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Impact of changing precipitation patterns on the plant-microbial response to rewetting

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Abstract

Water availability governs terrestrial nutrient cycles by impacting the functioning of both plants and of soil microorganisms. The predicted changes in precipitation patterns (i.e. the magnitude and frequency of precipitation events) associated with climate change, will thus likely have important consequences on ecosystem functioning. Dry and seasonally dry ecosystems are particularly vulnerable to changes in precipitation patterns, as they are already constrained to a large extent by water availability. However, more mesic systems may also experience dry periods that may impact plant-soil functions. In this thesis, experiments in soil-only systems and plant-soil systems were used to gain insight into how the legacy effects of several weeks of exposure to contrasted precipitation patterns set the scene for the rewetting response of the system. First, in an experiment using soil-only mesocosms, we evaluated the effects of contrasting precipitation regimes on the actively growing as well as the inactive bacterial and fungal communities 2 and 5 days after rewetting, using an ^{18}O-SIP (stable isotope probing) approach by applying $H_2^{18}O$ followed by metagenomics targeting soil bacteria and fungi. Second, we performed two separate and complementary experiments using plant-soil mesocosms with wheat plant cover. The first plant-soil experiment focused on soil depth. It determined the effects of contrasting precipitation patterns on the flux of C from plants to microbes and the microbial response to rewetting at different soil depths, using a heavy isotope tracer approach (^{13}C-CO_2) and ^{18}O-SIP with metagenomics respectively. The second plant-soil experiment evaluated the effects of a history of contrasting precipitation patterns on the dynamics of the rewetting response of the plant-soil system over time (over 29 hours post-rewetting). In addition, two levels of N inputs allowed to determine how N availability modulated plant-soil responses. The response of the potentially active soil bacterial and fungal communities to rewetting was assessed using targeted metagenomics. The responses of

biogeochemical cycles were evaluated using heavy isotope tracers (^{13}C-CO_2 and ^{15}N-NO_3^-) to quantify C flux from plants to soil microorganisms and plant-microbial competition for N over time post-rewetting.

We found that precipitation patterns shaped plant morphology and physiology, microbial community composition as well as soil N cycling in our systems, which set contrasting scenes for the rewetting responses in our systems. In particular, infrequent precipitation patterns (cycles of longer dry periods followed by larger magnitude rain events) resulted in increased microbial N transformation potentials and smaller inorganic N pools. The rewetting responses were determined by evaluating C dynamics (plant-microbial coupling and soil CO_2 efflux rate), N dynamics (plant-microbial competition for N and soil N_2O efflux rate) and microbial dynamics (composition of active and potentially active bacterial and fungal communities after rewetting). First, we found that plant-microbial coupling (i.e the microbial assimilation of C from fresh photosynthate) may be reduced under more infrequent precipitation patterns, especially near the soil surface, and under conditions of low N availability. Our findings also suggest that whilst in soil-only systems, dead microbial cells appear to be a major source fuelling soil CO_2 efflux pulse upon rewetting, in plant-soil systems root respiration plays an important role in the magnitude of the CO_2 efflux upon rewetting. Second, concerning soil N dynamics, we found, in concurrence with previous studies, that soil microorganisms were the stronger competitor for N over short time scales, likely due to their overall fast response rates and high affinity for substrate, whilst plants outcompeted soil microbes for soil N assimilation, over longer time scales likely taking advantage of the fast microbial turnover. In addition, a history of plant-favourable conditions, resulting in larger plant biomass, significantly enhanced the overall competitiveness of plants for soil inorganic N upon rewetting. Third, our findings strengthen the existing theory of contrasting water-related strategies between bacteria and fungi. We showed that infrequent precipitation patterns increased bacterial dominance over

fungi, thereby highlighting the potential consequences this may have for food web stability. The active bacterial response to rewetting was driven by a few phylogenetically clustered operational taxonomic units (OTU) which responded similarly over time and along the soil profile, regardless of precipitation pattern history. Contrastingly, the active fungal response was delayed, with no significant response for up to 5 days post-rewetting, regardless of precipitation pattern history. The evenness of the active fungal community decreased with depth, suggesting that fungal activity may be shaped more by the availability of plant derived C than water. Finally, the impact of infrequent precipitation patterns on the composition and evenness of the soil microbial community which was inactive upon rewetting (i.e. the microbial seed bank) could indicate a loss of functional potential under changing environmental conditions, with consequences for future ecosystem processes. Furthermore, surface soils were the most vulnerable to changes in precipitation pattern, with infrequent precipitation patterns leading to reduced bacterial but increased fungal evenness, whilst communities in deeper soil horizons were left unaffected. These findings highlight the need for considering the whole soil profile when relating soil microbial communities and ecosystem processes.

In conclusion, our results suggest that due to effects on plant function, contrasting bacterial and fungal water-related strategies as well as soil C and N dynamic responses, shifts in precipitation patterns, even under temperate conditions, will likely have important consequences for ecosystem processes. Based on this work, we propose that biophysical aspects of microbial ecology, activity- as well as trait-based approaches in future research to further advance our understanding of the links between soil microbial communities and ecosystem processes.

