e. plant residue, root exudates etc), involved in desiccation tolerance (osmolytes like sucrose, trehalose and glycine). Water was also added to assess basal respiration. A stock solution was made from 25 µL of each substrate that was added to the 96-deep-well plate to deliver 42 mg per mL of soil water to each one. To obtain the catabolic fingerprint of the fungal communities, we added 25 µL of the bactericide bronopol with a concentration of 78 $\mu g.g^{-1}$ soil in each deep-well. However, to obtain the catabolic fingerprint of the whole microbial communities we added 25 µL of water instead of bronopol. Soil (≈ 40% of WHC) was then added using a volumetric dispenser system in each deep well of the microplate, following Campbell et al. (2003). After 1 h pre-incubation with the bacterial inhibitor (to let the toxicant penetrate the cells and induce maximal inhibition), each deep-well microplate was sealed to the microplate containing a gel with an indicator dye that responds to the gel pH and, indirectly, to $CO_2$, *via* a silicone seal (MicroResp™, UK) and incubated in the dark at 23°C (±2) for 6 h. Gel absorbance was measured at 570 nm (Bioteck L-800 spectrophotometer) immediately before sealing to the soil deep-well plate, and after 6 h incubation.

When using MicroResp$^{TM}$ technique as described by Campbell et al. (2003), absorbance values were converted to $CO_2$ concentration after the construction of a calibration curve of absorbance *versus* headspace equilibrium $CO_2$. Doing so, researchers assume generally that the microbially produced $CO_2$ accumulates in the gaseous headspace and that this $CO_2$ is of microbial origin exclusively. However, $CO_2$ is also trapped within the Agar gel and the soil solution. Moreover, the soil we used in our study is a calcareous soil with pH>7 exacerbating the risk of $CO_2$ retention in soil solution and the risk of abiotic $CO_2$ emission with acidic substrates. Thus, absorbance values were converted to $CO_2$ concentration after correction using a new geochemical model that assesses the biases in microbial respiration measurements resulting from the $CO_2$ solubilisation and dissociation in the soil solution as in the Agar gel and the effect of substrate supply on $CO_2$ physicochemical equilibriums (Renault et al., unpublished, Chapter II PhD).

Microbial and fungal biomasses were estimated by the glucose-induced respiration (Anderson and Domsch, 1978; Ben Sassi et al., 2012).

*Microbial community structure (PLFA analysis)*

Lipids were extracted using the procedure described previously by Frostegård et al. (1993) from samples at 2 sampling dates (1 day and 42 days after restored conditions). Briefly, 5 g (wet wt) samples of soil were extracted in a one-phase mixture consisting of chloroform, methanol and citrate buffer (1: 2:0.8, v/v/v). After splitting the extracts into two phases by adding chloroform and buffer, the lipid containing phase was dried under a stream of $N_2$. Lipids were fractionated on silicic columns and then the phospholipid fraction was dried under a stream of $N_2$. The samples were then subjected to a mild alkaline methanolysis and the resulting fatty acid methyl esters were separated on an Elite-5MS capillary column (30 m, 0.25 mm, 0.25 µm) in gas chromatograph equipped with a mass spectrometer (GCMS-QP 2010, Shimadzu). The injector temperature was 250°C.

The PLFA peaks were identified by comparing retention times with peaks of standard mixture of BAME (Bacterial Acid Methyl Ester Mix, 47080-U, SUPELCO Analytical, USA and Grain FAME Mix (47801, SUPELCO Analytical, USA) and specific fatty acid markers. The relative abundance of PLFA is expressed in µg $g^{-1}$ soil and specific lipid indicators are used to assess the abundance of fungal and bacterial structural groups. The specific lipid indicators used to assess the abundance of fungi were 18:2ω6,9 and 18:1ω9 (Klamer and Bååth 1998). Gram-negative bacteria were characterized by 16:1ω7, 16:1ω5, 17:1ω8, 18:1ω7,

cyclo17:0 and cyclo19:0 (Kaur et al., 2005). However, Gram-positive bacteria were characterized by i14:0, 14:0, i15:0, a15:0, 15:0, i16:0, i17:0 and a17:0 (Klamer and Bååth 1998; Feng and Simpson 2009) and gram positive thermophile bacteria were characterized by i16:0, a17:0 and i17:0 (Klamer and Bååth, 1998). Actinomycetes were characterized by 10Me16:0 and 10Me18:0 (Williams and Rice, 2007).

### 2.5. Data analysis

The values of microbial and fungal chemical group SIR for the different treated soils were normalized to the control values % change from control (Chaer et al., 2009). The significance of differences between the data obtained with the different perturbation was tested using XLSTAT (Addinsoft, Paris, France). Community level physiological profiles (corresponding to substrate-induced respiration measurements normalized to the sum of all SIR measurements; 4 replicates), PLFA profiles after normalization of each PLFA biomarker to the sum of the total PLFAs (3 replicates) were carried out with principal component analyses (PCA). Non-parametric partial redundancy analysis (RDA), followed by a Monte Carlo permutation test (5000 permutations) were then used on CLPPs and PLFAs data to test the statistical significance of each explanatory variable (factor): amendment type and the period duration between compost amendment of the field and drought-heat perturbation.

## 3. Results

### 3.1. Physicochemical parameters

The soil water holding capacity (WHC) and pH were higher in amended soils (M, NMb, NMa) than in the unamended reference (R) soil (Tab.1). Non-methanized compost amended soils (NMa and NMb) showed slightly higher organic carbon, organic matter content, total N and different mineral N forms and exchangeable cations than M and R soils. The non-methanized compost “latterly amended” soil (NMb) presented however high concentrations of $NO_3^-$. DOC concentration was lower in R soil compared to amended soils (NMa, NMb and M; Table 1).

**Table 1:** Soil characteristics before drought-heat perturbation.

| Parameters | R | NMa | NMb | M |
|---|---|---|---|---|
| WHC (%) | 60.81 | 61.16 | 62.17 | 63.30 |
| pH $H_2O$ | 8.51 | 8.54 | 8.54 | 8.54 |
| pH KCl | 7.75 | 7.75 | 7.83 | 7.72 |
| Organic Carbon (g $Kg^{-1}$) | 15.1 | 15.2 | 16.0 | 14.1 |
| Organic matter (g $Kg^{-1}$) | 26.1 | 26.4 | 27.6 | 24.4 |
| DOC (µgC $g^{-1}$ soil) | 87.20 | 91.00 | 101.37 | 100.09 |
| Total N (g $Kg^{-1}$) | 1.42 | 1.48 | 1.56 | 1.40 |
| $NH_4^+$ N (mg $Kg^{-1}$) | 1.22 | 1.37 | 1.40 | 1.28 |
| $NO_3^-$ N (mg $Kg^{-1}$) | 18.3 | 19.0 | 35.6 | 16.9 |
| Exchangeable $Ca^{2+}$($cmol^+$/Kg) | 16.3 | 16.0 | 16.1 | 16.5 |
| Exchangeable$Mg^{2+}$($cmol^+$/Kg) | 1.47 | 1.52 | 1.50 | 1.49 |
| Exchangeable $Na^+$($cmol^+$/Kg) | 0.04 | 0.06 | 0.31 | 0.04 |
| Exchangeable $K^+$($cmol^+$/Kg) | 1.00 | 1.12 | 1.19 | 1.02 |

The DOC concentration in control microcosms (RC, NMaC, NMbC and MC) increased during the experiment (Table 2). After DH perturbation (1 day after restored conditions), the DOC and Mn concentrations were higher in DH soils (compared to control ones; Tables 2, 3). At the same experiment day, the increase of Mn concentration was similar in non methanized compost amended soils impacted by drought-heat (NMaDH and NMbDH) and was lower in methanized compost amended soils impacted by drought-heat (MDH). During the recovery time, the DOC and Mn concentrations decreased in the different treated soils (RDH, NMaDH, MDH and NMbDH), but remained higher than controls for DOC concentrations (except for MDH; Tables 2, 3).

**Table 2:** DOC, Mn concentrations and microbial biomass in the control soils during the experiment.

| Parameter | Recovery time (days) | RC | NMaC | NMbC | MC |
|---|---|---|---|---|---|
| DOC (µgC $g^{-1}$soil) | 1 | 46.31 | 75.04 | 62.57 | 58.45 |
| | 42 | 89.38 | 81.71 | 88.59 | 120.11 |
| Mn (µg $g^{-1}$ soil) | 1 | 1.01 | BDL | 0.68 | 0.35 |
| | 42 | 4.38 | BDL | 6.09 | 1.04 |
| Microbial biomass (µg biomass-C $g^{-1}$ soil) | 1 | 109.15 (10.74) | 122.60 (9.91) | 125.50 (30.28) | 88.06 (4.88) |
| | 42 | 32.84 (3.30) | 103.99 (4.02) | 217.88 (39.73) | 108.18 (6.49) |

BDL: Below detection limit

**Table 3:** DOC and Mn concentrations in dried-heated soils 1 and 42 days after restored conditions.

| Parameters | Recovery time (days) | RDH | NMaDH | NMbDH | MDH |
|---|---|---|---|---|---|
| DOC (µgC $g^{-1}$ soil) | 1 | 113.21 | 186.07 | 142.10 | 115.78 |
| | 42 | 94.77 | 106.00 | 121.75 | 110.31 |
| Mn (µg $g^{-1}$ soil) | 1 | 36.39 | 34.65 | 36.54 | 14.50 |
| | 42 | 0.34 | 1.33 | 0.68 | 1.04 |

### 3.2. Basal Respiration and microbial and fungal SIR measurements

Microbial and fungal biomasses (SIR glucose) were higher in amended soils (NMa, NMb and M) than in the reference soil (R) whatever the field sampling dates (Table 4) and microbial biomass showed a drastic decrease in unamended reference control soil during the experiment (Table 2).

The percentages of change from control for microbial basal respiration (BR), microbial SIR glucose and microbial SIR groups (carbohydrates, amino acids, carboxylic acids), were calculated for all treatments and during the recovery time (results are more detailed for the dates day 1 and day 42 after restored conditions).

**Table 4:** Microbial and fungal biomasses measured in the field amendment experiment.

| Parameter | Date | RC | NMaC | NMbC | MC |
|---|---|---|---|---|---|
| Microbial biomass (μg biomass-C $g^{-1}$ soil) | April 2011 | 119.60 (17.65) | 186.69 (10.22) | 260.79 (23.01) | 132.21 (6.39) |
| | June 2011* | 147.93 (4.89) | 274.25 (4.92) | 334.37 (19.50) | 188.89 (17.16) |
| | August 2011 | 111.52 (19.98) | 234.65 (2.39) | 187.13 (2.65) | 175.32 (15.14) |
| | October 2011 | 59.27 (7.57) | 146.90 (1.27) | 110.10 (14.55) | 88.12 (1.12) |
| Fungal biomass (μg biomass-C $g^{-1}$ soil) | April 2011 | 47.93 (8.34) | 79.64 (14.85) | 92.35 (12.46) | 62.67 (2.50) |
| | June 2011* | 50.23 (4.79) | 151.63 (6.55) | 186.72 (11.13) | 67.20 (4.03) |
| | August 2011 | 37.09 (2.44) | 64.86 (7.92) | 65.93 (5.38) | 43.31 (2.81) |
| | October 2011 | 35.29 (2.20) | 46.12 (5.62) | 42.31 (1.48) | 72.42 (2.20) |

*****field sampling date for drought-heat perturbation

**Table 5:** Mean percentage of change relative to control for microbial and fungal chemical group SIR, microbial BR and microbial and fungal SIR glucose (biomass) in dried-heated soils 1 day after of restored conditions (standard deviation in brakets; n=4).

| Parameters | | RDH | NMaDH | NMbDH | MDH |
|---|---|---|---|---|---|
| Microbial | SIR Glucose | -36.47 (10.99) | 47.89 (4.80) | 66.35 (16.84) | -7.50 (6.67) |
| | BR | -60.73 (2.14) | 116.21(13.46) | -36.70 (1.16) | -48.53 (2.61) |
| Fungal | SIR Glucose | -74.07 (0.56) | -41.49 (2.81) | -72.57 (1.64) | -71.77 (2.76) |
| Microbial SIR | Carbohydrates | -25.01 (3.10) | 81.64 (6.70) | 81.15 (5.04) | -12.94 (2.26) |
| | Amino acids | 20.01 (6.60) | 32.41 (6,34) | 43.28 (5.22) | -11.60 (6.46) |
| | Carboxylic acid | -3.00 (2.09) | 86.7 (13.86) | 5.14 (7.38) | 11.94 (17.20) |
| Fungal SIR | Carbohydrates | -67.11 (0.68) | -30.26 (2.28) | -66.63 (3.17) | -66.94 (0.92) |
| | Amino acids | -17.45 (1.56) | -34.91 (2.56) | -45.17 (11.82) | -48.88 (2.00) |
| | Carboxylic acid | -64.35 (1.18) | -13.25 (2.64) | -88.74 (0.74) | -69.45 (1.52) |

Just after the end of perturbations (day1), BR and microbial SIR glucose of NMa soil were positively impacted by DH treatment (Table 5). However, BR was negatively impacted by DH treatment in the 3 other soils (NMbDH, MDH and RDH). Microbial SIR glucose was negatively impacted by DH treatment in the reference soil (RDH), while it was positively impacted by DH treatment in the NMb soil (NMbDH). In the M treated soil (MDH), microbial SIR glucose was near to its control value. At the end of the experiment (day 42), while microbial BR of "latterly amended" soils (MDH and NMbDH) was strongly negatively impacted by DH treatment, the oldest amended and the reference dried-heated soils (NMaDH and RDH) showed microbial BR near to their controls (Table 6). At the same experiment day (day42), the microbial SIR glucose was strongly negatively impacted in the dried-heated "latterly amended" soils (Table 6). Just after the end of perturbation (day1) the results showed that microbial SIR carbohydrates were strongly positively impacted by the drought-heat treatment in soils previously amended with non methanized compost (NMaDH and NMbDH) but slightly negatively impacted in the soil previously amended with methanized compost and the soil reference (Table 5). At the same experiment day (day1) and except for NMaDH soil, microbial SIR carboxylic acids of NMbDH, MDH and RDH were near their respective control values. Except for MDH soil, microbial SIR amino acids of NMa, NMb and R soils were slightly positively impacted by drought-heat treatment. Although the fluctuation observed in the changes from control for microbial SIR groups in all the dried-heated soils (data not shown), these soils were negatively impacted after 42 days of recovery (Table 6).

Just after the end of perturbations (day1) and at the end of the experiment (day42), the fungal SIR glucose and all fungal SIR groups were negatively impacted by drought-heat perturbation whatever the soil amended or non-amended with compost (Tables 5, 6). However, the fungal SIR glucose and all fungal SIR groups of the oldest amended NMaDH soil were less impacted by drought-heat treatment than fungal SIRs of the other soils (RDH, NMbDH and MDH).

**Table 6:** Mean percentage of change relative to control for microbial and fungal chemical group SIR, microbial RB and microbial and fungal SIR glucose in dried-heated soils 42 days after of restored conditions (standard deviation in brakets; n=4).

| Parameters | | RDH | NMaDH | NMbDH | MDH |
|---|---|---|---|---|---|
| Microbial | SIR Glucose | -45.98 (4.53) | -42.60 (2.53) | -93.27 (0.13) | -84.61 (0.52) |
| | BR | 16.83(16.90) | -2.29 (3.20) | -98.00 (1.87) | -92.08 (2.16) |
| Fungal SIR | SIR Glucose | -30.26 (2.26) | -33.51 (6.64) | -91.42 (0.47) | -86.11 (0.95) |
| Microbial SIR | Carbohydrates | -38.99 (1.90) | -39.54 (3.21) | -89.75 (0.72) | -82.08 (1.36) |
| | Amino acids | -50.02 (5.10) | 67.55(16.68) | -77.13 (1.24) | -72.44 (2.11) |
| | Carboxylic acid | -57.55 (1.68) | -46.76 (3.10) | -94.44 (0.57) | -87.66 (0.97) |
| Fungal SIR | Carbohydrates | -20.43 (3.34) | -35.14 (4.09) | -82.42 (1.51) | -83.74 (0.18) |
| | Amino acids | -42.15 (3.16) | -64.15 (1.73) | -71.25 (1.40) | -71.28 (2.47) |
| | Carboxylic acid | -22.84 (2.88) | -25.58 (3.44) | -89.76 (8.18) | -85.84 (1.15) |

### 3.3. Microbial community level physiological profiles (CLLPs)

The first two axes of the PCA performed on microbial CLLPs accounted for more than 79% of the variability (Fig. 1). Just after the end of the perturbation (1 day after restored conditions), the PC1 axis (accounting for more than 43%) highly separated reference dried-heated soil (RDH1) from its control (RC1) and during the recovery time RDH and RC presented opposite directional trajectories. However, 1 day after restored conditions there were similarities between amended dried-heated soils (NMaDH1, NMbDH1, MDH1) and their respective controls on PC1, while PC2 axis (accounting for more than 36%) separated between these DH soils and their controls. At the end of the experiment (day42), while the PC1 axis highly separated "latterly amended" dried-heated soils (MDH42 and NMbDH42) from their respective controls (MC42 and NMbC42), there was a similarity of the ordination of the microbial catabolic structure between the "oldest amended" dried-heated soil (NMaDH42) and its control (NMaC42) on axis 1 and 2. The PC1 separated, NMbDH1, RDH7 soils and NMaDH soils during the recovery time (which had negative ordinates)

compared to the other dried-heated soils (which had positive ordinates on this axis). The former group having negative ordinates was characterized by the microbial mineralization of mannose, cellobiose and trehalose. However, the latest group having positive ordinates on PC1 was characterized by the microbial mineralization of D+Glucosamine, alanine and glycine. Except for NMaC42, NMaC and NMbC (which had negative ordinates on axis 2) were characterized by the microbial mineralization of malate, glucose and sucrose during the experiment. Partial redundancy analysis (RDA) performed on microbial CLPPs showed that the type of compost (methanized or not methanized) and the period duration between compost amendment of the field soil and drought-heat perturbation had no significant effects (F=0.44, p=0.26 and F=0.081, p=0.081 respectively) on the catabolic functions of the soil microbial communities.

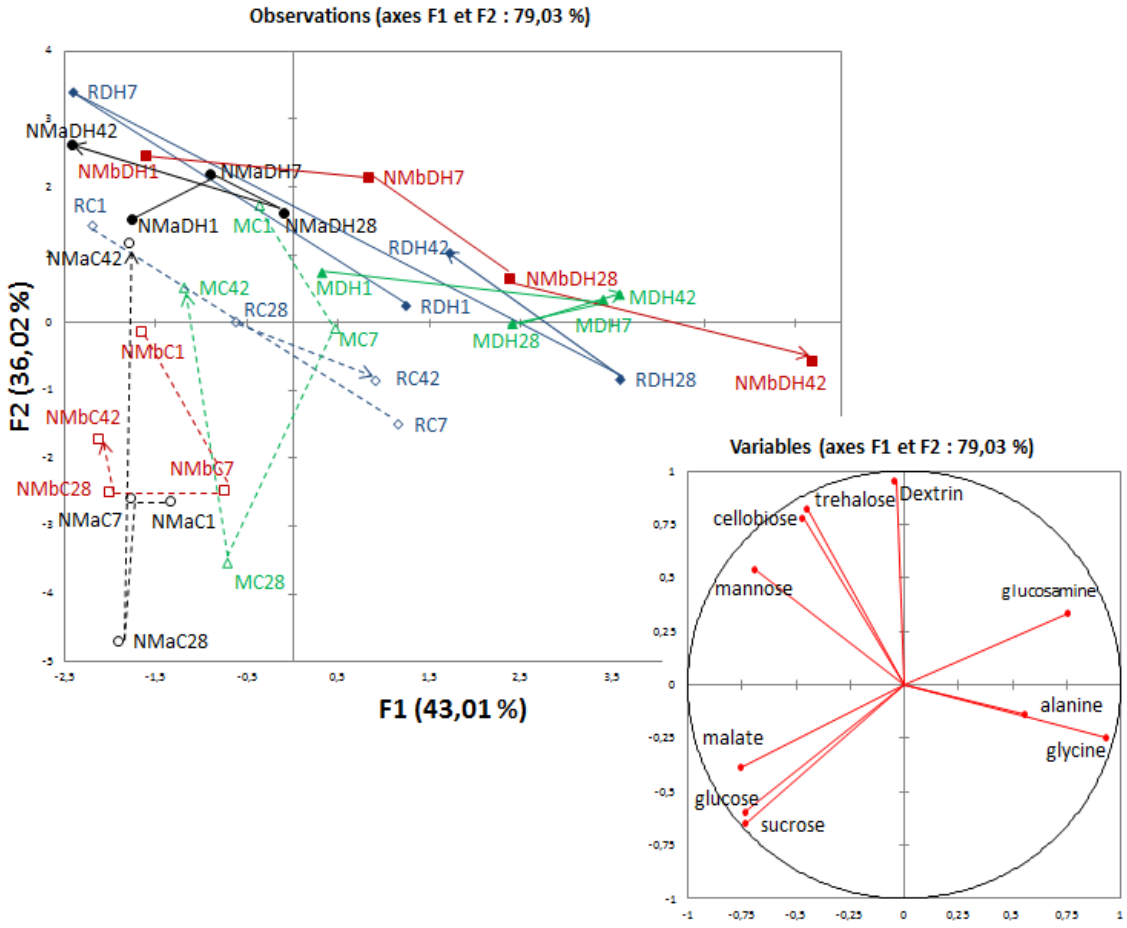


**Fig.1.** Principal component analysis of the microbial CLPP measurements during the recovery time after perturbation of 21 days (1: 1 day, 7: 7 days, 28: 28 days and 42: 42 days). Amended dried-heated soils (NMaDH, NMbDH, MDH) an unamended dried-heated soil (RDH), solid lines. Controls (NMaC, NMbC, MC and RC), hatched lines.

### 3.4. Fungal community level physiological profiles (CLLPs)

The first two axes of the PCA performed on fungal CLLPs accounted for more than 86% of the variability (Fig. 2). Just after the end of perturbations (day1), there was a high degree of similarity of the ordination of the fungal catabolic structure between dried-heated NMa soil (NMaDH1) and its control (NMaC1) within PC1 and PC2 accounting for more than 74% and 12% of the overall variation respectively. However, the PC1 separated "latterly amended" and reference dried-heated soils (MDH1, NMbDH1 and RDH1) from their respective controls (MC1, NMbC1 and RC1). At the end of the experiment (day42), while the PC1 axis highly separated "latterly amended" dried-heated soils (MDH42 and NMbDH42) from their respective controls (MC42 and NMbC42), there was a similarity of the ordination of the fungal catabolic structure between the "oldest amended" dried-heated soil (NMaDH42) and its control (NMaC42) on one hand and between the reference RDH42 soil and its control (RC42) on the other hand. During the recovery time, "latterly amended" and reference dried-heated soils (MDH, NMbDH and RDH, except for RDH7) were more and more characterized by the fungal mineralization of D+Glucosamine, alanine and glycine. However, control soils (RC, NMaC, NMbC and MC) and NMaDH soils were characterized by the fungal mineralization of malate, mannose, cellobiose and trehalose. Partial redundancy analysis (RDA) performed on fungal CLPPs showed that the type of compost had no significant effects ($F=0.620$, $p=0.12$) on the catabolic functions of the soil fungal communities. However, the period duration between compost amendment of the field soil and drought-heat perturbation had significant ones ($F=1.30$, $p=0.012$).

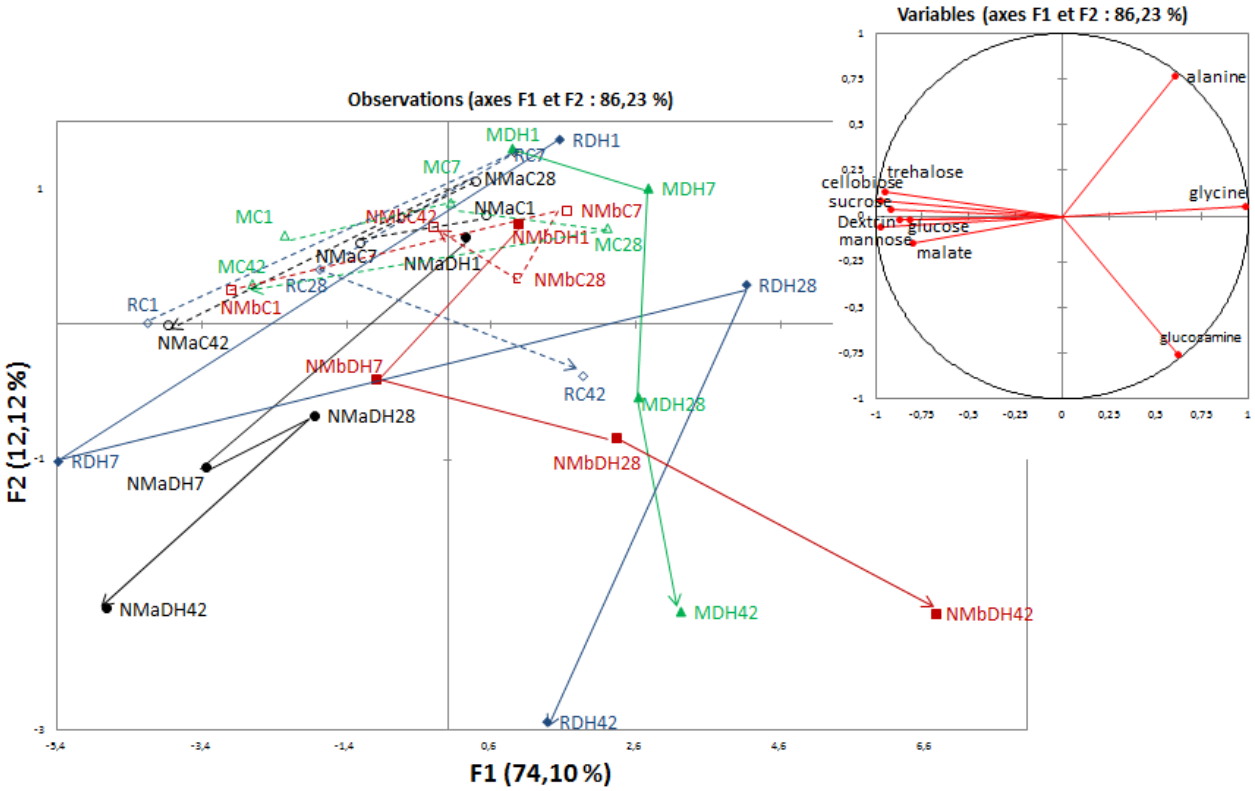


**Fig.2.** Principal component analysis of the fungal CLPP measurements during the recovery time after perturbation of 21 days (1: 1 day, 7: 7 days, 28: 28 days and 42: 42 days). Amended dried-heated soils (NMaDH, NMbDH and MDH) and unamended dried-heated soil (RDH), solid lines. Controls (NMaC, NMbC, MC and RC), hatched lines.

### 3.5. Microbial community structrure

PLFA analyses were performed at 2 sampling dates (day1 and day42). About 55% of the total variability in the PLFA profiles was represented by a PCA plot (39.66% along axis 1, and 15.87% along axis 2; Fig. 3). The first axis PC1 separated “latterly amended” and reference dried-heated soils (MDH, NMbDH and RDH) from their respective controls (MC, NMbC and RC). However, while one day after restored conditions the PC2 axis separated NMaDH1 from NMaC1, at the end of the experiment (day42) NMaDH42 and NMaC42 had similar ordinates on both PC1 and PC2 axis. The third axis of the PCA accounted for about 12% of the overall variation and highly separated MDH from MC during recovery time. However, NMaDH42 and NMaC42 had also similar ordinates on PC3 axis. NMbDH1 and MDH1 were characterized by the dominance of G+ bacteria biomarkers (a15 and i14). MC42

and RDH42 were characterized by the dominance of fungal biomarkers (18:2ω6,9 and 18:1ω9). NMa soils were characterized by the dominance of G+ bacteria biomarkers (i16 and i17) and *Actinobacteria* biomarkers (10Me16 and 10Me18). Partial redundancy analysis (RDA) performed on PLFA profiles showed that the type of compost and the period duration between compost amendment of the field soil and drought-heat perturbation had no significant effects (F=0.57, p=0.258 and F=0.47, p=0.108) on the microbial structure of the soil.

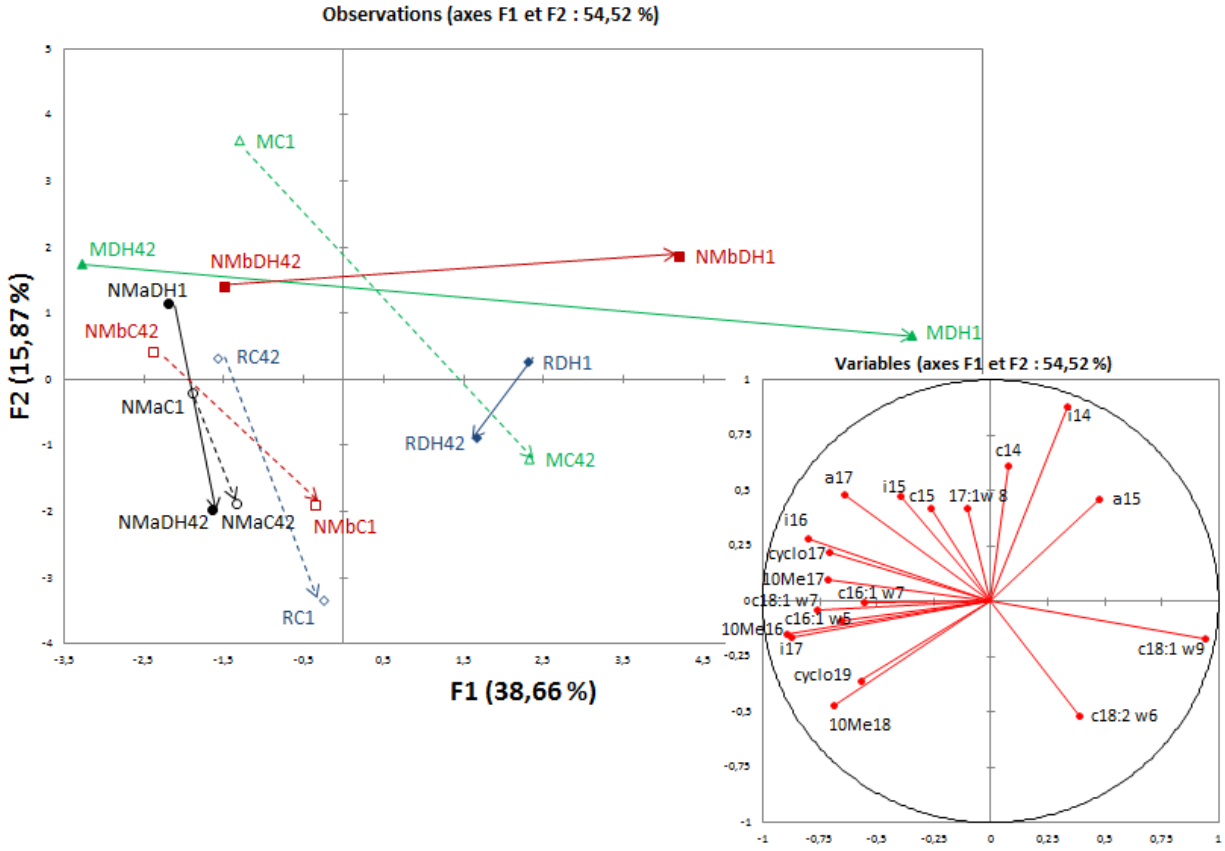


**Fig.3.** Principal component analysis of the PLFA profiles 1 day and 42 days after perturbation of 21 days. Amended dried-heated soils (NMaDH, NMbDH and MDH) and unamended dried-heated soil (RDH), solid lines. Controls (NMaC, NMbC, MC and RC), hatched lines.

### 3.6. Microbial and fungal soil functions and microbial taxonomic structure after 1 and 42 days restored conditions

To highlight the effects of the 21 days perturbation duration on the resistance (1 day after restored conditions) and on the last resilience date fixed in our study (42 days after restored conditions) and to asses fungal functions within microbial community functions, we focused on the PCA performed on microbial and fungal CLLPs at both 1 and 42 days after restored conditions. For further highlight on the microbial community structure PCA was also performed on PLFA profiles at 1 day and at 42 days after restored conditions separately, and fungal to microbial biomass ratios were calculated.

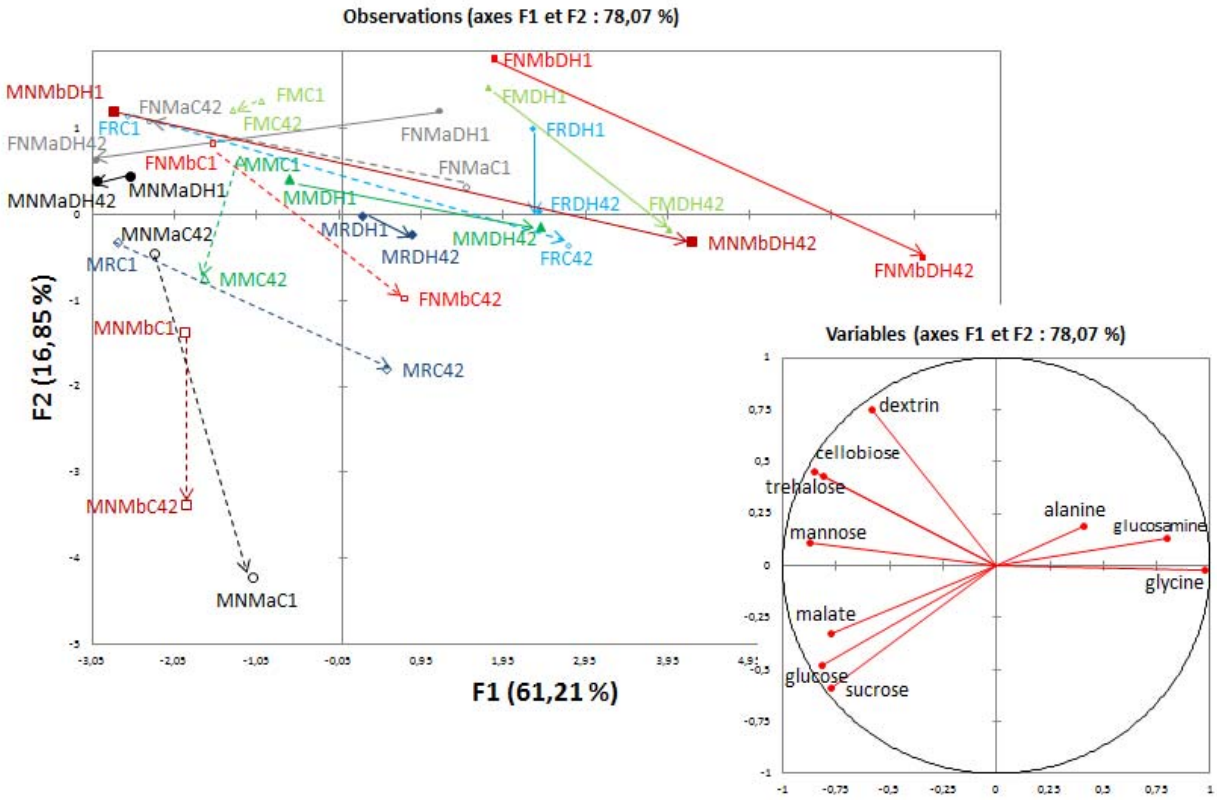


**Fig.4.** Principal component analysis of the microbial (M, dark colors with "big symbols") and the fungal (F, Light colors with "small symbols") CLPP measurements 1 day and 42 days after perturbation of 21 days. Amended dried-heated soils (NMaDH, NMbDH and MDH) and unamended dried-heated soil (RDH), solid lines. Controls (NMaC, NMbC, MC and RC), hatched lines.