Résumé

La disponibilité en eau exerce un contrôle majeur sur les cycles des nutriments terrestres, à travers ses impacts sur le fonctionnement des plantes et des microorganismes du sol. Les changements de magnitude et de fréquence des épisodes de pluie (c'est-à-dire les régimes de précipitations) prédits par les modèles et associés au changement climatique vont ainsi avoir des conséquences importantes sur le fonctionnement des écosystèmes. Les écosystèmes arides et semi-arides sont particulièrement vulnérables à des changements de régime de précipitations, car ils sont déjà contraints par la disponibilité en eau. Cependant, des systèmes plus tempérés peuvent aussi être soumis à des périodes sèches qui peuvent affecter le fonctionnement plante-sol. Dans la présente thèse, les effets d'un historique de régimes de précipitations contrastés ont été étudiés dans des systèmes sol seul et plante-sol, afin de déterminer dans quelle mesure plusieurs semaines de régime hydrique peuvent moduler la réponse des écosystèmes à une réhumectation lors d'un événement pluvieux important. Premièrement, nous avons évalué les effets de régimes de précipitations contrastés dans des mésocosmes de sol seul, sur les communautés bactériennes et fongiques actives et inactives dans le sol, 2 et 5 jours après réhumectation. Nous avons employé une approche de ^{18}O-SIP (stable isotope probing), en réhumectant le sol avec $H_2^{18}O$ puis en utilisant la métagénomique ciblée sur les bactéries et champignons du sol. Deuxièmement, nous avons mis en place deux expériences séparées en mésocosmes plante-sol avec couvert de blé. La première expérience sol-plante s'est intéressée à la profondeur de sol. Nous avons évalué les effets de régimes de précipitations contrastés sur le flux de C depuis les plantes vers les microorganismes du sol ainsi que la réponse des microorganismes à différentes profondeurs de sol (de 0 à 35 cm) en utilisant des approches de traceur isotopiques stables (^{13}C-CO_2) et ^{18}O-SIP, respectivement. La deuxième expérience plante-sol a évalué les effets de régimes de précipitations contrastés sur la dynamique temporelle (durant 29h) de la réponse du système plante-sol à la réhumectation. En outre, deux

niveaux de fertilisation azotée ont permis de déterminer l'éventuelle modulation de la réponse par la disponibilité en N dans le sol. La réponse des communautés bactériennes et fongiques potentiellement actives dans le sol a été évaluée par métagénomique ciblée. La réponse de cycles biogéochimiques a été évaluée à l'aide de traceurs isotopiques stables (^{13}C-CO_2 et ^{15}N-NO_3^-) pour quantifier le flux de C des plantes vers les microorganismes du sol et déterminer la compétition plantes-microorganismes du sol au cours du temps après réhumectation.

Nos résultats ont montré un contrôle du régime de précipitation sur la morphologie et physiologie des plantes, les communautés microbiennes du sol ainsi que sur le cycle de l'azote du sol dans nos systèmes. En particulier, des régimes de précipitations peu fréquentes (cycles de périodes sèches longues suivies de périodes de pluie plus importantes) se sont traduits par une augmentation des potentiels de transformation de l'azote dans le sol et une réduction des stocks d'azote minéral dans le sol. Ceci a façonné l'environnement de la réponse de nos systèmes à la réhumectation, que nous avons évaluée en déterminant les dynamiques du C (couplage plantes-microbes et émissions de CO_2 du sol), de l'azote du sol (compétition plantes-microorganismes du sol pour le N et émissions de N_2O) et de la composition des communautés microbiennes du sol (bactéries et champignons actifs et potentiellement actifs) après réhumectation. Tout d'abord, nous avons montré que le couplage plante-microorganismes (c'est-à-dire l'immobilisation microbienne de C organique provenant de photosynthats récents) pouvait être réduite en régime de précipitations moins fréquentes, en particulier dans les couches de sol les plus superficielles, et en conditions de faible disponibilité en N dans le sol. Nos résultats suggèrent également que dans les systèmes sans plante, les cellules microbiennes mortes sont un des substrats principaux du flux de CO_2 émis par le sol après réhumectation, tandis que dans les systèmes plante-sol, la respiration racinaire joue un rôle majeur dans l'amplitude de ce flux. Deuxièmement, concernant la dynamique du N dans le sol, nous avons mis en évidence, en concordance avec des études précédentes, que les microorganismes du sol

étaient de meilleurs compétiteurs à court terme pour le N que les plantes, probablement en lien avec leur réponse généralement rapide aux changements environnementaux et leur forte affinité pour le substrat, tandis que ces dernières profitaient vraisemblablement du turnover microbien rapide pour surpasser les microorganismes du sol sur des pas de temps plus longs. Par ailleurs, nos résultats montrent qu'un historique de conditions environnementales favorables à la croissance des plantes a stimulé la compétitivité de celles-ci pour l'azote du sol par rapport aux microorganismes du sol. Troisièmement, nos résultats renforcent la théorie existante de stratégies contrastées entre les bactéries et les champignons du sol par rapport aux conditions hydriques environnementales. Nous avons montré que des régimes de précipitations moins fréquentes sont susceptibles d'augmenter la dominance des bactéries par rapport aux champignons du sol, avec des conséquences potentielles pour la stabilité du réseau trophique édaphique. La réponse des bactéries actives du sol à la réhumectation était menée par quelques unités taxonomiques opérationnelles (OTU), qui présentaient une réponse similaire dans le temps et le long du profil de sol, indépendamment de l'historique de régime de précipitations. En revanche, la réponse des champignons actifs était décalée dans le temps, avec une absence de réponse jusqu'à cinq jours après réhumectation, indépendamment de l'historique de régime de précipitations. L'équité de la communauté fongique active a diminué avec la profondeur de sol, suggérant son contrôle plus par disponibilité en C labile que par la disponibilité en eau. En outre, les effets de régimes de précipitations moins fréquentes sur la composition et l'équité de la communauté microbienne du sol qui était inactive lors de la réhumectation (c'est-à-dire la banque de graines microbiennes du sol) pourraient indiquer une perte de potentiel fonctionnel lors de modifications de conditions environnementales, avec des conséquences négatives pour les processus écosystémiques dans des conditions futures. En outre, nos travaux ont montré que les microorganismes des couches les plus superficielles du sol étaient les plus vulnérables à des changements de régimes de précipitations, des régimes de précipitations moins fréquentes

entraînant une diminution de l'équité de la communauté bactérienne mais une augmentation de celle de la communauté fongique, tandis que les communautés plus profondes n'étaient pas affectées. Ces résultats soulignent l'importance de considérer le profil de sol dans sa totalité pour relier les communautés microbiennes du sol aux processus écosystémiques.