The first two axes of the PCA performed on both microbial and fungal CLLPs after 1 and 42 days of restored conditions accounted for more than 78% of the variability (Fig. 4). Just after the end of DH perturbation (day1), the first axis PC1 (accounting for more than 61%) separated microbial catabolic structure of all dried-heated soils (MNMaDH1, MNMbDH1, MMDH1 and MRDH1) from their respective fungal catabolic structures (FNMaDH1, FNMbDH1, FMDH1 and FRDH1). At the end of the experiment (day42) PC1 separated microbial catabolic structure of "latterly amended" and reference dried-heated soils (MMDH42, MNMbDH42 and MRDH42) from their respective fungal catabolic structures (FMDH42, FNMbDH42 and FRDH42). This separation was not as pronounced as that observed 1 day after restored conditions. However, at the same last experiment date there was a high degree of similarity of the ordination of the microbial and the fungal catabolic structure of the "oldest amended" dried-heated soil (MNMaDH42 and FNMaDH42) on both PC1 and PC2.

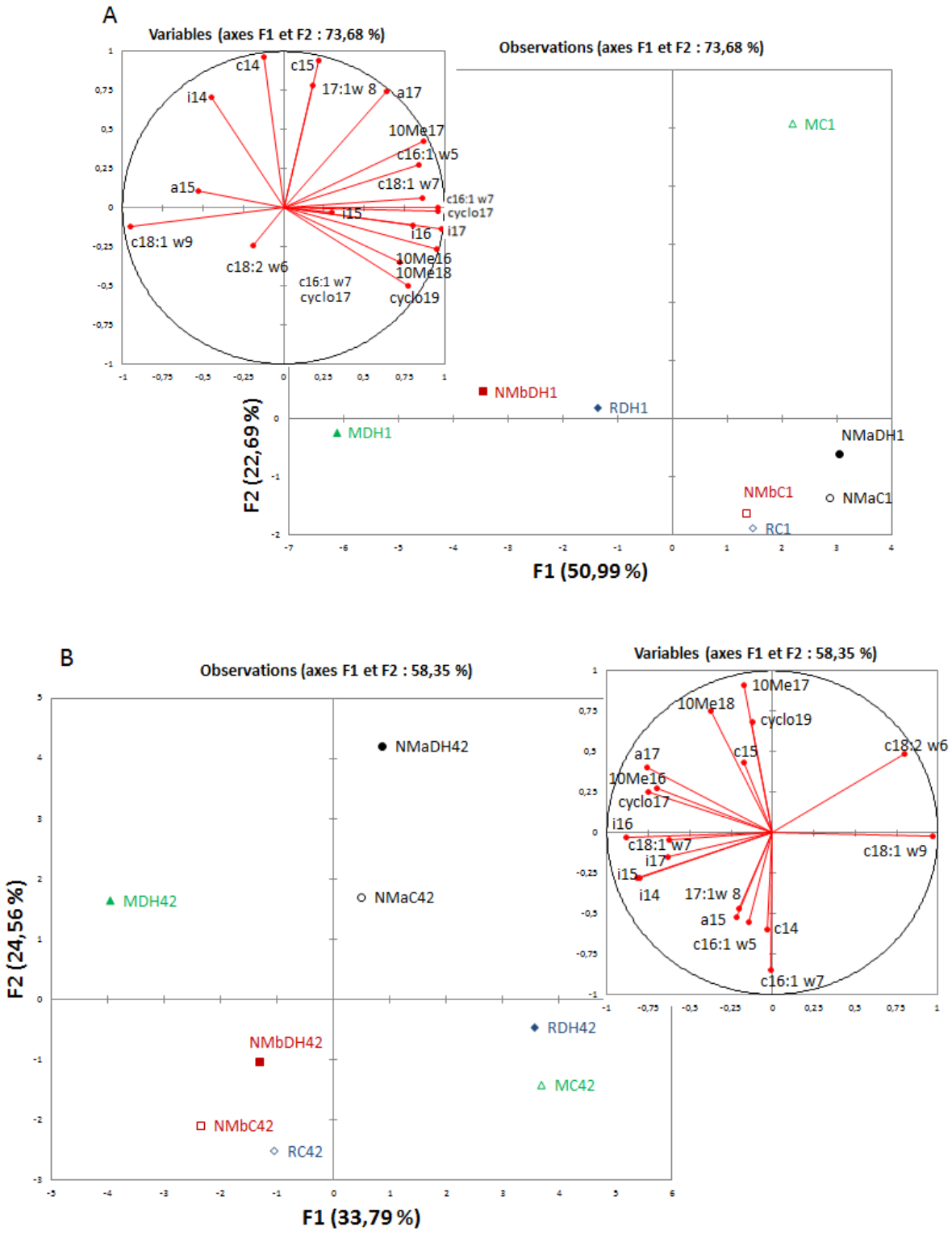


**Fig.5. A-B.** Principal component analysis of the PLFA profiles 1 day (A) and 42 days (B) after perturbation of 21 days. Amended dried-heated soils (NMaDH, NMbDH and MDH) and unamended dried-heated soil (RDH), solid lines. Controls (NMaC, NMbC, MC and RC), hatched lines.

PCA performed on PLFA profiles at 1 day and at 42 days after restored conditions separately (Fig. 5A and Fig. 5B respectively) showed that RDH1, NMbDH1 and MDH1 were characterized by the dominance of G+ bacteria biomarkers (i14) and fungal biomarkers (18:1ω9). RC1, NMbC1, NMaC1 and NMaDH1 were characterized by the dominance of G+ bacteria biomarkers (i16 and i17) and *Actinobacteria* biomarkers (10Me16 and 10Me18). However, MC42, NMaC42, RDH42 and NMaDH42 were more characterized by the dominance of fungal biomarkers (18:2ω6,9 and 18:1ω9).

Moreover, fungal to microbial biomass ratios based on PLFA analysis were similar between the 1$^{st}$ and the 42$^{th}$ day after restored conditions in the NMb and R dried-heated soils. However, while this ratio increased during the recovery time in the NMa dried-heated soil it decreased in the M dried heated soil (Table 7). Nevertheless, fungal to microbial biomass ratios based on SIR glucose analysis increased during the recovery time (Table 7). These fungal to microbial biomass ratios were near to 1 in the oldest amended soil impacted by heat-drought (NMaDH) at the end of the experiment.

**Table 7:** Fungal to microbial SIR glucose ratio and Fungal to microbial PLFA ratios in dried-heated soils 1 and 42 days after restored conditions.

| Parameters | Recovery time (days) | RDH | NMaDH | NMbDH | MDH |
|---|---|---|---|---|---|
| Fungal to microbial PLFA ratio | 1 | 0.25 (0.11) | 0.12 (0.00) | 0.27 (0.10) | 0.35 (0.13) |
| | 42 | 0.36 (0.28) | 0.21 (0.06) | 0.18 (0.11) | 0.12 (0.04) |
| Fungal to microbial SIR glucose ratio | 1 | 0.32 (0.05) | 0.14 (0.01) | 0.10 (0.01) | 0.25 (0.03) |
| | 42 | 0.72 (0.06) | 0.92 (0.01) | 0.54 (0.03) | 0.63 (0.04) |

## 4. Discussion

### 4.1. Do previous compost amendments have an influence on the resilience of biological soil properties after 21 days of drought-heat perturbation?

Compost amendments had an influence on the physico-chemical soil properties. The soil water holding capacities (WHC) were higher in amended soils (M, NMb, NMa) than in the unamended reference (R) soil. This is consistent with several studies, which showed that

the use of organic amendments improves the physical properties of soils by increasing soil porosity and water holding capacity (Shiralipour et al., 1992; Giusquiani et al, 1995). However, while amended soils showed similar organic carbon, organic matter content and total N than unamended soil, the previous addition of compost increased the DOC concentration of amended soils. The DOC concentration remained higher in the control compost amended soils (NMaC, NMbC and MC) compared to the reference unamended control soil (RC) during the experiment. This increase during microcosm experiment in DOC amounts in previously compost amended control soils could be related to degradation of residual compost nutrients. Whereas, we also observed an increase in DOC amounts in unamended control soil during microcosm experiment. These high DOC concentrations could be related to dead and lysed microbial cells in this Reference soil. Indeed, while microbial biomass increased or remained stable in compost amended control soils during the experiment probably due to enhancement of substrate accessibility for microorganisms, a drastic decrease of microbial biomass was observed in unamended control soil probably due to limited substrate availability because of artefacts due to confinement conditions. Thus, application of compost improves the physicochemical soil properties by for example increasing C availability (Gomez et al. 2006) and then enhances biological properties (Saison et al., 2006).

Moreover, several studies showed that the organic amendment incorporation in soils resulted in significant increase in microbial biomasses (Garcia-Gil et al., 2000; Ros et al., 2003; Laudicina et al., 2011) and stimulated microbial activities (Garcia-Gil et al., 2000; Bastida et al., 2008). Such changes in activities and in the size of the microbial community could result in changes in the community composition and an enhancement in biodiversity (Bossio et al., 1998; Bastida et al., 2008), which could then increase resistance and resilience to perturbation (Griffiths et al. 2008). In our case compost amendments also increased soil microbial and fungal biomasses as observed in field measurements. Moreover one day after restored conditions, drought-heat perturbation positively impacted microbial biomass in compost amended soils (SIR glucose compared to control) and drought-heat impacts on microbial catabolic structures were lower in amended soils in comparison to the Reference soil. This is consistent with Hueso et al. (2012) study, which showed that perturbation (e.g. drought) induced microbial community structure changes which were less pronounced in compost amended than unamended soils, they suggested that the organic amendment helped the soil to retain moisture and encouraged the growth and activity of soil microbial populations. Thus, by increasing microbial biomass, the organic amendment of composts

seemed to have a positive influence on microbial biomasses and activities just after the 21 day of drought-heat perturbation, whereas unamended soil was negatively impacted. This is in accordance with Banning and Murphy (2008) study, who suggested that there was a threshold content below which the microbial biomass was no longer able to grow even if it was supplied with available substrates. Whereas, it seemed that the fungal part of the microbial biomass was drastically impacted by the DH perturbation whatever the soil (amended or unamended).

Another hypothesis that could explain the positive impact of compost amendment on microbial resistance to drought-heat perturbation is the role of compost microbial inoculum. While there is controversy about this role (Saison et al., 2006), compost amendments were suggested to be a source of exogenous microorganisms in amended soils (Pérez-Piquerez et al., 2006). Moreover, the composting process comprises mesophilic and thermophilic phases involving successions of numerous microorganisms (Ryckeboer et al., 2003). Compost-born thermophiles or thermotolerant species incorporated as microbial inoculum after amendment, which in the context of a not drought-heat perturbed soil would not be active and competitive (Saison et al. 2006), could have then been selected by the DH perturbation, resulting in more tolerant microbial communities in amended soils to the drought-heat perturbation (Norris et al., 2002). Our PLFA measurements did not clearly indicated the higher presence of thermopiles in soil amended microcosms (controls at day 1). However, while PLFA analysis give some information about thermophiles (i16:0, a17:0 and i17:0, especially for NMaDH1), they could only describe the dominant groups within species may be thermophile or not (Griffiths et al., 2000).

Independently of soil (amended or not), the DOC and Mn concentrations were higher in dried-heated soils than in control soils 1 day after restored conditions. This is consistent with our previous study on different drought-heat perturbation durations, which described an increase of DOC and Mn concentrations compared to the control soil 1 day after restored conditions (Ben Sassi et al. in prep, Chapter III PhD). Indeed, rewetting of dry soils caused a flush of carbon and nitrogen mineralization (Birch, 1958; Bloem et al., 1992) and Mn concentration in soil increased under dried conditions (Bartlett and James, 1980; Makino et al., 2000; Ross et al., 2001). Moreover, it is known that Mn (II) co-factor is required for a large number of microbial cellular functions (Krishnan et al., 2007). We then suggested that Mn solubilised during heat-drought disturbance, may have stimulate the revival soil microbial communities rendered inactive during the perturbation (Ben Sassi et al. in prep, Chapter III PhD). In the case of this study Mn dissolution after DH perturbation coupled with the

stimulation of microbial biomass before DH perturbation could have governed together resistance of microbial biomass in the amended soils. During the recovery time after DH perturbation, DOC and Mn concentrations decreased in the different soils. These DOC and Mn concentrations evolutions are consistent with our previous observations on the resilience of soil microbial community impacted by drought-heat perturbation of 21 days (Ben Sassi et al., in prep, Chapter III PhD). This microbial biomass stimulated just after the end of perturbation by Mn and organic matter availability, oxidized soluble Mn (II) during the recovery time and then was more and more limited by low concentrations of the trace metal decrease (Ben Sassi et al., in prep, Chapter III PhD). In this study, microbial biomass was negatively impacted by drought-heat treatment 42 days after restored conditions and there was no resilience of microbial catabolic structure whatever the soil (amended or not). Particularly, for all soils, microbial activities (SIR) were negatively impacted at the end of the experiment (42 days after restored conditions). Indeed, short-term shifts observed in microbial biomasses (i.e. fungal biomass impacted one day after restored conditions), changes in physico-chemical soil parameters (i.e. Mn concentrations decrease) and rewetting shock could then have governed together the resilience of these communities to the drought-heat perturbation (Bérard et al. 2011).

In conclusion we suggest that compost amendment (by influencing the physico-chemical soil structure, supplying organic matter, and maybe microbial inoculum adapted to high temperatures) may have governed soil microbial biomass and activities resistance to the drought-heat perturbation. Whereas, resilience of these microbial communities seems to have been governed by short-term shifts in microbial structure and changes in physico-chemical parameters during recovery time.

### 4.2. Is there resilience of biological soil properties related to the type of compost amended?

We observed some differences in physico-chemical characteristics between amended soils. Indeed, soil amendment with non-methanized compost (NMb) was matched by an increasing of organic carbon, DOC and $NO_3^-$ content and these concentrations were higher than in soil amended at the same period with methanized compost (M). Moreover, microbial and fungal biomasses were higher in non-methanized compost amended soil (NMb) than in methanized compost amended soil (M). Indeed, it is well known that the methanization steps enhance the mineralization and produce less decomposable organic matter (Tambone et al.,

2010) and thus remaining pool of easily degradable organic compounds could be higher in the non-methanized compost stimulating growth of microbial community. However, if we observed differences in drought-heat impacts just after the end of the perturbation on microbial biomass between non methanized soil (NMb positively impacted) and methanized soil (M less impacted), at the end of the experiment (42 days after restored conditions) microbial and fungal biomasses were all highly negatively impacted by the DH perturbation regardless of compost type amended soil. Even the enhancement in microbial biomass at short-term after DH perturbation, the type of compost amendment seemed to have no significant effects on the resilience of microbial communities after 21 days of drought-heat perturbation.

### 4.3. Is there resilience of biological soil properties related to the period duration between compost amendment of the field soil and drought-heat perturbation?

The microbial community structure measured with PLFA analysis and the fungal catabolic structure of dried-heated "latterly amended" soils (MDH and NMbDH) were neither resistant nor resilient. However, the microbial community structure and the fungal catabolic structure of the dried-heated "oldest amended" soils (NMaDH) seemed to be more resistant and resilient. Moreover, whatever the recovery time studied, dried-heated "latterly amended" soils (MDH and NMbDH) showed higher negatively percentages of change for fungal SIR groups and fungal biomass (fungal SIR glucose) from their respective control than the dried-heated "oldest amended" soil (NMaDH). However, the fungal to microbial biomass ratios (SIR glucose and PLFA) increased only in the dried-heated "oldest amended" soil (NMaDH) during the recovery time. Indeed, while NMaDH community structure was characterized by the dominance of G+ bacteria biomarkers (i16 and i17) and *Actinobacteria* biomarkers (10Me16 and 10Me18) 1 day after restored conditions, it was characterized by the dominance of fungal biomarkers (18:2ω6,9 and 18:1ω9) at the end of the experiment (42 days after restored conditions). Therefore, despite that heat in drought-heat perturbation (DH) induced killing of fungal biomass (fungal SIR glucose measured one day after restoration of environmental conditions), some fungal species may have developed highly resistant forms such as conidia (Dose et al., 2001) and some species inside fungal community that are known to be heat tolerant (Bollen, 1969) may have survived. These resistant forms and tolerant species could have been stimulated after rewetting, increasing fungal to microbial biomass

during recovery time. However, fungal growth could require longer time than some bacterial species, which may explain the dominance of G+ bacteria biomarkers and *Actinobacteria* 1 day after restored conditions and later dominance of fungi in the dried-heated "oldest amended" soil (NMaDH). This fungal dominance could explain the high similarity between fungal catabolic structure of NMa soil impacted by drought-heat perturbation and its microbial catabolic structure. This is consistent with our previous study on the resilience of fungal and microbial community structure of dried-heated soil, which showed that changes in fungal biomasses during recovery time could explain changes in microbial community functions (Ben Sassi et al., in prep, Chapter III PhD). Moreover, these structural and functional changes of drought-heat impacted "oldest amended soil" (NMaDH42 compared to its control) were similar to those of the Reference soil (RDH42). We could thus suggest that the period separating the NMa compost amendment and the DH perturbation may be sufficient for microbial community to be structured by seasonal variations particularly to dry-wet events (compared to the period separating NMb and M compost amendments and the DH perturbation). These NMa communities restructured by soil environmental parameters in the field, could be more adapted to water perturbation than NMb and M soil communities, which less experienced field water stress events and which may have been less adapted to drought perturbation and rewetting shock as observed during the recovery time. Therefore, it seemed that the duration time in term of seasons and climatic environmental conditions and then period duration between compost amendment of the field soil and drought-heat perturbation, rather than the type of compost impacts the resilience of the soil microbial community structure and fungal catabolic functions to a drought-heat perturbation. This is in accordance with amendment field experiments considering temporal (e.g. seasonal variations) and organic amendment type effects on the microbial structure and which suggested that temporal effects were greater than organic amendment on the microbial structure (Bossio et al., 1998; Calbrix et al., 2007). Indeed, the period duration between NMa compost amendment of the field and drought-heat perturbation was about 20 months including 2 autumns, 2 winters, 2 springs and 1 summer. However, the "latterly amended" soils spent only one winter and spring in the soil before drought-heat perturbation. Thus, the "oldest amended" soils (NMa) in term of physico-chemical characteristics and microbial communities, had experienced more variable soil environment than the "latterly amended" soils, particularly the latter soils had not experienced summer season characterized by high temperatures and drought. In the light of physico-chemical properties, the "oldest amended soil" (NMa), which had experienced summer season

could have induced a persistent and more stable structure than the "latterly amended soils" (Annabi, 2005) and then may have enhanced resilience of microbial communities after a drought-heat perturbation. In the light of biological properties, microbial communities in the "latterly amended" soils, which experienced more stable environmental conditions (in term of temperature and humidity), may be more directly sensitive to drought-heat perturbation than those from the "oldest amended" soil (NMa), which had experienced a more variable soil environment (Waldrop and Firestone, 2006; Bérard et al., 2012 Annexe 2).

## 5. Conclusion

Compost amendments improved not only the physico-chemical soil properties but also enhanced biological properties. Moreover, our results showed changes in fungal and microbial catabolic structure and microbial community structure between amended and unamended control soils indicating that compost amendment induced shifts in taxonomic and catabolic structure. However, these shifts in taxonomic and catabolic structures could be linked to either physico-chemical soil improvement and to the field environmental context prevailing perturbation which could thus improve or not resilience to drought-heat perturbation. Thus whatever the soil type amendment, the biological soil properties showed similar but not identical results after drought-heat perturbation. However, resilience of the microbial and fungal communities after a drought-heat perturbation differed between "latterly amended" and "oldest amended" soils. These results suggest that period duration between compost amendment of the field soil and drought-heat perturbation rather than the type of compost influences the responses of the soil microbial taxonomic and catabolic structure to heat-wave. This was particularly highlighted in term of resilience (Philippot et al., 2008; Bérard et al., 2012 Annexe 2). We could suggest that amended soils, which experienced more environmental climatic shifts showed less sensitivity to drought-heat perturbation. Our field/microcosms study illustrated the conclusions of Griffiths and Philippot review (2012): "Biological stability is governed by the physico-chemical structure of the soil (in our case, partially governed by compost amendment) through its effect on microbial community composition and microbial physiology" and that "stability is particular to the disturbance (in our case, drought-heat) and soil history (in our case, seasonal climatic events)".

In this study, we assessed the effects of perturbation after 20 months of amendment for only one type of compost (i.e. the non methanized compost). However, we did not compare

these effects of perturbation to a “same (i.e. 20 months) and identical (i.e. seasons) previously residence time” methanized compost amended soil. Further studies after long-term and repeated amendments of different types of composts are required and would take into account the link between physic-chemical characteristics and microbial changes before and after heat-wave perturbation. More studies are also required to determine factors influencing soil biological parameters with considering the time separating the amendment and the extreme events occurrence in order to take these factors into account in amendment field practice within the future climatic change.

***Contexte experimental et analytique: contributions**

Les prélèvements d’échantillons sur la placette et la mise en place de l’expérimentation au laboratoire ont été réalisés avec l’aide de D. Renard, B. Bes, M. Debroux, P. Renault et A. Bérard (UMR EMMAH, Avignon).

Les analyses biochimiques (PLFA) ont été réalisées à l’UMR SQPOV, (INRA Avignon) encadrées et assistées par G. Sévenier.

L’amendement des placettes à été réalisé dans le cadre du programme ANR PRECODD (Programme de Recherche Ecotechnologie et Développement Durable) N° ANR-08-ECOT-004. Développement d'un cadre méthodologique pour l'évaluation des performances environnementales des technologies de prétraitement des déchets. http://cleanwast.brgm.fr/

**References**

Albiach, R., Canet, R., Pomares, F., Ingelmo, F., 2000. Microbial biomass content and enzymatic activities after the application of organic amendments to a horticultural soil.Bioresource Technology 75, 43-48.

Albiach. R., Canet. R., Pomares. F., Ingelmo. F., 2001. Organic matter components and aggregate stability after the application of different amendments to a horticultural soil. Bioresource Technology. 76, 125-129.

Anderson, J.P.E., and Domsch, K.H., 1978. A physiological method for the quantitative measurement of microbial biomass in soil. Soil Biology and Biochemistry 10, 215–221.

Annabi, M., 2005. Stabilisation de la structure d’un sol limoneux par des apports de composts d’origine urbaine: relation avec les caractéristiques de leur matière organique. Thèse Institut National Agronomique Paris-Grignon, 280p.

Arthur. E., Cornelis. W. M., Vermang. J., De Rocker. E., 2011. Amending a loamy sand with three compost types: impact on soil quality. Soil Use and Management. 27, 116–123.

Banning, N.C., and Murphy, D.V., 2008. Effect of heat-induced disturbance on microbial biomass and activity in forest soil and the relationship between disturbance effects and microbial community structure. Applied Soil Ecology 40, 109–119.

Bartlett, R., James, B., 1980. Studying dried, stored soil samples-Some pitfalls. Soil Science Society of America Journal 44, 721-724.

Bastida, F., Kandeler, E., Moreno, J.L., Ros, M., Hernandez, T., 2008. Application of fresh and composted organic wastes modifies structure, size and activity of soil microbial community under semiarid climate. Applied Soil Ecology 40, 318-329.

Ben Sassi, M., Dollinger, J., Renault, P., Tlili, A., Bérard, A., 2012. The FungiResp method: An application of the MicroResp$^{TM}$ method to assess fungi in microbial communities as soil biological indicators. Ecological Indicators 23, 482-490.

Bérard, A., Ben Sassi, M., Renault, P., Gros, R., 2012. Severe drought-induced community tolerance to heat wave. An experimental study on soil microbial processes. Journal of Soils and Sediments 12, 513-518.

Bérard, A., Bouchet, T., Sévenier, G., Pablo, A.L., Gros, R., 2011. Resilience of soil microbial communities impacted by severe drought and high temperature in the context of Mediterranean heat waves. European Journal of Soil Biology 47, 333-342.

Birch, H.F., 1958. The effect of soil drying on humus decomposition and nitrogen availability. Plant and Soil 10, 9-31.

Bloem, J., De Ruiter, P.C., Koopman, G.J., Lebbink, G., Brussaard, L., 1992. Microbial numbers and activity in dried and rewetted arable soil under integrated and conventional management. Soil Biology and Biochemistry 24, 655-665.

Bollen, G.J., 1969. The selective effect of heat treatment on the microflora of a greenhouse soil. Netherlands Journal of Plant Pathology 75, 157-163.

Borken. W., Muhs. A., Beese. F., 2002a. Changes in microbial and soil properties following compost treatment of degraded temperate forest soils. Soil Biology and Biochemistry. 34, 403-412.

Borken. W., Muhs. A., Beese. F., 2002b. Application of compost in spruce forests: effects on soil respiration, basal respiration and microbial biomass. Forest Ecology and Management. 159, 49-58.

Bossio, D.A., Scow, K.M., Gunapala, N., Graham, K.J., 1998. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. Microbial Ecology 36, 1–12.

Calbrix, R., Barray, S., Chabrerie, O., Fourrie, R., Laval, K., 2007. Impact of organic amendments on the dynamics of soil microbial biomass and bacterial communities in cultivated land. Applied Soil Ecology 35, 511–522

Campbell, C. D., Chapman, S. J., Cameron, C. M., Davidson, M. S., Potts, J. M., 2003. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Applied and Environmental Microbiology 69, 3593–3599.

Caravaca, F., Barea, J.M., Figueroa, D., Roldán, A. 2002. Assessing the effectiveness of mycorrhizal inoculation and soil compost addition for enhancing reafforestation with *Olea europaea* subsp. *sylvestris* through changes in soil biological and physical parameters. Applied Soil Ecology 20, 107–118.

Cellier, A., Francou, C., Houot, S., Ballini, C., Gauquelin, T., Baldy, V., 2012. Use of urban composts for the regeneration of a burnt Mediterranean soil: A laboratory approach. Journal of Environmental Management 95, S238-S244

Chaer, G., Fernandes, M., Myrold, D., Bottomley, P., 2009. Comparative resistance and resilience of soil microbial communities and enzyme activities in adjacent native forest and agricultural soils. Microbial Ecology. 58, 414-424.

Chenu, C., Le Bissonais, Y, Arrouays, D., 2000. Organic matter on clay wettability and soil aggregate stability. Soil Science Society of America Journal 64, 1479-1486.

Cordovil, C.M.d.S., de Varennes, A., Pinto, R., Fernandes, R.C., 2011. Changes in mineral nitrogen, soil organic matter fractions and microbial community level physiological profiles after application of digested pig slurry and compost from municipal organic wastes to burned soils. Soil Biology and Biochemistry 43, 845-852.

Crecchio, C., Curci, M., Pizzigallo, M.D.R., Ricciuti, P., Ruggiero, P., 2004. Effects of municipal solid waste compost amendments on soil enzyme activities and bacterial genetic diversity. Soil Biology and Biochemistry 36, 1595–1605.

Dalias, P., Anderson, J.M., Bottner, P., Coûteaux, M., 2001. Temperature responses of carbon mineralization in conifer forest soils from different regional climates incubated under standard laboratory conditions. Global Change Biology 6, 181-192.

Dose, K., Bieger-Dose, A., Ernst, B., Feister, U., Gomez-Silva, B., Klein, A., Risi, S., Stridde, C., 2001. Survival of microorganisms under the extreme conditions of the Atacama Desert. Origins of Life and Evolution of the Biosphere 31, 287-303.

Feng, X., and Simpson, M.J., 2009. Temperature and substrate controls on microbial phospholipid fatty acid composition during incubation of grassland soils contrasting in organic matter quality. Soil Biology and Biochemistry 41, 804-812.

Frostegard, A., and Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils 22, 59–65.

Gárcia-Gil, J.C., Plaza C., Soler-Rovira P., Polo A., 2000. Long-term effects of municipal solid waste compost application on soil enzyme activities and microbial biomass. Soil Biology and Biochemistry 32, 1173-1181.

Giusquiani, P.L., PagliaI, M., Gigliotti, G., Businelli, D., Benetti, A., 1995. Urban waste compost: effects on physical, chemical, and biochemical soil properties. Journal of Environmental Quality 24, 175-182.

Gomez, E., Ferreras, L., Toresani, S., 2006. Soil bacterial functional diversity as influenced by organic amendment application. Bioresource Technology 97, 1484–1489.

Griffiths, B.S., and Philippot, L., 2012. Insights into the resistance and resilience of the soil microbial community. FEMS Microbiol Reviews 1–18.

Griffiths, B.S., Hallett, P.D., Kuan, H.L., Gregory, A.S., Watts, C.W., Whitmore, A.P., 2008. Functional resilience of soil microbial communities depends on both soil structure and microbial community composition. Biology and Fertility of Soils 44, 745–754.

Griffiths, B.S., Ritz, K., Bardgett, R.D., Cook, R., Christensen, S., Ekelund, F., Sørensen, S.J., Bååth, E., Bloem, J., de Ruiter, P.C., Dolfing, J., Nicolardot, B., 2000. Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity–ecosystem function relationship. Oikos 90, 279–294.

Griffiths, I.R., Whiteley, A.S., O'Donnell, A.G., Bailey, M.J., 2003. Physiological and Community Responses of Established Grassland Bacterial Populations to Water Stress. Applied and Environmental Microbiology 69, 6961–6968.

Guerrero, C., Gomez, I., Moral, R., Mataix-Solera, J., Mataix-Beneyto, J., Hernandez, T., 2001. Reclamation of a burned forest soil with municipal waste compost: macronutrient dynamic and improved vegetation cover recovery. Bioresource Technology 76, 221–227.

Hamdi, S., Chevallier, T., Ben Aïssa N., Ben Hammouda, M., Gallali, T., Chotte, J.L., Bernoux, M. 2011. Short-term temperature dependence of heterotrophic soil respiration after one-month of pre-incubation at different temperatures. Soil Biology and Biochemistry 43, 1752–1758.

Hueso, S., García, C., Hernández, T., 2012. Severe drought conditions modify the microbial community structure, size and activity in amended and unamended soils. Soil Biology and Biochemistry 50, 167-173.

Hueso, S., Hernández, T., García, C., 2011. Resistance and resilience of the soil microbial biomass to severe drought in semiarid soils: The importance of organic amendments. Applied Soil Ecology 50, 27– 36.

IPCC 2007, Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. 21pp

Jensen, K. D., Beier, C., Michelsen, A., Emmett, B., 2003. Effects of experimental drought on microbial processes in two temperate heathlands at contrasting water conditions. Applied Soil Ecology 24, 165–176.

Kaur, A., Chaudhary, A., Kaur, A., Choudhary, R., Kaushik, R., 2005. Phospholipid fatty acid- A bioindicator of environment monitoring and assessment in soil ecosystem. Current Science. 89, 1103-1112.

Klamer, M., and Bååth, E., 1998. Microbial community dynamics during composting of straw material studied using phospholipid fatty acid analysis. FEMS Microb. Ecol. 27, 9-20.

Krishnan, K.P., Fernandes, S.O., Chandan, G.S., Loka Bharathi, P.A., 2007. Bacterial contribution to mitigation of iron and manganese in mangrove sediments. Marine Pollution Bulletin 54, 1427–1433.

Larchevêque, M., Baldy, V., Korboulewsky, N., Ormeño, E., Fernandez, C., 2005. Compost effect on bacterial and fungal colonization of kermes oak leaf litter in a terrestrial. Mediterranean ecosystem Applied Soil Ecology 30 79–89.

Laudicina, V.A., Badalucco, L., Palazzolo, E., 2011. Effects of compost input and tillage intensity on soil microbial biomass and activity under Mediterranean conditions. Biology and Fertility of Soils 47, 63–70.

Makino, T., Hasegawa, S., Sakurai, Y., Ohno, S., Utagawa, H., Maejima, Y., Momohara, K., 2000. Influence of Soil-Drying under Field Conditions on Exchangeable Manganese, Cobalt, and Copper Contents. Journal of Plant Nutrition and Soil Science 46, 581-590.

Norris, T.B., Wraith, J.M., Castenholz, R.W., McDermott, T.R., 2002. Soil microbial community structure across a thermal gradient following a geothermal heating event. Applied and Environmental Microbiology 68, 6300–6309.

Pérez-Piqueres, A., Edel-Hermann,V., Alabouvette, C., Steinberg, C., 2006. Response of soil microbial communities to compost amendments Soil Biology and Biochemistry 38, 460–470.

Philippot, L., Cregut, M., Cheneby, D., Bressan, M., Dequiet, S., Martin-Laurent, F., Ranjard, L., Lemanceau, P., 2008. Effect of primary mild stresses on resilience and resistance of the nitrate reducer community to a subsequent severe stress. FEMS Microbiology Letters 285, 51–57.