En conclusion, nos résultats suggèrent que, même en conditions tempérées, des changements de régimes de précipitations, à travers leurs effets sur le fonctionnement de la plante, sur les stratégies contrastées entre les bactéries et les champignons du sol ainsi que sur les réponses dynamiques du C et de l'azote du sol, seront susceptibles d'avoir des conséquences importantes pour les processus écosystémiques. A la suite de ce travail, nous proposons que des approches incluant les aspects biophysiques de l'écologie microbienne, l'activité et les traits des microorganismes soient utilisées dans les recherches futures afin de poursuivre les avancées dans la compréhension des relations entre communautés microbiennes du sol et fonctionnement des écosystèmes.

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List of publications

Chapter I: Engelhardt IC, Blazewicz SJ, Firestone MK, Barnard RL. Impact of contrasting moisture regimes on actively growing and inactive microbial communities is sustained up to 5 days after rewetting (Short communication: in preparation).

Chapter II: Engelhardt IC, Welty A, Blazewicz SJ, Bru D, Rouard N, Breuil MC, Gessler A, Galiano L, Miranda JC, Spor A and Barnard RL (2018) Depth matters: effects of precipitation regime on soil microbial activity upon rewetting of a plant-soil system, The ISME Journal. doi: 10.1038/s41396-018-0079-z.

Chapter III: Engelhardt IC, Niklaus PA, Bizouard F, Breuil MC, Bru D, Deau F, Mounier A, Philippot L, Barnard RL. Contrasting water and N-availability patterns have a legacy effect on plant-microbial response to rewetting and biogeochemical cycling (in preparation).

Abbreviations

IPCC	Intergovernmental Panel on Climate Change
C	Carbon
CO_2	Carbon dioxide
$^{13}C\text{-}CO_2$	Heavy carbon isotope labelled carbon dioxide
N	Nitrogen
NH_4^+	Ammonium
NO_3^-	Nitrate
NO	Nitric oxide
N_2O	Nitrous oxide
N_2	Di-nitrogen
$^{18}O\text{-}H_2O$	Heavy oxygen isotope labelled water
SOM	Soil organic matter
OM	Organic matter

1. General introduction

1.1 Climate change predictions: Precipitation patterns

Climate models predict that precipitation patterns will shift towards prolonged periods of drought followed by larger magnitude rain events (IPCC 2007). Ecosystems with strong seasonal precipitation such as those with a Mediterranean climate (reviewed by Giorgi et al., 2008, Barnard et al., 2015) or in dry ecosystems which are already frequently water-constrained such as those with hyper-arid, arid or even semi-arid climate (reviewed by Miranda et al., 2011) are likely the most sensitive to changes in the timing and magnitude of rain events. As 41% of all terrestrial ecosystems are currently classified as arid or semi-arid (IPCC 2007, Mortimore et al., 2009) this could have wide-reaching consequences for global ecosystem services. Additionally, the shift in precipitation patterns is likely to significantly increase the percentage of arid and semi-arid ecosystems (IPCC 2007, Feng and Fu, 2013, Fig. 1). The magnitude of expansion of semi-arid regions is in the range of 4-7% when comparing a 15-year time frame between 1948 and 1962 to a 15-year time frame between 1990 and 2004 (Feng and Fu, 2013, Huan et al., 2016).

Water availability, especially in these dry and seasonally dry ecosystems frequently constrains terrestrial nutrient cycles, due to the impact soil moisture has on plants and soil microorganisms, the key drivers of biogeochemical cycles. This study will focus on the effect of changing precipitation patterns on the carbon (C) and the nitrogen (N) cycle, which are not only tightly coupled to water availability but also to each other. Furthermore, imbalances in C and N budgets may negatively contribute towards the progression of climate change.
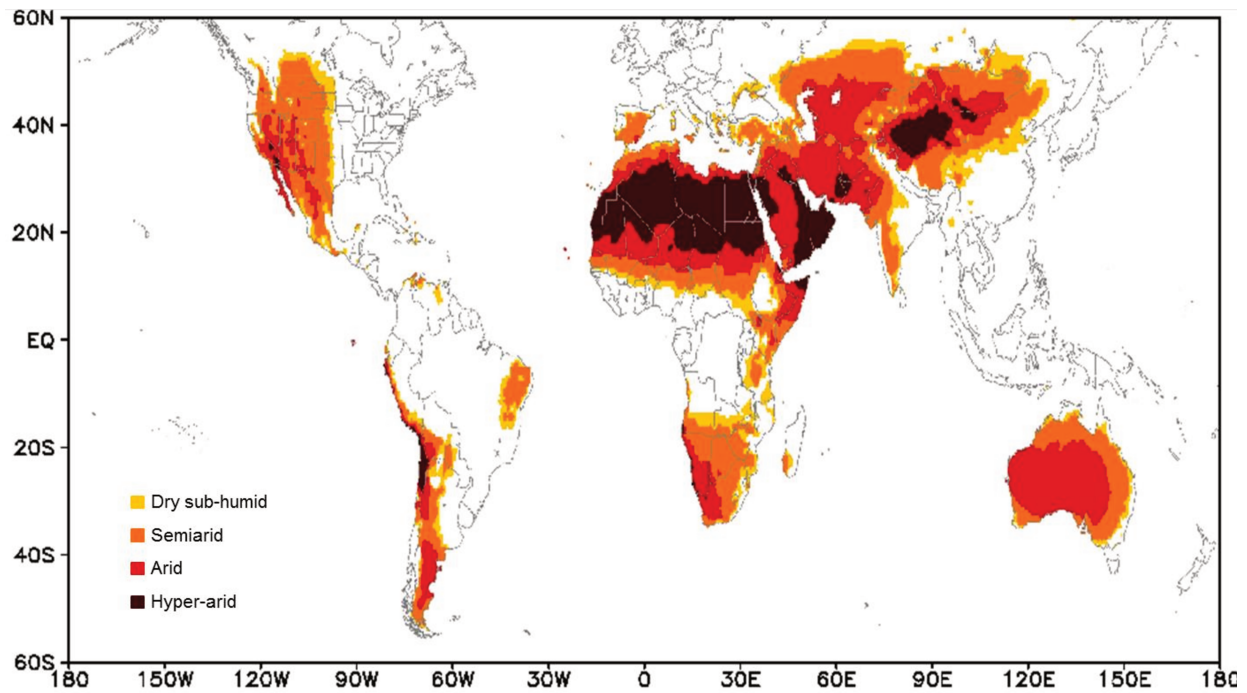
Fig. 1. Global distribution of drylands (dry sub-humid, semi-arid, arid and hyper-arid) for 1961–1990. Climatology is derived from observations of the ratio between precipitation and potential evapotranspiration (P/PET ratio). Image source: Feng and Fu, 2013.

1.2 Effects of changing precipitation patterns on ecosystem biogeochemical cycling

1.2.1 Carbon balance

CO_2 release into the atmosphere from anthropogenic sources has increased dramatically since the first industrial revolution in 1750 (Fig. 2). Since CO_2 is a potent greenhouse gas, this has been the main driver of the increasing earth surface temperatures (IPCC 2007). CO_2 is removed from the atmosphere by autotrophic plants and microbes and stored as soil organic matter (SOM) by the soil microbial communities acting as a continental ecosystem C-sink. Microbial SOM decomposition, microbial and plant respiration returns the C as CO_2 back to the

atmosphere (ecosystem C-source). Studies have shown that the elevated concentrations of CO_2 in the atmosphere may stimulate photosynthesis and growth in some plants (Vu et al., 1997, Thinh et al., 2017) but continuously rising atmospheric CO_2 levels suggest that this is not sufficient to counter the imbalances caused by anthropogenic input, particularly since deforestation is also a wide-spread phenomenon.

Furthermore, as the C-cycle is tightly coupled to water availability, the predicted changes in precipitation patterns may unbalance these processes even more. Upon rewetting of a dry soil, a large pulse of CO_2 is released, which has been termed the "Birch effect" in acknowledgement of the pioneering work of H. F. Birch in 1958 (Birch 1958). The Birch effect accounts for a significant amount of the annual CO_2 lost from arid, semi-arid and seasonally dry (Mediterranean) ecosystems (Schimel et al., 2007, Wang et al., 2015). Biotic (root respiration, microbial respiration and rapid organic matter turnover) as well as abiotic (desorption of accumulated C from soil surfaces, replacement of pore-space CO_2 by water) origins for the CO_2 flux have been described but the exact origins and mechanisms underlying this phenomenon are yet to be fully understood. The predicted changes in the global precipitation patterns have been shown to amplify the Birch effect which may thus have a strong impact on the ecosystem C balance. This includes the extent of the dry period preceding the rewetting event (Xiang et al., 2008) as well as both the frequency (Fierer and Schimel, 2002) and the magnitude (Lado-Monserrat et al., 2014) of precipitation events. As soil respiration accounts for the second largest flux of C between ecosystems and the atmosphere (Schimel et al., 1996), a more complete understanding of the mechanisms underlying this phenomenon is vital.
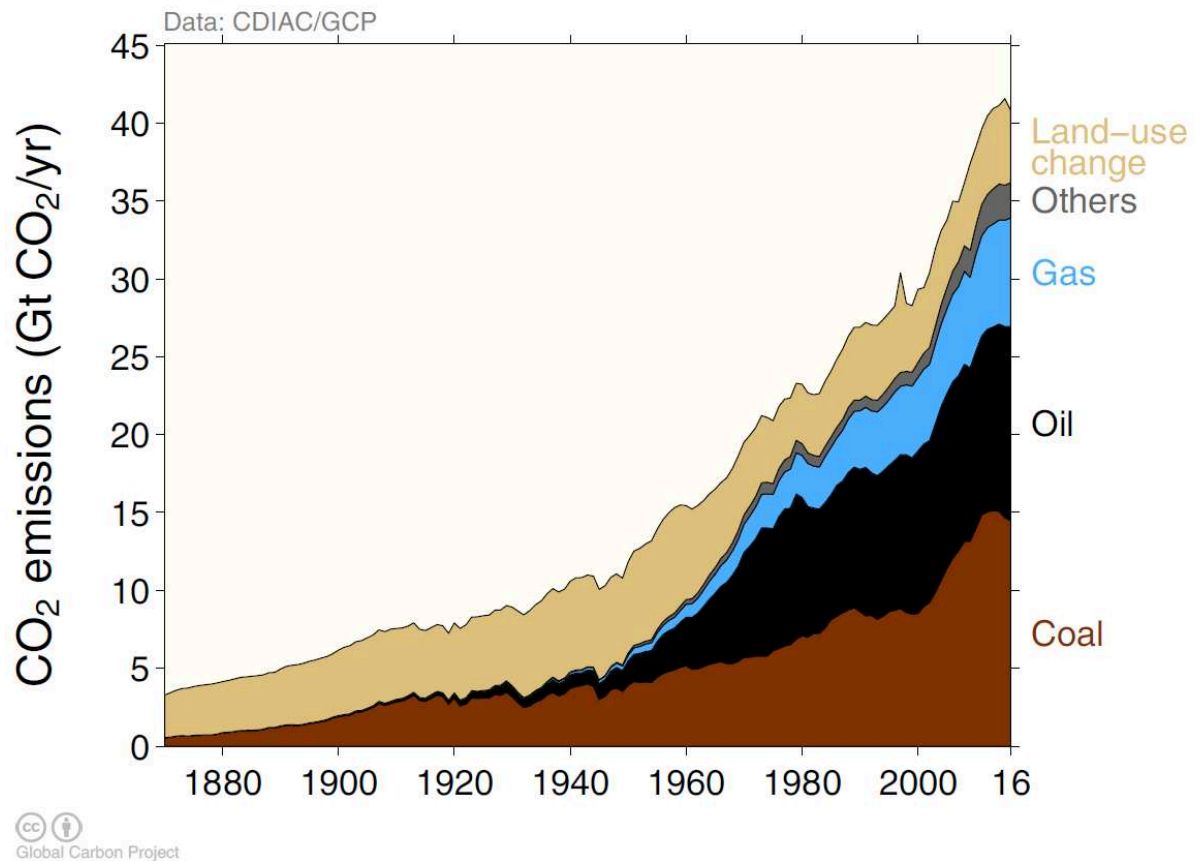
Fig 2. Global CO$_2$ emissions by source in gigatones per year. Others include emissions from cement production and gas flaring. Image source: Global Carbon Budget 2017.