Pimm, S. L., 1984. The complexity and stability of ecosystems. Nature 307, 321–326.

Planton, S., Déqué, M., Chauvin, F., Terray, L., 2008. Expected impacts of climate change on extreme climate events, C. R. Geoscience 340, 564-574.

Ros, M., Hernandez, M.T., Garcia, C., 2003. Soil microbial activity after restoration of a semiarid soil by organic amendments. Soil Biology and Biochemistry 35, 463-469.

Ros, M., Klammer, S., Knapp, B., Aichberger, K., Insam, H., 2006a. Long-term effects of compost amendment of soil on functional and structural diversity and microbial activity. Soil Use and Management 22, 209–218.

Ros, M., Pascual, J.A., Garcia, C., Hernandeza, M.T., Insam, H. 2006b. Hydrolase activities, microbial biomass and bacterial community in a soil after long-term amendment with different composts. Soil Biology and Biochemistry 38, 3443–3452.

Ryckeboer, J., Mergaert, J., Vaes, K., Klammer, S., De Clercq, D., Coosemans, J., Insam, H., Swings, J., 2003. A survey of bacteria and fungi occurring during composting and self-heating processes. Annals of Microbiology 53, 349-410.

Saison, C., Degrange, V., Olivier, R., Millard, P., Commeaux, C., Montange, D., Le Roux, X., 2006. Alteration and resilience of the soil microbial community folllowing compost amendment: effects of compost level and compost-borne microbial community. Environmental Microbiology 8, 247-257.

Sardans, J., and Peñuelas, J., 2004. Increasing drought decreases phosphorus availability in an evergreen Mediterranean forest. Plant and Soil 267, 367–377.

Sardans, J., and Peñuelas, J., 2007. Drought changes the dynamics of trace element accumulation in a Mediterranean Quercus ilex forest. Environmental Pollution 147, 567-583.

Seybold, C. A., Herrick, J. E., Brejda, J. J., 1999. Soil resilience: a fundamental component of soil quality. Soil Science 164, 224–234.

Shiralipour, A., McConnell, D.B., Smith, W.H., 1992. Physical and chemical properties of soils as affected by municipal solid waste compost application. Biomass and Bioenergy 3, 261-266.

Tabuchi, H., Kato, K., Nioh, K., 2008. Season and soil management affect soil microbial communities estimated using phospholipid fatty acid analysis in a continuous cabbage (*Brassica oleracea* var. *capitata*) cropping system. Soil Science and Plant Nutrition 54, 369–378.

Tambone, F., Scaglia, B., D'Imporzano, G., Schievano, A., Orzi, V., Salati, S., Adani, F., 2010. Assessing amendment and fertilizing properties of digestates from anaerobic digestion through a comparative study with digested sludge and compost. Chemosphere 81, 577–584.

Tejada, M., and Gonzalez, J.L., 2003. Effects of the application of a compost originating from crushed cotton gin residues on wheat yield under dryland conditions. European Journal of Agronomy 19, 357–368.

Waldrop, M.P., and Firestone, M.K., 2006. Response of microbial community composition and function to soil climate change. Microbial Ecology 52, 716–724.

Williams, M.A., and Rice, C.W., 2007. Seven years of enhanced water availability influences the physiological, structural, and functional attributes of a soil microbial community. Applied Soil Ecology 35, 535-545.

# CHAPITRE IV

## CONCLUSION

## SYNTHESES ET PERSPECTIVES

# Conclusion: Synthèse et perspectives

## 1. Synthèse

Rappel du contexte et des objectifs de la thèse :

Le sol, patrimoine non renouvelable, remplit des fonctions environnementales, sociales et économiques essentielles à la vie. La fonction écologique des sols est due aux fortes interactions entre la microflore, la réactivité géochimique et les transferts d'eau, de solutés, de gaz, de matière et de chaleur. En particulier, les microorganismes édaphiques contribuent à la qualité des sols, participant ainsi à leurs services écologiques (Millennium Ecosystem Assessment, 2005). Face aux changements climatiques actuels et à l'augmentation des populations, la vulnérabilité du sol et des services écosystémiques qu'il rend s'accroît. En particulier dans les zones climatiques Méditerranéennes, les modèles météorologiques prévoient une augmentation des sécheresses estivales et une augmentation des températures (Gibelin et Déqué, 2003) accompagnées par l'apparition plus fréquente d'évènements extrêmes de type canicule et sécheresse (IPCC, 2002 ; IPCC, 2012). Ces évènements pourraient accentuer l'appauvrissement de la teneur en carbone organique et de la diversité biologique des sols méditerranéens et entraineraient des modifications durables de leurs fonctionnements. Le maintien et l'amélioration de la qualité de ces sols sont donc essentiels pour assurer la durabilité de l'environnement et de la biosphère (Bastida et al., 2008b). Compte tenu de l'amélioration des propriétés physiques, chimiques et biologiques des sols après ajout de compost (Borken et al., 2002a,b ; Bastida et al., 1998a ; Ros et al., 2003), l'apport de compost au sol pourrait constituer une solution pour prévenir et atténuer les effets des sécheresses et des canicules dans les agrosystèmes Méditerranéens.

Dans ce contexte, les objectifs de la thèse étaient 1) d'étudier la réponse de la communauté microbienne édaphique (structures et fonctions) à une perturbation de type canicule et/ou sècheresse en fonction de la durée de la perturbation et de détecter l'existence éventuelle de seuils dans les réponses microbiennes, 2) d'aborder la résilience (structure et fonctions) des communautés microbiennes suite à une perturbation seuil, 3) d'étudier l'impact d'un apport préalable de compost sur les réponses des communautés microbiennes suite à une

perturbation de canicule et de sécheresse, et de comparer parallèlement l'influence de différents types de composts et 4) d'étudier les effets de la durée de la période entre l'épandage de compost et l'événement extrême appliqué et les effets de l'historique saisonnier de température et d'humidité sur les réponses à une perturbation de type canicule et de sécheresse appliquée.

Les principaux résultats de ces chapitres sont synthétisés ci-dessous. Nous les présentons sous la forme de trois questions : 1) quels sont les effets d'une canicule-sécheresse en terme d'une combinaison de deux perturbations température élevée et stress hydrique xérique ? 2) quels sont les effets d'une canicule-sécheresse en terme d'un accroissement de la durée de perturbation ? 3) quelles sont les influences d'un apport préalable de compost et des variations saisonnières du milieu sur les effets d'une canicule-sécheresse ?

### 1.1. La canicule et la sécheresse : prédominance, expression de deux facteurs ou nouvelle expression?

La capacité de la communauté microbienne à retrouver et à maintenir sa diversité structurale et fonctionnelle après une perturbation dépend non seulement de la communauté elle-même, de l'environnement, mais aussi de la nature de la perturbation appliquée (Griffiths et al., 2001b). Dans le contexte Méditerranéen, les sols seront de plus en plus exposés à des évènements extrêmes de type canicule-sécheresse impliquant des conditions simultanées de température élevée et de sécheresse intense. Nous avons abordé la "canicule-sécheresse" (température élevée à 50°C, potentiel hydrique xérique à -10MPa) comme la combinaison de deux perturbations : "canicule" (température élevée à 50°C, potentiel hydrique optimal à -0,01 MPa) et "sécheresse" (température optimale à 25°C, potentiel hydrique xérique à -10MPa). La comparaison de ces trois types de perturbations nous a ainsi permis de distinguer certains des effets des perturbations individuelles (canicule ou sécheresse) des effets de la combinaison de ces deux perturbations ("canicule-sécheresse"). Pour comparer ces différents types de perturbation, nous avons étudié non seulement la résistance mais aussi la résilience des communautés microbiennes vis-à-vis de ces perturbations. En écologie, la résistance est définie comme la capacité à supporter immédiatement une perturbation (Seybold et al., 1999 ; Griffiths et al., 2001a). Dans notre travail, nous avons défini la résistance comme la capacité de la communauté microbienne édaphique à retrouver dans les 24 heures après une

perturbation une biomasse et une structure taxonomique et fonctionnelle comparables à celles des communautés contrôles. Nous avons défini la résilience comme la capacité de la communauté microbienne édaphique à retrouver progressivement après la perturbation une biomasse et une structure taxonomique et fonctionnelle comparables à celles des communautés contrôles (Figure 1 du chapitre introductif). Durant cette expérimentation, des paramètres chimiques (COD et Mn), microbiologiques et biomoléculaires (biomasses microbienne, fongique, et bactérienne ; structure taxonomique microbienne et structure taxonomique bactérienne ; abondance des taxa bactériens les plus représentatifs de la communauté bactérienne du sol ; activités de respiration ; profils cataboliques) ont été choisis et analysés comme indicateurs de la qualité du sol.

Tandis qu'une perturbation de canicule (50°C) impacte négativement la biomasse microbienne, la sécheresse (-10MPa) semble avoir des effets moins délétères sur cette biomasse et induit une mobilisation du manganèse réduit (Makino et al., 2000 ; Ross et al., 2001). Quand nous appliquons une perturbation de canicule-sécheresse, combinant une température élevée et un stress hydrique (50°C et -10MPa), nos résultats suggèrent l'existence d'effets antagonistes (chapitre I ; Revue bibliographique) de la température élevée et du stress hydrique induisant à la fois des stimulations et des inhibitions du développement de la communauté microbienne. En effet, la perturbation de canicule et de sécheresse combinées induirait une mortalité microbienne liée en grande partie à la perturbation canicule et bénéficierait des effets de la sécheresse qui limiterait la transmission de la chaleur dans le sol et induirait une solubilisation du manganèse, qui par ailleurs stimulerait (co-facteur limitant des activités microbiennes) cette biomasse microbienne. De plus, 24 heures après retour aux conditions environnementales des contrôles, nous avons constaté une teneur en manganèse solubilisée plus importante dans les sols préalablement soumis à la perturbation canicule-sécheresse que dans les sols préalablement soumis à la perturbation sécheresse seule. La littérature rapporte que la plupart des microorganismes oxydent le Mn (Miyata et al., 2007). En conséquence, une biomasse faible (par exemple inhibée par la température élevée de la perturbation canicule-sécheresse) oxyderait le Mn moins rapidement qu'une biomasse microbienne importante (cas de la perturbation sécheresse ; chapitre III). Tandis que les structures microbiennes taxonomique et catabolique semblent peu impactées par la perturbation de sécheresse seule et fortement impactées par la perturbation canicule seule en termes de résistance et de résilience, nous avons observé que la combinaison des deux perturbations canicule-sécheresse a induit des changements structurels intermédiaires à ceux

induits par chacune des deux perturbations canicule et sécheresse séparées (chapitre III). Par exemple, la structure taxonomique de la communauté microbienne impactée par une canicule seule est caractérisée par la prédominance des bactéries G+ thermophiles, celle impactée par une sécheresse seule est caractérisée par la prédominance des champignons, tandis que celle impactée par la combinaison des deux perturbations est caractérisée par la dominance des bactéries G+ et G-. D'un point de vue fonctionnel, nous avons aussi observé une ressemblance entre la structure catabolique de la communauté fongique des sols impactés par la "canicule" seule et ceux impactés par la "canicule-sécheresse" sur le court-terme après la fin de la perturbation ; alors que sur le long-terme, la structure catabolique de la communauté fongique des sols impactés par la "canicule-sécheresse" ressemblerait à celle des sols impactés par la "sécheresse" seule (chapitre III). Ainsi la combinaison des deux perturbations canicule et sécheresse a induit des réponses similaires à l'une ou l'autre des perturbations appliquées individuellement en bénéficiant des effets positifs (par exemple solubilisation du Mn qui stimule la biomasse microbienne) et négatifs (par exemple mortalité microbienne induisant une chute de la biomasse microbienne) sur la communauté microbienne de chacune des perturbations appliquées individuellement. Bien que la résultante de ces effets positifs et négatifs ait induit des effets intermédiaires sur les communautés microbiennes, particulièrement en termes de structure taxonomique et catabolique, ces communautés n'ont pas montré de résistance ni de résilience après une perturbation de canicule-sécheresse (Figure 1).

D'un point de vue "stabilité" du système (c'est à dire résistance et résilience, Giffiths et al., 2001a), nos résultats corroborent avec la littérature en termes de différences observées dans les réponses à court-terme (résistance) et à long-terme (résilience) des communautés microbiennes face aux perturbations (Philippot et al., 2008 ; Bérard et al., 2012 Annexe 2). Nous avons par exemple mis en évidence un "effet-retard" de la réponse des communautés microbiennes face aux perturbations (Bérard et al., 2011). En effet, la résistance des communautés microbiennes face aux perturbations n'est pas forcément suivie d'une résilience (cas de la structure taxonomique de la perturbation sécheresse). De plus, les communautés fongiques préalablement soumises à une perturbation "canicule-sécheresse", à court-terme (résistance) semblent plus impactées par la température, alors qu'à long-terme (résilience), la perturbation induite par la sécheresse et la réhumidification influence leur structure catabolique. Nous avons aussi constaté que la solubilisation du manganèse au cours de la perturbation favorise la résistance de la biomasse microbienne et qu'au fur et à mesure

du temps de recouvrement le Mn est oxydé et devient limitant pour la biomasse, ce qui influe sur la résilience de celle-ci.

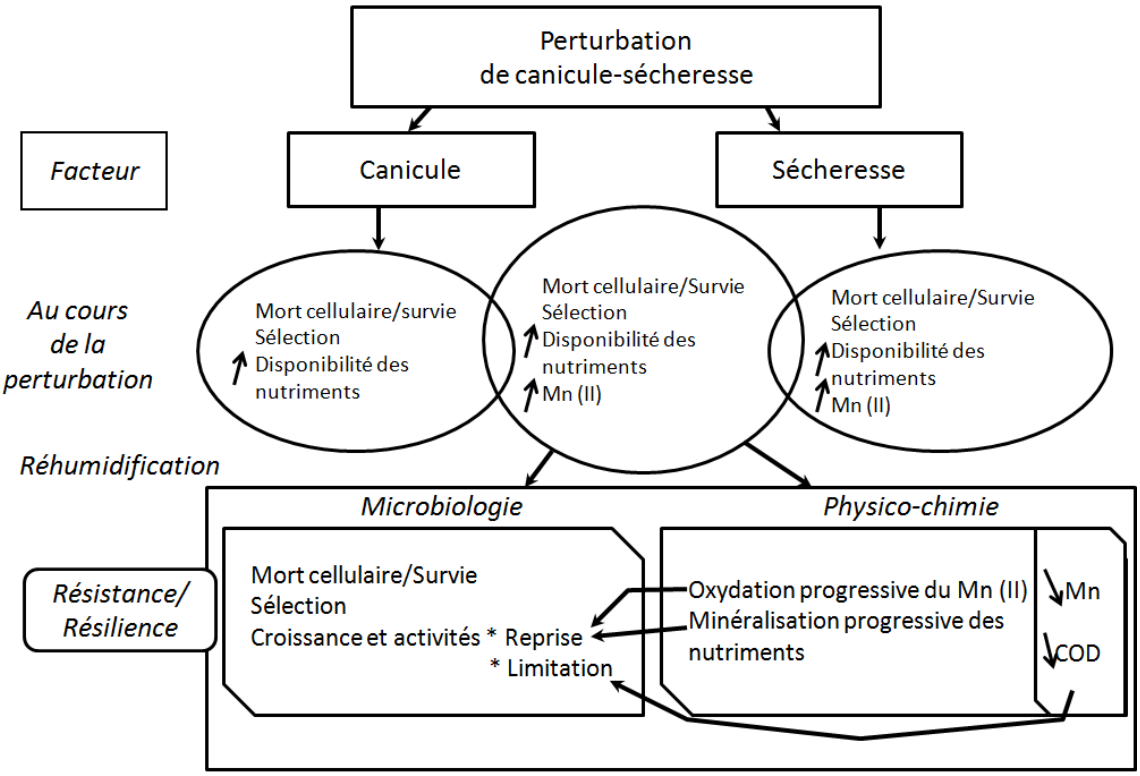


**Figure 1 :** Effets de la nature de la perturbation (canicule et/ou sécheresse) sur les propriétés physico-chimiques et biologiques du sol au cours de la perturbation et après retour aux conditions environnementales du contrôle (résistance et résilience).