1.2.2 Nitrogen balance

Rewetting of dry soils also releases a flux of N$_2$O, a potent greenhouse gas with a global warming potential 298 times that of CO$_2$ over a 100-year time period (Bates et al., 2008). N$_2$O is also currently the dominant ozone-depleting substance in the atmosphere (Ravishankara et al., 2009). N$_2$O is released into the atmosphere as a result of microbial N transformations, either as an end-product (denitrification) or as an intermediate by-product (DNRA, nitrification). As N is an essential and frequently limiting nutrient for plant growth in most terrestrial ecosystems, large amounts of industrially manufactured inorganic N is applied globally to crops, to keep up

with increased global demand. Indeed, since the development of the Haber-Bosch process of industrial nitrogen fixation in 1913, the anthropogenic N deposition into terrestrial ecosystems has increased dramatically (Galloway et al., 2002). The advantage of being able to industrially fix and apply plant-available inorganic N is that crop productivity has increased substantially. However, it is estimated that only about half of the applied fertilizer globally ends up being used by plants whilst the rest is used by soil microbes, lost in the form of trace gases to the atmosphere or lost through leaching of NO_3^- into aquatic systems (Galloway et al., 2004).

The predicted more intense precipitation patterns will likely further impact terrestrial N budgets by favouring one microbial N transformation process over another, governing microbial activity as well as access to substrate and altering the supply and demand of N between microbes and plants. Furthermore, larger magnitude rewetting events, particularly following extended dry periods are linked to increased run-off and thus the risk of N loss and contamination of groundwater and estuaries, but leaching was not tackled in the context of the present work.

The interactive effect of changing precipitation patterns and increased inorganic N availability on overall ecosystem health and functioning is frequently highlighted but as yet is only poorly understood. On one hand, research suggests that on top of being essential to meet production demand of crops, high N availability may additionally improve plant resistance to drought and thus might support plant survival and productivity under the predicted increased variability in precipitation patterns (Yang et al., 2012, Abid et al., 2016). On the other hand, high inorganic N application has also been linked to a loss of C storage (Malik et al., 2016) and N retention capability of ecosystems due to a relative loss of fungal food webs (Van Diepen et al., 2017).

1.3 Soil microbes and soil moisture

1.3.1 Cycles of drying and rewetting: How microbes survive, thrive and adapt

Drying and rewetting of soil results in contrasting environments for soil microbial communities and their response to these changes is a frequently studied topic (Fierer et al., 2003, Bapiri et al., 2010, Evans and Wallenstein, 2012, Barnard et al., 2015, Averill et al., 2016). Fluctuations in soil moisture not only leads to contrasting osmotic potentials which the cells have to adjust to, but also changes in soil aeration status and nutrient availability.

Dry soils are characterized by conditions of increasing substrate limitation due to spatial isolation which is brought on by the discontinuity of soil water films (Schimel and Bennett, 2004, Or et al., 2007, Moyano et al., 2012). Additionally, as microbes have semi-permeable membranes and live in close contact (fungi) or within (bacteria) water films, they need to prevent cellular dehydration when the osmotic potential of the soil increases. Different microbes may use different mechanisms of osmoregulation and may also show varied tolerance levels (reviewed by Borken and Matzner, 2009). Typically, this includes intracellular accumulation of osmolytes (Bonaterra et al., 2005) or secretion of protective mucilage (Chenu and Roberson, 1996; Schimel et al., 2007), both of which are expensive in terms of energy and C, at a time of increasing nutrient limitation. Eventually some microbes may take on resistant forms such as cysts and spores, in order to survive extended periods of intense drought, especially once nutrients become severely limited.

The rewetting of dried soils is characterised by a very acute change in osmotic potential which can rupture microbial cells if they are not able to regulate in time by pumping out or metabolising the intracellular osmolytes. However, this is also a time when microbial activity flourishes, and extremely rapidly (Fierer et al., 2003, Iovieno and Bååth, 2008, Placella et al., 2012; Barnard et al., 2013). Soil microbial response to rewetting is gaining a lot of attention at

the community level (Placella et al., 2012) as well as the ecosystem scale, commonly measured by the flux of CO_2 released from the soil (Birch effect). Microbes which are able to respond quickly can take advantage of the abundance of accumulated and due to continuity of water films now accessible labile nutrients (Griffiths and Philippot, 2013). Different water-related microbial strategies have come to light, depending on whether microbes track soil water availability and gain advantage by their fast response, or they resist the dry conditions and remain well-established (Placella et al., 2012, Barnard et al., 2013).

Repeated cycles of drying and rewetting thus cause a shift in the composition of soil microbial communities towards those which are more resistant and more resilient and thus adapted to these conditions (Owens et al., 2012; Sistla and Schimel, 2012). Microbes which are able to withstand longer drying and are able to rapidly respond to improved nutrient accessibility will flourish over more sensitive or slow-growing organisms. Fungi in general have been described as more resistant to desiccation than bacteria, due to their ability to access more wide-reaching pore spaces in search of water, with their extensive hyphal network. An exception to this are bacteria belonging to the phylum Actinomycetes, which have been described as exhibiting fungal-like growth with a high tolerance to drought (de Boer et al., 2005). Additionally, Gram positive bacteria such as Actinomycetes have a thick peptidoglycan cell wall which may also convey increased resistance to drying and rewetting, but this is expensive in terms of C, N and energy (Schimel et al., 2007, Manzoni et al., 2012, Fuchslueger et al., 2016). On the other hand, though, bacteria generally have faster response and growth rates than fungi, which allows them to flourish when conditions become more favourable. This is particularly true for the typically fast-growing but drought-sensitive Gram-negative bacteria (Steenwerth et al., 2005). Fungi and bacteria also have different preferences for osmolytes, as bacteria tend to accumulate nitrogenous osmolytes such as amino acids or amines whilst fungi use C-rich sugar alcohols (Csonka 1989, Boot et al., 2013). Thus, the availability of suitable osmolytes can also influence

the resistance of different domains. Shifts in the microbial composition, particularly in the fungal:bacterial ratio can modify the functional potentials of the communities (Evans and Wallenstein, 2012) and thus have consequences for ecosystem function (Lennon and Jones 2011, Wallenstein and Hall, 2012). Increased fungal dominance has been linked to a higher C storage potential (Malik et al., 2016) of the soil as well as an improved N retention within the food web. However, no clear consensus has been reached on the impact of repeated cycles of drying and rewetting on the relative proportion of fungi and bacteria in the soil. Contrasting results have been reported and are predominantly due to the length of the cycles, the number of cycles and the ecosystem type.

1.3.2 Microbial N transformations

All living cells require N for the synthesis of vital bioorganic molecules, including proteins and nucleic acids. However, even though 80% of the atmosphere is di-nitrogen (N_2), this gas is inert and therefore inaccessible to most living organisms, which are thus dependent on bioavailable forms of N such as ammonium (NH_4^+), nitrate (NO_3^-) or amino acids. Soil microbes drive N transformations in soil, including processes which make N bioavailable (Fig. 3). N transformations in soil are tightly coupled with moisture availability, not only due to the impact on microbial activity and access to substrate but also by determining the aeration status of the soil, resulting in conditions which may favour one process over another.

Biological N-fixation is the conversion of biologically unavailable N_2 gas to biologically available ammonium by a group of specialized prokaryotes which carry the nifH gene cluster. Most commonly studied N-fixing bacteria live in symbiotic relationships with plants, but free-living N-fixers have been shown to significantly contribute to N-budgets of soils (Zhan et al., 2012). N-fixation is limited to bacteria and archaea, but within these groups there is

considerable phylogenetic diversity (Young 1992) which includes diverse physiologies, including heterotrophs, phototrophs and chemolithotrophs with varying O_2 requirements. Another biologically-mediated pathway in which N is released as NH_4^+ or small bioavailable N-containing compounds (e.g. amino acids), is the microbial decomposition or mineralisation of organic matter by a sequence of extracellular and intracellular enzymatic reactions. N mineralisation rates are generally highest in moist but well-aerated soils and is performed by a large and diverse group of prokaryotes and fungi. However, the decomposition of recalcitrant organic matter such as lignin (de Boer et al., 2006) or compounds released from senescent roots (Hegde and Fletcher, 1996) is generally considered a fungal niche. The resulting NH_4^+ in soil is bioavailable for plant assimilation and microbial immobilisation.

NH_3 is the substrate for microbial nitrification, long-believed to be a 2-step oxidation reaction, ammonia oxidation to nitrite then nitrite oxidation to NO_3^-, which is performed by separate, specialized groups of chemolithoautotrophs to obtain energy for growth. Ammonia oxidation can be performed by bacteria or archaea (AOB and AOA) whilst nitrate oxidation is performed only by bacteria (NOB). Additionally, recent discoveries have confirmed the presence of bacteria (Nitrospira species) which are capable of both oxidation reactions (Daims et al., 2015, van Kessel et al., 2015) by a process known as "Complete ammonia oxidation" (Comammox). Costa et al. (2006) first coined the term when they hypothesized that such a complete oxidation would be more energetically efficient and beneficial particularly in resource-limited environments where growth yield is more important than growth rate. Nitrification is an aerobic process, and thus aeration of the soil is vital, and this process is reduced or even inhibited in flooded soils. Additionally, N_2O may be produced as a by-product of nitrification and the amount of N_2O produced is positively correlated with soil water content (Smith et al., 2003). However, even though nitrification is an aerobic process which is favoured in dryer, well aerated soils, there is a threshold after which soils are too dry and nitrifier activity decreases

due to diffusional limitations and thus lack of access to substrate (Stark and Firestone, 1995). Like NH_4^+, the resulting NO_3^- is available to both plant and microbes for growth and function, but unlike NH_4^+, NO_3^- does not have an affinity for binding to soil particles and is thus more mobile within the soil matrix which increases the risk of N loss from the system through leaching.

NO_3^- is also the substrate for denitrification, an alternative anaerobic respiratory pathway in which nitrogen oxides are used as electron receptors instead of O_2. Nitrate is sequentially reduced to greenhouse gases, nitric oxide (NO) and nitrous oxide (N_2O) and inert N_2. Denitrification can be performed by a highly diverse group of bacteria, archaea and fungi, using organic C as an energy source. N_2O can be released as an intermediate or as an end-product as it has been demonstrated that not all microorganisms which are capable of denitrification harbour the gene encoding for nitrous oxide reductase (Wood et al., 2001). In general, denitrification rates are higher under anaerobic conditions or very low oxygen conditions, such as water saturation, and when there is an abundance of readily available C (reviewed by Philippot et al., 2009). A wide range of environmental factors, including the extent of water saturation, structure of the soil and soil depth (reviewed by Smith et al., 2003) have been implicated in driving the $N_2O:N_2$ ratio of gas emissions from soils. In short, any situation in which the N_2O molecule can diffuse readily from an anaerobic into an aerobic site increases the chance of it being released into the atmosphere rather than being further reduced to N_2. Additionally, recent studies have shown the denitrifier diversity as playing a role in determining how much N_2O is produced relative to N_2 (Philippot et al., 2011; Jones et al., 2014).