### 1.2. Effets de la durée de la perturbation

Nous avons étudié les durées de perturbation (2, 7, 14, 21 et 28 jours) et leurs effets sur la résistance des communautés microbiennes (chapitre III). Deux situations contrastées ont été observées dans la littérature : 1) plus la durée de la perturbation est longue, plus la communauté est impactée (Schimel et al., 1999 ; Bérard et al., 2011) ; 2) dans le cas de courtes durées de perturbation, la communauté microbienne n'ayant probablement pas un temps suffisant pour s'acclimater aux nouvelles conditions du milieu, celle-ci est alors fortement impactée par la perturbation (Bérard et al., 2011). Dans notre travail, les observations ont permis de surligner que les courtes durées de perturbation de canicule et de

sécheresse ont des effets moins marqués que les longues durées de perturbation sur certaines caractéristiques physico-chimiques des sols, plus précisément la solubilisation du manganèse. De plus, l'augmentation de la durée de la perturbation de canicule et de sécheresse s'est traduite par une modification de la structure taxonomique et fonctionnelle de la communauté microbienne. En effet, si d'un côté la biomasse microbienne active semble moins inhibée par l'augmentation de la durée de la perturbation de canicule-sécheresse, car probablement stimulée par une teneur en manganèse soluble (co-facteur limitant les activités microbiennes) croissante avec la durée de la perturbation ; d'un autre côté l'impact de cette perturbation sur la structure taxonomique microbienne et plus particulièrement bactérienne s'accroît avec la durée de la perturbation. De plus, nous avons constaté une déstructuration prononcée de la communauté microbienne après 21 jours de perturbation de canicule-sécheresse et qui présente une même "intensité" au-delà de cette durée. Un changement brusque et rapide dans la réponse à une perturbation définit le seuil de résistance (Clements et Rohr, 2009). Ainsi nous avons considéré 21 jours de durée de perturbation (comparable à la durée de la canicule observée en 2003 en France) comme une durée seuil à partir de laquelle les communautés microbiennes ne sont plus en mesure de répondre efficacement à une perturbation de canicule-sécheresse telle que nous l'avons expérimentée, et un changement brusque particulièrement de la structure taxonomique de la communauté bactérienne a été observé.

### 1.3. Résilience des communautés microbiennes : rôle de la matière organique et des variations saisonnières

L'expérimentation réalisée *in situ* sur des placettes amendées en compost nous a permis de vérifier l'amélioration des caractéristiques physico-chimiques et biologiques imputables à l'apport de matière organique exogène au sol et de montrer que les variations climatiques prévaleraient sur le type de la matière organique apportée en termes de modifications de la biomasse microbienne. Ainsi, nos observations de terrain ont mis en évidence une biomasse microbienne active plus importante dans les sols amendés en comparaison avec le sol non amendé le jour même de l'épandage et cela jusqu'à 2 ans après l'épandage. Des travaux sur l'évolution temporelle des communautés microbiennes dans les sols ont montré que les variations saisonnières influencent la disponibilité des substrats et probablement leurs qualités (Bastida et al., 2008c) ; les variations saisonnières participeraient ainsi à la sélection des communautés microbiennes non seulement *via* les paramètres

pédoclimatiques modifiés mais aussi *via* la capacité de ces communautés à dégrader ces substrats disponibles (Bastida et al., 2008c ; Oren et Steinberger, 2008 ; Bell et al., 2009). Dans le cadre de notre étude les variations saisonnières semblent avoir eu un impact majeur sur la biomasse microbienne et la dynamique structurale fonctionnelle de la communauté microbienne des sols, que ceux–ci aient été amendés en compost ou non (chapitre III).

Il semblerait que la biomasse microbienne active et le pool de matières organiques facilement dégradables soient plus importants dans les composts non méthanisés que dans le compost méthanisé. Cependant, malgré la différence de caractéristiques entre les composts méthanisés et non méthanisés (i.e. biomasse microbienne active et pool de matières organiques facilement dégradables), nos résultats montrent que les caractéristiques microbiologiques du sol sont régies par l'historique saisonnier et répondent de manière similaire à une perturbation de canicule-sécheresse de longue durée quelque soit le type d'amendement organique préalablement appliqué au sol (type de compost). En effet, après une perturbation de longue durée (21 jours) et sur le long-terme (résilience) après la perturbation, tandis que la biomasse microbienne est fortement inhibée dans les sols amendés mais n'ayant pas passé après l'amendement de période estivale au champ, la biomasse microbienne est faiblement impactée dans le cas du sol amendé ayant vécu une période estivale au champ. De plus, la structure taxonomique de la communauté microbienne semblerait résistante et résiliente uniquement dans le sol amendé ayant vécu une période estivale (chapitre III). Des manipulations complémentaires de perturbation de canicule et de sécheresse de courte durée simulée en microcosmes à différentes saisons confirment que l'historique saisonnier du sol a un effet majeur sur la résistance des communautés microbiennes édaphiques à une perturbation de température et de sécheresse élevées (Annexe 5). Ainsi, l'apport de compost semble améliorer les caractéristiques physico-chimiques du sol sans pour autant améliorer la résistance et la résilience de la communauté microbienne après une perturbation de type canicule et sécheresse.

En conclusion, dans ce contexte Méditerranéen où la température et la teneur en eau sont des facteurs environnementaux primordiaux affectant la croissance et l'activité microbienne dans les sols, l'augmentation de la durée de ces deux perturbations combinées lors d'évènements extrêmes de canicule-sécheresse pourrait induire un changement de structure taxonomique et fonctionnelle de la communauté microbienne de manière durable, affectant ainsi la qualité des sols. Bien que l'apport d'amendement organique au sol (sous

forme de compost) permette d'améliorer sa structure physico-chimique et de stimuler les microorganismes indigènes, il semble que cet amendement n'ait pas d'effets majeurs sur l'amélioration de la qualité du sol en termes de résistance et de résilience de ses propriétés microbiologiques face à des perturbations de type canicule-sécheresse. Il semble par contre que l'historique saisonnier "conditionnerait" la réponse de la communauté microbienne à ce type d'événement extrême.

## 2. Perspectives

Les résultats et les limites de ce travail permettent de proposer de nouvelles pistes de recherche.

- Vers une confirmation d'hypothèses énoncées

Nous avons suggéré que le type de compost n'a pas d'effet ni sur la résistance ni sur la résilience de la communauté microbienne après une perturbation de canicule-sécheresse. Cependant, la durée séparant l'épandage de l'évènement extrême aurait un effet sur la réponse de la communauté microbienne sujette à cette perturbation. Toutefois, la première année nous avons épandu un seul type de compost : le compost non méthanisé seul. Nous n'avons pas pu ainsi comparer les effets de l'épandage de différents types de composts ayant passé la même période et au moins une période estivale au champ sur les réponses de la communauté microbienne (structure et fonctions) après une perturbation de canicule et de sécheresse. Il serait donc intéressant de comparer les effets de différents types de composts ayant passé la même période (incluant une période estivale) pour vérifier notre hypothèse.

Nous avons aussi suggéré une influence de l'apport d'inoculum par le compost (avec des espèces adaptées à des températures élevées, préalablement sélectionnées lors de la phase thermophile du compostage) sur la réponse des communautés microbiennes du milieu à une perturbation de canicule-sécheresse. Afin de vérifier notre hypothèse, il serait envisageable de suivre au cours d'expérimentations de type "canicule-sécheresse" la dynamique de certains microorganismes thermophiles prédominants dans le compost tel que *Thermus thermophilus* qui semble être une bactérie dominante durant les phases thermophiles du compostage (Blanc et al., 1999).

- Vers la proposition d'un modèle de dynamique microbienne

D'un point de vue modélisation, nous proposons en premier lieu de définir des groupes microbiens "homogènes" en termes des caractéristiques de croissance et d'activités, ainsi qu'en termes de réaction à la perturbation. Puis en second lieu, nous proposons de développer un modèle décrivant la dynamique des groupes microbiens basée pour leur mortalité sur leur réaction simulée à une perturbation (de type canicule-sécheresse), et pour leur croissance après un retour à des conditions non perturbées, sur les activités des survivants qui peuvent être dopées par les composés organiques libérés par les mortalités microbiennes et par toute autre modification de contexte affectant ces activités, notamment l'accroissement de mobilité du manganèse. Le modèle permettrait de tester certaines hypothèses, et une étude de sensibilité du modèle aux facteurs du milieu (canicule et sécheresse) permettrait d'évaluer sa pertinence.

- Vers des systèmes expérimentaux plus complexes, mais plus réalistes

Pour caractériser les facteurs liés aux évènements extrêmes de type canicule-sécheresse et leurs conséquences sur les microorganismes, nous avons du simplifier le système, et notre étude a été réalisée sur des petits volumes de sol nu sans végétation. Cependant, la composition et la richesse de la végétation affectent les propriétés chimiques et microbiennes du sol (Orwin and Wardle, 2005). Nous avons ainsi occulté le phénomène de "rhizodéposition" (chapitre I ; Revue bibliographique) et les interactions entre les microorganismes et les plantes qui peuvent varier en fonction des conditions climatiques (Henry et al., 2007). Pour les mêmes raisons de simplification, nous nous sommes limités à l'étude des effets de perturbations physiques de nature différente appliquées sur un seul type de sol. Cependant, les réponses physico-chimiques et biologiques à des perturbations peuvent être non seulement liées à la nature de la perturbation mais aussi au type de sol (Bárcenas Moreno et al., 2011). De plus, afin de contrôler les facteurs de perturbation et de multiplier les réplicas, nous avons simulé les perturbations de canicule et/ou de sécheresse dans des microcosmes au laboratoire. Cependant, certains résultats des manipulations en microcosmes suggèrent des problèmes de confinement (chapitre III). Ainsi, ces phénomènes de confinement ne permettraient ni de prendre en considération les possibilités de recolonisation microbienne ni la diffusion de nutriments provenant de zones moins perturbées, liées par exemple à l'hétérogénéité verticale des facteurs physico-chimiques du milieu et à l'atténuation des variations pédoclimatiques avec la profondeur (Fierer et al. 2003). Il serait

donc intéressant d'étudier dans des systèmes expérimentaux plus complexes et/ou *in situ*, les impacts d'une perturbation de canicule-sécheresse sur les réponses de la communauté microbienne de différents types de sols en interaction avec la végétation et les possibles effets de diffusions de nutriments et de recolonisation microbienne.

**Références**


Bárcenas-Moreno, G., Rousk, J., Bååth, E., 2011. Fungal and bacterial recolonisation of acid and alkaline forest soils following artificial heat treatments. Soil Biology and Biochemistry 43, 1023-1033.

Bastida, F., Barbera, G.G., Garcia, C., Hernandez, T., 2008c. Influence of orientation, vegetation and season on soil microbial and biochemical characteristics under semiarid conditions. Applied Soil Ecology 38, 62 – 70.

Bastida, F., Kandeler, E., Hernandez, T., Garcià, G., 2008a. Long-term effect of municipal solid waste amendment on microbial abundance and humus-associated enzyme activities under semiarid conditions. Microbial Ecology 55, 651–661.

Bastida, F., Zsolnay, A., Hernández, T., García, C., 2008b. Past, present and future of soil quality indices: A biological perspective. Geoderma 147, 159–171.

Bell, C.W., Acosta-Martinez, V., McIntyre, N.E., Cox, S., Tissue, D.T., Zak, J.C., 2009. Linking Microbial Community Structure and Function to Seasonal Differences in Soil Moisture and Temperature in a Chihuahuan Desert Grassland. Microbial Ecology 58, 827–842.

Bérard, A., Ben Sassi, M., Renault, P., Gros, R., 2012. Severe drought-induced community tolerance to heat wave. An experimental study on soil microbial processes. Journal of Soils Sediments 12, 513-518.

Bérard, A., Bouchet, T., Sévenier, G., Pablo, A.L., Gros, R., 2011. Resilience of soil microbial communities impacted by severe drought and high temperature in the context of Mediterranean heat waves. European Journal of Soil Biology 47, 333-342.

Blanc, M., Marilley, L., Be¡a, T., Aragno, M., 1999. Thermophilic bacterial communities in hot composts as revealed by most probable number counts and molecular (16S rDNA) methods. FEMS Microbiology Ecology 28, 141-149.

Borken. W., Muhs. A., Beese. F., 2002a. Changes in microbial and soil properties following compost treatment of degraded temperate forest soils. Soil Biology and Biochemistry. 34, 403-412.

Borken. W., Muhs. A., Beese. F., 2002b. Application of compost in spruce forests: effects on soil respiration, basal respiration and microbial biomass. Forest Ecology and Management. 159, 49-58.

Clements, W.H., and Rohr, J.R., 2009. Community responses to contaminants: using basic ecological principles to predict ecotoxicological effects. Environmental Toxicology and Chemistry 28, 1789–1800.

Fierer, N., Schimel, J.P., Holden, P.A., 2003. Variations in microbial community composition

through two soil depth profiles. Soil Biology and Biochemistry 35, 167–176.

Gibelin, A. L., Déqué, M., 2003. Anthropogenic climate change over the Mediterranean region simulated by a global variable resolution model. Climate Dynamics 20, 327–339.

Griffiths, B.S., Bonkowski, M., Roy, Ritz, J.K., 2001a. Functional stability, substrate utilisation and biological indicators of soils following environmental impacts. Applied Soil Ecology 16, 49–61.

Griffiths, B.S., Ritz, K., Wheatley, R., Kuan, H.L., Boag, B., Christensen, S. et al. 2001b. An examination of the biodiversity– ecosystem function relationship in arable soil microbial communities. Soil Biology and Biochemistry 33, 1713–1722.

Henry A., Doucette W., Norton J., Bugbee B., 2007. Changes in Crested Wheatgrass Root Exudation Caused by Flood, Drought, and Nutrient Stress. Journal of Environmental Quality 36, 904-912.

IPCC, 2002. Climate Change and Biodiversity. Technical paper V: 46pp. (http://www.ipcc.ch/pub/tpbiodiv.pdf)

IPCC, 2012. Summary for Policymakers, in *Managing the risks of extreme events and disasters to advance climate change adaptation*, Cambridge University Press., C. B. Field et al. , ed. Cambridge, UK, and New York, NY, USA, 19 p.

Makino, T., Hasegawa, S., Sakurai, Y., Ohno, S., Utagawa, H., Maejima, Y., Momohara, K., 2000. Influence of Soil-Drying under Field Conditions on Exchangeable Manganese, Cobalt, and Copper Contents. Journal of Plant Nutrition and Soil Science 46, 581-590.

Millennium Ecosystem Assessment, 2005. Ecosystems and Human Well-being: Synthesis. Island Press, Washington, DC.

Miyata, N., Tani, Y., Sakata,M., Iwahori, K., 2007. Microbial manganese oxide formation and interaction with toxic metal ions. Journal of Bioscience and Bioengineering 104, 1-8.

Oren, A., and Steinberger Y., 2008. Catabolic profiles of soil fungal communities along a geographic climatic gradient in Israel. Soil Biology and Biochemistry 40, 2578–2587.

Orwin, K., and Wardle, D., 2005. Plant species composition effects on belowground properties and the resistance an resilience of the soil microflora to a drying disturbance. Plant and Soil 278, 205-221.

Philippot, L., Cregut, M., Cheneby, D., Bressan, M., Dequiet, S., Martin-Laurent, F., Ranjard, L., Lemanceau, P., 2008. Effect of primary mild stresses on resilience and resistance of the nitrate reducer community to a subsequent severe stress. FEMS Microbiology Letters 285, 51-57.

Ros, M., Hernandez, M.T., Garcia, C., 2003. Soil microbial activity after restoration of a semiarid soil by organic amendments. Soil Biology and Biochemistry 35, 463-469.

Ross, D.S., Hales, H.C., Shea-McCarthy, G.C., Lanzirotti, A., 2001. Sensitivity of Soil Manganese Oxides: Drying and Storage Cause Reduction. Soil Science Society of America Journal 65, 736–743.

Schimel ,J.P., Gulledge, J.M., Clein-Curley, J.S., Lindstrom, J.E., Braddock, J.F., 1999. Moisture effects on microbial activity and community structure in decomposing birch litter in the Alaskan taiga. Soil Biology and Biochemistry 31, 831–838.

Seybold, C.A., Herrick, J.E., Brejda, J.J., 1999. Soil resilience: a fundamental component of soil quality. Soil Science 164, 224–234.

# ANNEXES

## Annexe 1

**Réponse de la biomasse microbienne à une perturbation de type canicule et sécheresse de courte durée, appliquée sur des sols amendés ou non en compost et prélevés au cours de trois saisons différentes**

L'annexe concerne des expérimentations complémentaires à l'expérimentation principale réalisée l'été 2011 (Chapitre III.2).

Trois bioessais de perturbation de canicule-sécheresse (-10 MPa, 50°C) de 48 heures ont été réalisés au cours de trois saisons (printemps : 21/04/2011, été : 17/08/2011 et automne : 11/10/2011), sur des sols prélevés dans les 4 placettes (R, NMa, NMb et M). L'évaluation de la résistance de la biomasse microbienne à cette perturbation de canicule et de sécheresse (DH) de courte durée a été réalisée 24h après retour aux conditions des contrôles. Les protocoles détaillés de ces bioessais sont présentés dans le chapitre II.

Les biomasses microbiennes mesurées à l'aide de la méthode SIR, 24h après retour aux conditions initiales de température et d'humidité des sols ayant subi la perturbation DH de courte durée ont été exprimées en pourcentage de leurs contrôles respectifs (Figure 1 ; Chaer et al., 2009).

Les bioessais d'été et d'automne suggèrent que quel que soit le type de sol amendé ou non avec du compost, la biomasse microbienne résiste à la perturbation DH de courte durée (avec une légère inhibition de la biomasse du sol NMb et une stimulation variable de la biomasse du sol non amendé R). Au printemps, nous constatons que la biomasse microbienne est impactée négativement par la perturbation DH de courte durée simulée, ceci quel que soit le type de sol amendé ou non avec du compost.