Finally, the dissimilatory reduction of NO_3^- to NH_4^+ (DNRA) is another important N transformation which is generally considered a process which aids to retain N within the ecosystem. Evidence suggests that a wide range of bacteria and fungi are able to perform

DNRA and many of them may produce N_2O as a by-product of this process. DNRA is generally an anaerobic process but the ratio of NO_3^- substrate to C in the soil has been suggested to be more influential in driving this process than the O_2 concentrations (reviewed by Rütting et al., 2011). The importance of DNRA across ecosystems is still unclear.
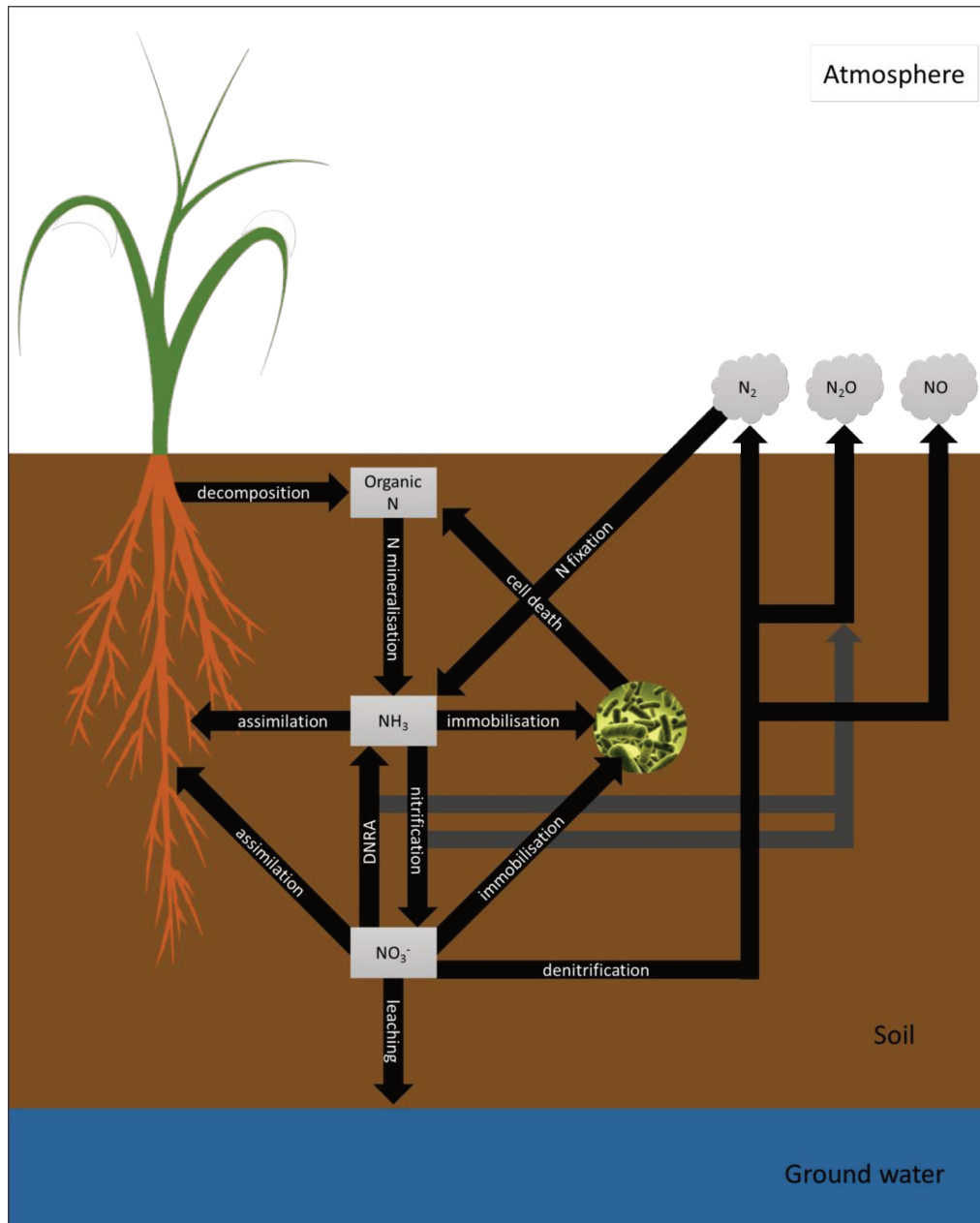
Fig. 3. Schematic outline of the terrestrial N cycle, including microbial-driven transformations (N-fixation, OM mineralisation, nitrification, denitrification and DNRA), N uptake into plant and microbial biomass (assimilation and immobilisation), N re-entering the cycle as dead OM (plant and microbial cell death) and N losses from the system as gas (N_2, NO or N_2O) or through leaching. Microbial picture credit: https://www.gettyimages.fr/.

1.3.3 Active versus present microbial communities

It has been shown that although a huge diversity of microbes is present in soil, only a fraction of them are active or growing and thus of interest on a functional level (Jones and Lennon 2010, Blagodatskaya and Kuzyakov, 2013, Blazewicz et al., 2014). This is also reinforced by the finding that during the well-documented burst of microbial activity post-rewetting, the present microbial community composition remains relatively unchanged (Fierer et al., 2003, Placella et al., 2012). This is due to the fact that the present microbial communities include not only the actively responding groups but also large amounts of (a) dormant microbes and (b) relic DNA (Fig. 4).