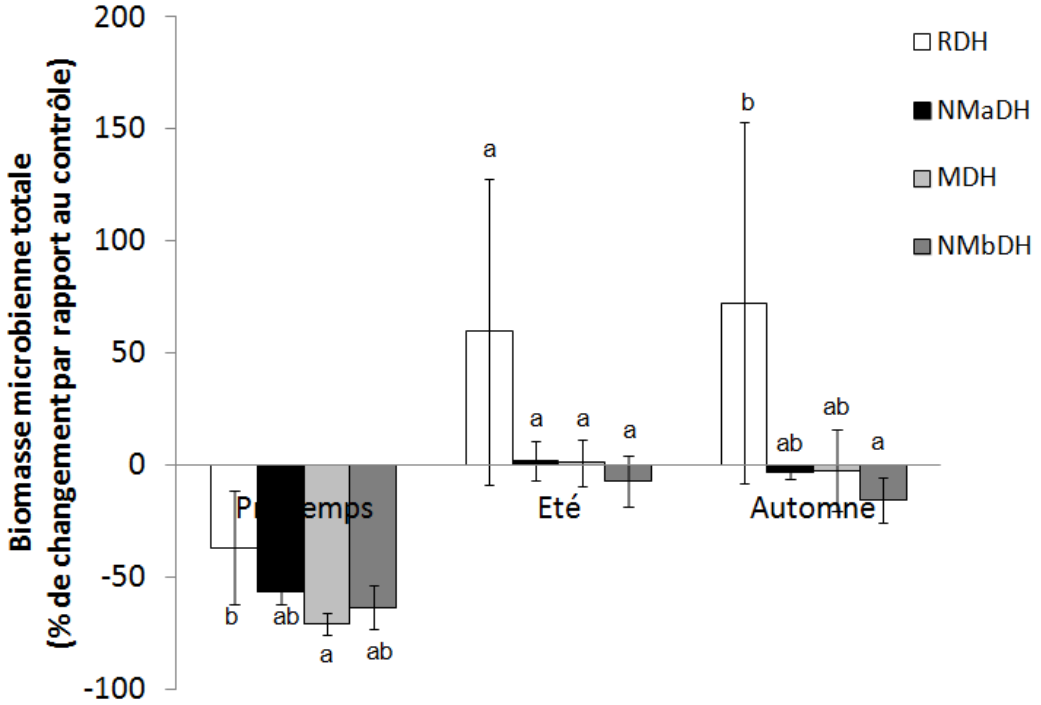


**Figure 1:** Résistance de la biomasse microbienne des sols amendés (NMa, M et NMb) ou non (R) après une perturbation de canicule et de sécheresse (DH) simulée le Printemps, l'Eté et l'Automne.

Au cours de notre expérimentation précédente réalisée sur les même types de sols prélevés l'été (le 10/06/2011) mais ayant subi une perturbation DH d'une durée plus longue (21j), nous avions observé une stimulation de la biomasse microbienne dans les sols amendés avec du compost et une inhibition de la biomasse microbienne dans le cas du sol référence (R) non amendé. Il semble cependant dans le cas de cette série de bioessais, que l'apport de compost n'a pas d'effet sur la résistance de la biomasse microbienne à une perturbation DH de courte durée (48h), nos résultats suggèrent une inhibition de la biomasse microbienne dans le cas du bioessai réalisé au printemps. Nous avions déjà observé que les réponses des communautés microbiennes à des perturbations varient en fonction de leur durée (Chapitre III.1).

Cependant, il semble aussi que les capacités de résistance différeraient selon les saisons. Les températures de l'air et les précipitations mensuelles moyennes mesurées avant les dates de prélèvement des échantillons de sols le printemps (le 21/04/2011), l'été (le 17/08/2011) et l'automne 2011 (le 11/10/2011) sur les différentes placettes sont de 14,53 ;

22,24 et 19,87°C et de 0,94 ; 2,87 et 0,18mm respectivement. Ainsi, nos résultats suggèrent que la biomasse microbienne serait impactée négativement par la perturbation DH de courte durée appliquée à des échantillons de sols ayant vécu préalablement une saison froide en comparaison avec des échantillons de sols ayant vécu préalablement une saison chaude (échantillons prélevés l'été et l'automne) et présentant une résistance à cette même perturbation.

Par ailleurs, Bérard et al., (2011) ont constaté sur un sol comparable, prélevé en fin d'hiver mais non amendé, qu'une perturbation identique d'une durée de 2 jours avait des effets délétères sur les biomasses plus marqués qu'une perturbation de plus longue durée (7 jours) ; ces auteurs suggèrent que la durée de stress de 2 jours n'était probablement pas suffisante pour permettre aux communautés du sol de développer des stratégies d'adaptation au stress appliqué. Cette hypothèse pourrait s'appliquer dans le cas de nos sols prélevés aussi au printemps et n'ayant pas connu les mois précédents des conditions variables de perturbation hydrique et thermique (sécheresse et températures élevées); en particulier dans le cas des sols amendés l'hiver 2010-2011 dont les communautés microbiennes sont "plus jeunes" et non-structurées par une saison chaude et sèche (Chapitre III.2, Article en préparation ; Bérard et al., 2012 Annexe 2) en comparaison avec ces mêmes sols prélevés pendant et après l'été.

En résumé, indépendamment du sol amendé ou non avec du compost, l'historique saisonnier semble avoir un effet majeur sur la résistance des communautés microbiennes vis-à-vis d'une perturbation de canicule et de sécheresse de courte durée. Il serait intéressant de répéter ces manipulations sur plusieurs années afin de confirmer les hypothèses suggérées par ces résultats.

**Références**

Bérard, A., Ben Sassi, M., Renault, P., Gros, R., 2012. Severe drought-induced community tolerance to heat wave. An experimental study on soil microbial processes. Journal of Soils and Sediments 12, 513-518.

Bérard, A., Bouchet, T., Sévenier, G., Pablo, A.L., Gros, R., 2011. Resilience of soil microbial communities impacted by severe drought and high temperature in the context of Mediterranean heat waves. European Journal of Soil Biology 47, 333-342.

Chaer, G., Fernandes, M., Myrold, D., Bottomley, P., 2009. Comparative resistance and resilience of soil microbial communities and enzyme activities in adjacent native forest and agricultural soils. Microbial Ecology 58, 414-424.

**Annexe 2**

J Soils Sediments (2012) 12:513–518
DOI 10.1007/s11368-012-0469-1



# Severe drought-induced community tolerance to heat wave. An experimental study on soil microbial processes

**Annette Bérard · Meriem Ben Sassi · Pierre Renault · Raphael Gros**

Received: 9 September 2011 / Accepted: 9 January 2012 / Published online: 24 January 2012


**Abstract**
*Purpose* The purposes of this study were to identify the influence of a severe drought period on the impact of a subsequent heat–drought disturbance on the microbial community of a Mediterranean agricultural soil and particularly to highlight the long-term effects on the microbial catabolic profiles.
*Materials and methods* We performed an experiment in microcosms and applied the MicroResp™ method on soil microbial communities.
*Results and discussion* A 21-day combined heat–drought disturbance had less impact on soil microbial communities pre-exposed to a 73-day severe drought than on those that were not pre-exposed. These differences were observed not only for biomass and physiological traits (basal respiration, $qCO_2$), but also for catabolic microbial structure evolution during the recovery time.
*Conclusions* These observations suggest that the physiological stress imposed by the initial severe drought changed the microbial catabolic structure or physiological state and favoured a portion of the microbial community best adapted to cope with the final heat–drought disturbance. Consequently, the initial severe drought may have induced a community tolerance to the subsequent heat wave. In this study, we also note that resilience was, more than resistance, an indicator of pre-exposure to stress. In the context of assessing the effects of extreme climatic events on soil microbial processes, these results suggest that future studies should take into account the historic stress of habitats and resilience parameters.

**Keywords** Drought · Heat wave · Resilience · Soil microbial catabolic structure · "Stress-induced community tolerance" · Substrate-induced respiration

Responsible editor: Chengrong Chen

A. Bérard (✉) · M. Ben Sassi · P. Renault
UMR INRA/UAPV 1114 EMMAH (Environnement Méditerranéen et Modélisation des Agro-Hydrosystèmes), Site Agroparc,
84914 Avignon Cedex 9, France
e-mail: annette.berard@paca.inra.fr

R. Gros
Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE), Equipe Vulnérabilité des systèmes microbiens, Faculté des Sciences et Techniques de Saint-Jérôme, UMR CNRS IRD Aix-Marseille Université,
13397 Marseille Cedex 20, France

## 1 Introduction

Soil microbial communities present a great diversity, and their activities play a critical role in the decomposition and stabilization of organic matter (Six et al. 2006) as well as in the immobilization and mineralization of nutrients, which are important processes for the regulation of plant growth (Reynolds et al. 2003). General circulation models predict changes in spatial and temporal patterns of precipitation, including shifts in the frequency and intensity of drought events and heat waves (IPCC 2007). This is especially true in Mediterranean-type ecosystems (Gibelin and Déqué 2003), where surface soils undergo periods of prolonged drying combined with heat wave events interspersed with relatively rapid rewetting events. Drought and high temperature affect substrate availability and microbial cell physiology, and by killing biomass and community selection, these stresses may affect soil microbial processes and carbon allocation in soil microbial communities (Fierer et al. 2003; Bérard et al. 2011). Moreover, temperature stress can lead to cell stress, increased mineralization and cell lysis and through these mechanisms may increase substrate availability but reduce biomass (Hamdi et

al. 2011). Historical events and current environmental conditions may influence the composition and adaptation of microbial communities (Martiny et al. 2006; Schimel et al. 2007).

The purpose of this study was to determine how a severe drought period might influence the ability of the soil microbial community to cope with a subsequent heat wave event (i.e. heat–drought disturbance). We hypothesised that microbial activities of a disturbed community will be more resistant and/or resilient to a subsequent disturbance. To test this hypothesis, we performed an experiment in microcosms to identify the impacts of drought and high temperatures on a disturbed (severe drought) and undisturbed microbial community of a Mediterranean agricultural soil, highlighting the long-term effects on the microbial catabolic capabilities, biomass and physiological traits (basal respiration, $qCO_2$).

## 2 Material and methods

A fine calcareous silty clay loam (Cambisol; see Table 1) was sampled (0–20-cm horizon with 20 soil cores) in March 2008 from a conventionally farmed pea field (INRA-Avignon, South Eastern France), with no chemical treatment during the month preceding soil sampling. The soil was sieved (2–3-mm mesh) and pre-incubated at −0.01 MPa (corresponding to an average gravimetric water content of 21.4%) on a suction table for 2 weeks at ambient temperature (25±2°C) and in the dark.

An aliquot of this soil (1.2 kg) was then submitted to a 73-day period of severe drought by air drying at laboratory temperature (25±2°C) in a microcosm (50×30×6-$cm^3$ box) (microcosm D): during the first 24 days; the soil dried linearly to approximately 4% gravimetric water content corresponding to a water potential of approximately −10 MPa; it was maintained at this moisture level. Another aliquot of the soil was maintained at an average gravimetric water content of 21.4% in a control microcosm (microcosm C) for the same period (73 days). To simulate 21 subsequent/additional days during which the soil was submitted to combined drought and heat disturbance, microcosms C and D were split into nanocosms (50 g of soil aggregates in Petri boxes), and the following treatments were defined:

C–C: 73 days in "control condition" + 21 days in the same "control condition": some nanocosms initially filled with soil issued from microcosm C were maintained in the same temperature and moisture conditions.

C–HD: 73 days in "control condition" + 21 days of drought and heat; some nanocosms initially filled with soil issued from microcosm C were subjected to high temperature and drying (50°C, −10 MPa corresponding to 4% of gravimetric water)

D–HD: 73 days of drought + 21 days of drought and heat; some nanocosms initially filled with soil issued from microcosm D were subjected to high temperature and drying (50°C, −10 MPa)

D–C: 73 days of drought + 21 days with a return to "control conditions": some nanocosms initially filled with soil issued from microcosm D were rewetted to a gravimetric water content of 21.4% and incubated at 25±2°C.

Each treatment was replicated four times (four nanocosms per treatment). The four sampling dates were day 75 (i.e. 2 days after the nanocosm filling for C–C and D–C soils), day 96 (i.e. 2 days after the end of the subsequent heat–drought disturbance 21-day period), days 137 and 157 (i.e. respectively 43 and 63 days after the subsequent heat–drought disturbance 21-day period). Soil samples were then analysed for dissolved organic carbon (DOC, 0.5 M $K_2SO_4$ extractions). Community Level Physiological Profiles (CLPP) and basal respiration (BR) were measured using the MicroResp™ system (Campbell et al. 2003), consisting of a 96-deep-well microplate (1.2-ml volume) filled with soil and with the addition of water only (BR) or aqueous carbon substrates (SIR), sealed individually to a colorimetric $CO_2$-trap microplate and incubated in the dark at laboratory temperature (23±1°C) for 6 h. Mineralization of eight carbon substrates (30 mg $g^{-1}$ soil water, corresponding to 6.7 mg $g^{-1}$dw soil) was tested for CLPP: glucose, sucrose, trehalose, cellulose, glycine, alanine, malic acid and catechol. In each deep well, the carbon substrates were dispensed before (25 μl), and the soil (40% of WHC) was added by way of a volumetric (300 μl, corresponding to 0.35 g dry soil) dispenser system. The duration of MicroResp™ incubation protocol is 6 h, and it is possible that a start of biomass synthesis may occur during the incubation (we have done preliminary measurements from 1 to 8 h, and at 6 h of incubation, we are at the beginning of the increase of $CO_2$ production). In consequence, we calculated the ratio between BR and glucose-induced respiration (GIR) as an index of metabolic quotient ($qCO_2$), following the method described in Saul-Tcherkas and Steinberger (2009) and used by Bérard et al. (2011); this index

**Table 1** Selected physical and chemical characteristics of the soil/texture analysis performed on decarbonated samples

| Parameters | WHC[a] (%) | pH (water) | $CaCO_3$ (g $kg^{-1}$) | Clay (g $kg^{-1}$) | Silt (g $kg^{-1}$) | Sand (g $kg^{-1}$) | Organic C (g $kg^{-1}$) | Total N (g $kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| Values | 31.4 | 8.37 | 365 | 326 | 270 | 33 | 11.6 | 1.42 |

[a] water-holding capacity

was assumed to be proportional to active microbial biomass (Anderson and Domsch 1985; Chapman et al. 2007), but unitless without the coefficient of 40 proposed by Anderson and Domsch (1978).

BR and $qCO_2$ were considered as physiological traits of the microbial communities. The functional stability of the microbial communities was analysed following Griffiths et al.'s (2001) approach, in terms of resistance (to withstand the immediate effects of stress) and resilience (to recover from stress) of the microbial communities' physiological traits in C–HD and D–HD soils compared to the controls C–C and D–C, respectively, at the same dates. We studied the short-term impacts on GIR, BR, $qCO_2$ and DOC of the treatments 2 days after the end of the stress as an evaluation of the resistance and studied the longer-term impacts of the treatments at the end of the experiment as an evaluation of the resilience (63 days after the subsequent heat–drought disturbance and 82 days after the end of the initial severe drought). The values of the microbial parameters (BR, GIR, $qCO_2$) and DOC for the different treated soils were normalized to their respective control values' percent change from control (Chaer et al. 2009). We applied the non-parametric statistical Mann–Whitney test to assess the differences between the treated microcosms and their respective controls ($n=4$ for BR, GIR and $qCO_2$; $n=3$ for DOC; the significance level was set to 5%).

The effect of treatments (drought and heat–drought) on CLPP at day 96 was assessed using non-parametric redundancy analysis (RDA). A total RDA was performed, using drought and heat–drought as independent dummy variables and CLPP as dependent variables. All variables were centred and reduced prior to analysis. Partial RDAs were then performed using either the drought or heat–drought variable as co-variable in order to estimate their respective influence on CLPP (and their interaction) and to partition the total variance. Monte Carlo permutation test was used to determine the statistical significance of the relation between the CLPPs and the treatments. Patterns of recovery were also assessed on CLPP during the course of recovery time till the end of the experiment using principal component analysis (PCA). All statistical treatments were performed with XLSTAT® (Addinsoft, Paris, France) under EXCEL 7® (Microsoft Corporation, Redmond, WA).

## 3 Results and discussion

### 3.1 Direct impacts of initial drought and heat–drought on microbial parameters

Soils subjected initially to the 73 days of initial drought (D–C) showed no increase of $qCO_2$ but a slight increase in their BR and DOC (significant for DOC, Table 2) 2 days after the end of this first stress (day 75). This increase in C substrate availability, suggesting a slight "Birch effect" (Borken and Matzner 2009), may provide from a physical disruption of soil aggregates due to drying–rewetting disturbance and/or directly from microbes (dead and lysed microbes due to drought and

**Table 2** Mean percentage change relative to the controls C–C and D–C and to C–HD (standard deviation in brackets) for the rate of soil basal respiration ($n=4$), glucose-induced respiration ($n=4$), $qCO_2$ ($n=4$) and DOC ($n=3$), measured at two dates: 2 days after the end of each stress for resistance and at the end of the experiment (day 157) for resilience

| Type of stress | | 73 days of drought disturbance | 21 days of heat–drought disturbance | | |
|---|---|---|---|---|---|
| | Functional stability | D–C treatment relative to C–C | D–HD treatment relative to D–C | C–HD treatment relative to C–C | D–HD treatment relative to C–HD |
| Dates of measurements (days after the beginning of experiment) | Resistance | 75 | 96 | 96 | 96 |
| | Resilience | 157 | 157 | 157 | 157 |
| Basal soil respiration | Resistance | 9.6 (5.3) | 27.8 (12.0)* | 51.6 (12.0)* | −13.7 (0.5)* |
| | Resilience | 3.0 (6.1) | 3.8 (4.8) | −13.0 (3.3)* | 22.9 (5.7)* |
| Glucose-induced respiration | Resistance | 7.6 (6.2) | −23.0 (10.0)* | −18.9 (5.3)* | −5.2 (3.4) |
| | Resilience | 4.8 (3.4) | −14.3 (3.0)* | −34.03 (2.8)* | 30.4 (4.5)* |
| $qCO_2$ | Resistance | 2.0 (3.1) | 66.2 (7.0)* | 86.5 (1.7)* | −10.0 (3.8)* |
| | Resilience | −1.9 (6.4) | 21.0 (4.0)* | 31.6 (5.4)* | −22.5 (2.5)* |
| DOC | Resistance | 19.2 (2.9)* | nm | nm | nm |
| | Resilience | −2.9 (4.9) | 10.3 (4.5)* | 27.8 (5.1)* | −13.2 (3.6)* |

The non-parametric statistical Mann–Whitney test was applied to assess the differences between the treated microcosms and their relatives ($n=4$ for RB, GIR and $qCO_2$; $n=3$ for DOC; the significance level was set to 5%)

*nm* non-measured

*significant ($p<0.05$)

drying–rewetting and/or osmolyte intracellular compounds synthesized during drying stress) (Chaer et al. 2009; Fierer et al. 2003). But at the end of the experiment, these parameters recovered. Concerning CLPP measured at day 96, the first two axes of RDA (F1 and F2) extracted 100% of the explained variance (90.5% for F1 and 9.5% for F2) (Fig. 1), indicating that the samples were fully discriminated on F1 and F2. Partial RDAs showed that the variance explained by treatments represented 59.99% of the total variance and that initial drought and heat–drought did not contribute to the same extent to the CLPP variation. Heat–drought explained a large and significant part (57.55%) of CLPP variation (Monte Carlo permutation test, pseudo-$F$=10.84, $P$<0.0001), while a much lower (12.61%) but significant part of CLPP variation was explained by initial drought (pseudo-$F$=1.15, $P$=0.044). The interaction between heat–drought and initial drought was close to 10%. Residual (unexplained) variation amounted to 40%. The ordination obtained from a principal component analysis (Fig. 2) illustrates that the previously dried soils (D–C and D–HD) were first separated on the second axis (16.1%), in their CLPP, from the not dried ones (C–C and C–DH) at day 96.

The initial severe drought-induced low impacts in terms of resistance and resilience on microbial physiological traits

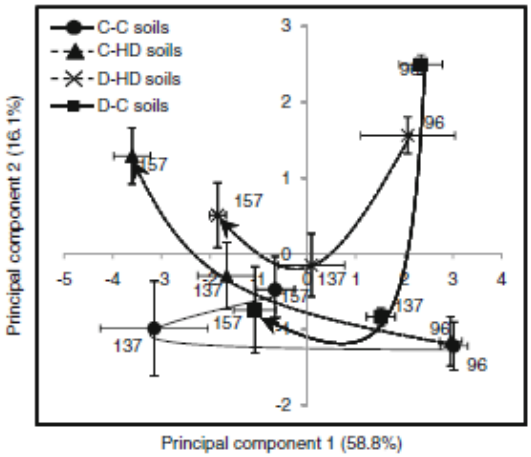


**Fig. 2** PCA of treatments in CLPP space over the time course of the experiment. *Circles*, *triangles*, *crosses* and *squares* illustrate the mean coordinates (±standard errors, $n$=4) for the treatments CC, C–HD, D–HD and D–C, respectively. Values indicate the number of days between the beginning of the experiment and the measurement

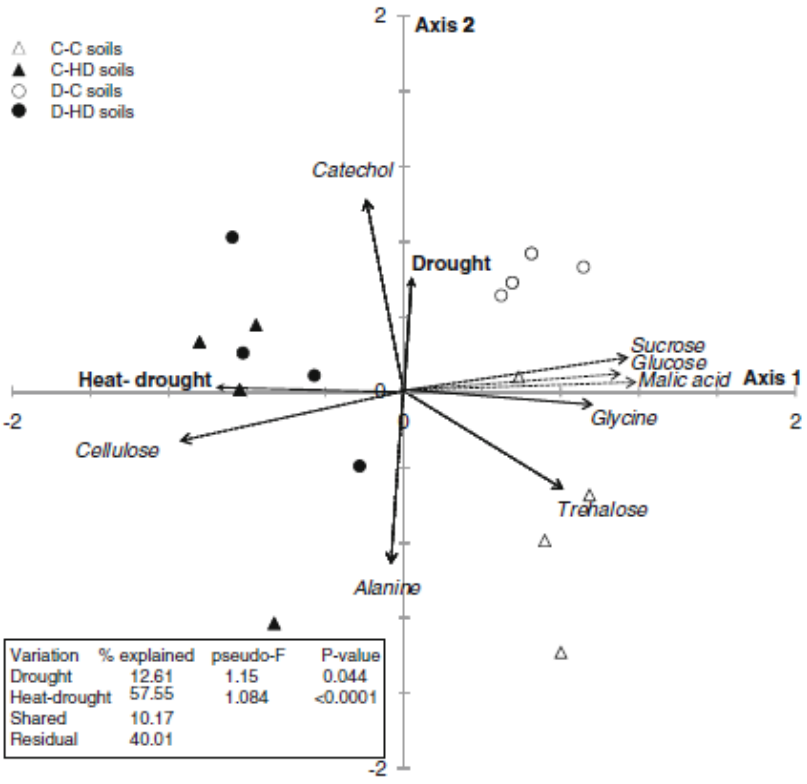


**Fig. 1** Non-parametric redundancy analysis (RDA) of treatments in CLPP space and factor scores for the effects of the two disturbance treatments (73 days of severe drought; 21 days of heat–drought). The RDA was performed on CLPP measured at day 96 with 5,000 random Monte Carlo permutations

(as shown in Table 2, D–C treatment relative to C–C) for this Mediterranean soil, which in situ is frequently exposed to water shortage. If catabolic profiles were significantly structured by this severe drought (as shown in Fig. 1, 12.61% of CLPP variation was explained by the initial drought), they were much more structured by the heat–drought disturbance (as shown in Fig. 1, 57.55% of CLPP variation was explained by the heat–drought disturbance). A previous study showed the higher impact of combined heat–drought disturbance compared to drought disturbance on functional structure, concomitant to taxonomic shifts of soil microbial communities (Bérard et al. 2011), suggesting that functional resistance to a disturbance like heat may be related to microbial community composition (Griffiths et al. 2001).

Two days after the end of subsequent heat–drought disturbance (day 96), the C–HD and D–HD soils showed a significant increase in their BR and $qCO_2$ compared to the respective controls (C–C and D–C), especially for the C–HD soils (see Table 2), suggesting a subsequent "Birch effect". At the end of the experiment (63 days after the end of the subsequent heat–drought disturbance), these physiological traits did not recover to control values, especially for the C–HD soils that presented a collapse of GIR and BR ($qCO_2$ remained significantly higher), compared to C–C control. The higher impact of heat–drought disturbance in terms of resistance and resilience compared to initial severe drought stress on these physiological traits may be the consequence of killing biomass (confirmed by an important decrease in GIR, see Table 2) associated to some thermal denaturation of microbial enzymes (Bérard et al. 2011; Joergensen et al. 1990).

### 3.2 Initial severe drought impacts on the sensitivity to a subsequent heat–drought

Moreover, direct comparison between D–HD and C–HD microbial parameters and DOC confirms a higher impact of the 21-day subsequent heat–drought disturbance on microbial communities and carbon allocation for soil that has not been subjected to drought prior to heat–drought disturbance (C–HD, see Table 2).

The PCA (see Fig. 2) shows change over time in the catabolic microbial structure of the control along the first axis (58.8%), probably corresponding not only to successional stages of microbial communities, but also to a possible carbon source starvation and confinement taking place in the nanocosms when isolating soils from the field (Joergensen et al. 1990). In the case of the three treatments, and especially of the heat–drought treatments, the "Birch effect" may have induced a later turnover of more recalcitrant soil organic matter (Kuzyakov 2010), probably partly associated to different catabolic profile successional stages illustrated in the PCA. The second axis (16.1%) distinguishes progressively between soils that had been subjected to a heat–drought (C–HD, D–HD) and those that had not (C–C, D–C) during the course of recovery time till 63 days after the end of the heat–drought disturbance.

No complete resilience was then observed for soil microbial communities that have been subjected to heat–drought disturbance (D–HD, C–HD). Moreover, soil that has not been subjected to drought prior to heat–drought disturbance presented microbial parameters dramatically altered by the end of the experiment. In the context of our study, C–HD soil microbial communities may have undergone a drastic shift in physiological traits (e.g. allocation of carbon to energy requirements instead of growth) and catabolic structure, probably associated with a shift in composition as measured in a previous study (Bérard et al. 2011). As a result, no resilience seems to be possible.

These results showed that the 21-day heat–drought disturbance had less impact on soil microbial communities pre-exposed to an initial 73-day severe drought than on those not pre-exposed. These differences were observed not only for GIR, for $qCO_2$ that indicates a higher maintenance-energy requirement in microbial communities not pre-exposed to drought (Schimel et al. 2007), but also for catabolic microbial structure evolution during the recovery time. These observations suggest that the initial severe drought imposed changes in microbial catabolic structure (Degens et al. 2001) or physiological state (Bär et al. 2002) and favoured a portion of the microbial community best adapted to cope with the final heat wave event.

Ecosystem resistance and resilience to a stressor relies on tolerant species or individuals that can compensate for sensitive competitors and maintain ecosystem processes, such as catabolic activities, in the soil (Williams 2007; Fierer et al. 2003). Resistance and resilience to additional stressors may depend on these tolerances being positively correlated (called "positive species co-tolerance" by Vinebrooke et al. (2004). Initial exposure to a stressor combined with positive species co-tolerance should reduce the impacts of other stressors, which Vinebrooke et al. (2004) term "stress-induced community tolerance". This concept was largely developed in the case of chemical stressors (Pollution-Induced Community Tolerance, e.g. Dorigo et al. 2007; Bérard et al. 2004; Brandt et al. 2009) and has been studied to a lesser extent with physicochemical stressors such as pH (Pettersson and Baath 2004) and temperature (Ranneklev and Baath 2001). In our study, the severe drought may have induced a community tolerance to the subsequent heat–drought disturbance. Moreover, our results pointed out that this influence of the previous severe drought on the subsequent heat–drought disturbance impacts on microbial processes, and carbon allocation was better enlightened during the course of the experiment and at the end (resilience) than just after the end of the disturbance (resistance). Resilience was thus, more than resistance, an indicator of pre-exposure to stress (Philippot et al. 2008; Bérard et al. 2011).

## 4 Conclusions

We have shown by way of the MicroResp™ method the drastic impacts of a 21-day heat–drought disturbance on soil microbial community catabolic functions and carbon allocation. In terms of resilience, these impacts were lower on soil microbial communities pre-exposed to an initial 73-day severe drought than on those not pre-exposed. In the context of climate extreme event assessment on soil microbial processes, we suggest to develop studies that take into account the historic stress of habitats and resilience parameters.

**Acknowledgements** This study benefited from the support of the "Soil-Aquifer Flux Experimental Facility" at INRA-Avignon. The authors wish to thank the UE-EA (INRA-Avignon) for technical assistance in the agricultural management of the crop field and D. Renard, B. Bes, T. Bouchet and G. Sévenier for their support with suction table manipulations and biochemical analysis. The project received funding from the INRA Division Environment and Agronomy (Projets Innovants). The English text was edited by Harriet Coleman and checked by Eric Michel.

## References

Anderson JPE, Domsch KH (1978) A physiological method for the quantitative measurement of microbial biomass in soil. Soil Biol Biochem 10:215–221

Anderson JPE, Domsch KH (1985) Maintenance carbon requirements of actively metabolizing microbial populations under in situ conditions. Soil Biol Biochem 17:197–203

Bär M, von Hardenberg J, Meron E, Provenzale A (2002) Modelling the survival of bacteria in drylands: the advantage of being dormant. Proceedings of the Royal Society of London, B 269:937–942

Bérard A, Rimet F, Capowiez Y, Leboulanger C (2004) Procedures for determining the pesticide sensitivity of indigenous soil algae—a possible bioindicator of soil contamination? Arch Environ Contam Toxicol 46(1):24–31

Bérard A, Bouchet T, Sévenier G, Pablo AL, Gros R (2011) Resilience of soil microbial communities impacted by severe drought and high temperature in the context of Mediterranean heat waves. Eur J Soil Biol 47:333–342

Borken W, Matzner E (2009) Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. Global Change Biol 15:808–824

Brandt KK, Sioholm OR, Krogh KA, Halling-Sorensen B, Nybroe O (2009) Increased pollution-induced bacterial community tolerance to sulfadiazine in soil hotspots amended with artificial root exudates. Environ Sci Technol 43:2963–2968

Campbell CD, Chapman SJ, Cameron CM, Davidson MS, Potts JM (2003) A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Appl Environ Microbiol 69:3593–3599

Chaer G, Fernandes M, Myrold D, Bottomley P (2009) Comparative resistance and resilience of soil microbial communities and enzyme activities in adjacent native forest and agricultural soils. Microb Ecol 58(2):414–424

Chapman SJ, Campbell CD, Artz RRE (2007) Assessing CLPPs using MicroRespTM. A comparison with biolog and multi-SIR. J Soils Sediments 7(6):406–410

Degens BP, Schipper LA, Sparling GP, Duncan LC (2001) Is the microbial community in a soil with reduced catabolic diversity less resistant to stress or disturbance? Soil Biol Biochem 33:1143–1153

Dorigo U, Leboulanger C, Bérard A, Bouchez A, Humbert J-F, Montuelle B (2007) Lotic biofilm community structure and tolerance along a pesticide contamination gradient in a vineyard area. Aquat Microb Ecol 50:91–102

Fierer N, Schimel JP, Holden PA (2003) Influence of drying-rewetting frequency on soil bacterial community structure. Microb Ecol 45:63–71

Gibelin AL, Déqué M (2003) Anthropogenic climate change over the Mediterranean region simulated by a global variable resolution model. Clim Dyn 20:327–339

Griffiths BS, Bonkowski M, Roy J, Ritz K (2001) Functional stability, substrate utilisation and biological indicators of soils following environmental impacts. Appl Soil Ecol 16:49–61

Hamdi S, Chevallier T, Ben Aïssa N, Ben Hammouda M, Gallali T, Chotte JL, Bernoux M (2011) Short-term temperature dependence of heterotrophic soil respiration after one-month of pre-incubation at different temperatures. Soil Biol Biochem 43:1752–1758

IPCC (2007) Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) Cambridge University Press, Cambridge, United Kingdom, and New York, NY, USA, 996 pp

Joergensen RG, Brookes PC, Jenkinson DS (1990) Survival of the soil microbial biomass at elevated temperatures. Soil Biol Biochem 22 (8):1129–1136

Kuzyakov Y (2010) Priming effects: interactions between living and dead organic matter. Soil Biol Biochem 42:1363–1371

Martiny JB, Bohannan BJM, Brown JH, Colwell RK, Fuhrman JA, Green JL, Horner-Devine MC, Kane M, Krumins JA, Kuske CR, Morin PJ, Naeem S, Øvreås L, Reysenbach A, Smith VH, Staley JT (2006) Microbial biogeography: putting microorganisms on the map. Nature 4:102–112

Pettersson M, Baath E (2003) The rate of change of a soil bacterial community after liming as a function of temperature. Microb Ecol 46:177–186

Philippot L, Cregut M, Cheneby D, Bressan M, Dequiet Martin-Laurent F, Ranjard L, Lemanceau P (2008) Effect of primary mild stresses on resilience and resistance of the nitrate reducer community to a subsequent severe stress. FEMS Microbiol Let 285:51–57

Ranneklev SB, Baath E (2001) Temperature-driven adaptation of the bacterial community in peat measured by using thymidine and Leucine Incorporation. Appl Environ Microbiol 67:1116–1122

Reynolds HL, Packer A, Bever JD, Clay K (2003) Grassroots ecology: plant-microbe-soil interactions as drivers of plant community structure and dynamics. Ecology 84(9):2281–2291

Saul-Tcherkas V, Steinberger Y (2009) Substrate utilization patterns of desert soil microbial communities in response to xeric and mesic conditions. Soil Biol Biochem 41:1882–1893

Schimel J, Balser TC, Wallenstein M (2007) Microbial stress-response physiology and its implications for ecosystem function. Ecology 88(6):1386–1394

Six J, Frey SD, Thiet RK, Batten KM (2006) Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Sci Soc Am J 70:555–569

Vinebrooke RD, Cottingham KL, Norberg J, Scheffer M, Dodson SI, Maberly SC, Sommer U (2004) Impacts of multiple stressors on biodiversity and ecosystem functioning: the role of species co-tolerance. Oikos 104:451–457

Williams MA (2007) Response of microbial communities to water stress in irrigated and drought-prone tallgrass prairie soils. Soil Biol Biochem 39:2750–2757