(a) When environmental conditions are unfavourable, microbes enter a state of dormancy, surviving as resting structures with minimal energy expenditure until conditions become more favourable (reviewed by Lennon and Jones, 2011). This creates a large reservoir of functional potential referred to as the microbial seed bank. The seed bank may determine how a microbial community responds to disturbance. Cycles of drying and rewetting creates alternating very contrasting environments which may lead to re-activation of different individuals from the seed bank at different times.

(b) Relic DNA originates from the lysis of dead cells and may persist in the environment for extended periods of time (Nielsen et al., 2007, Levy-Booth et al., 2007, Pietramellara et al., 2009). Especially in complex soil structures such as soil, relic DNA may bind inorganic or organic substances which may slow down its degradation. Aside from representing a pool of genetic information which may be incorporated into the genomes of organisms by transformation, relic DNA can also bias microbial community analysis (Carini et al., 2016). However, contrasting reports of the magnitude of the bias have been reported, even on similar relic DNA pool sizes. Lennon et al. (2017) suggest that the amount of bias created may depend

on how similar the relic pool is to the intact community rather than the amount of relic DNA present.
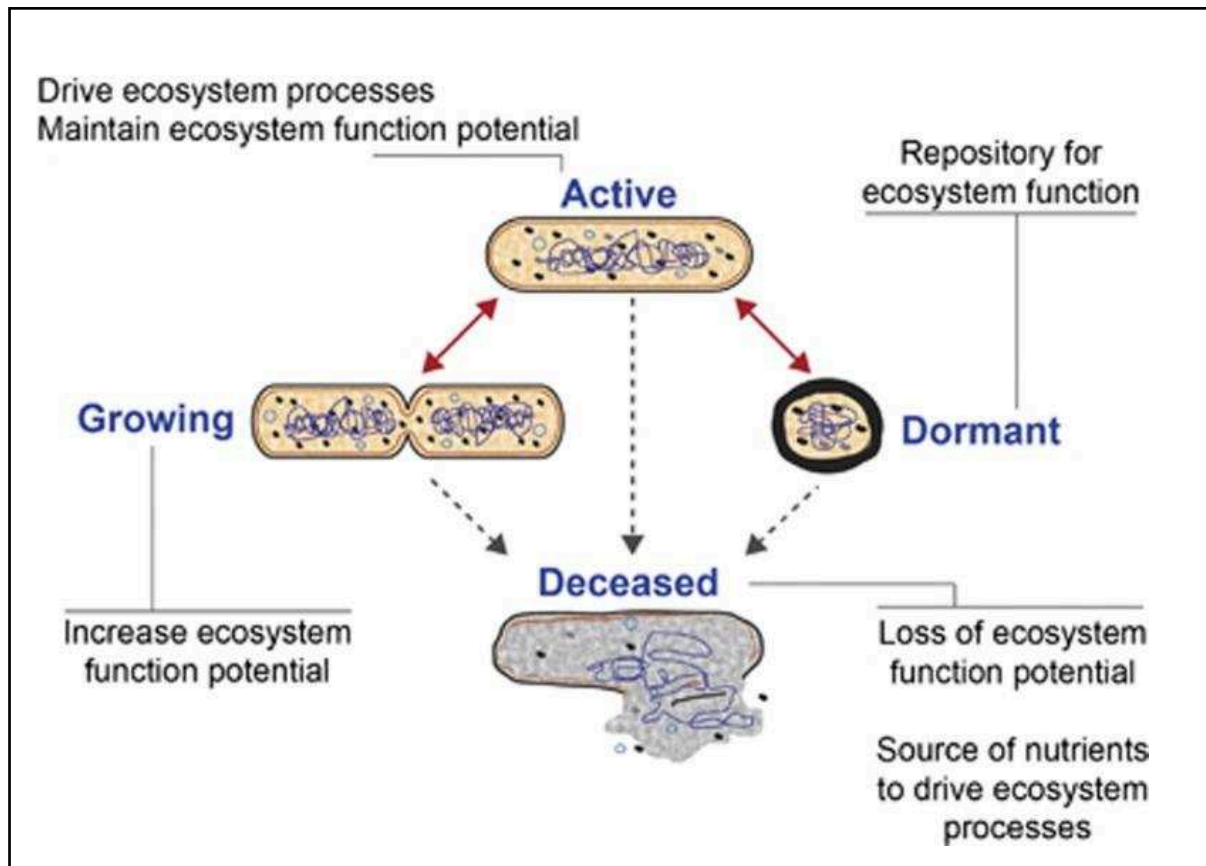
Fig. 4. Schematic representation of the different metabolic states of microbial cells and their contributions to ecosystem functioning. Solid red arrows indicate reversible states and dotted black arrows show irreversible states. Source of image: Blazewicz et al. 2013.

As important as the active microbial fraction in soil is at a given time point, the inactive seed pool likely contains a wide range of functional potential which may become active, often within a very short time frame, once environmental conditions change. During the dynamic changes associated with drying and rewetting of soil systems, it is likely that the communities which are inactive in response to rewetting, may indeed be active and thus functionally significant

during the contrasting conditions associated with the dry period or due to slower activation rates, at a time post-rewetting which is not routinely characterized in rewetting studies.

Advances in the field of molecular biology has opened up a large repertoire of methods which can be applied to describe whole microbial communities, including metagenomics, metatranscriptomics and metaproteomics. Metagenomics is the extraction of the DNA and high throughput sequencing of microbial communities. Metagenomics allows the characterisation of the present microbial community composition but does not discriminate between metabolically active and dormant microbes and can also include significant amounts of relic DNA. Additionally, information may be gained about the functional potential of the communities but not the actual expression. One of the currently available methods to analyse the potentially active microbial communities is metatranscriptomic techniques with high throughput sequencing. The sequencing of total RNA allows the inclusion of both the functionally (mRNA) as well as the taxonomically (rRNA) relevant molecules and thus asses the metabolically active microbial communities (Urich et al., 2008), without the influence of dormant microbes and relic DNA. However, additional post-translational modifications are common, which can be taken into account when using metaproteomics and high-performance mass spectrometry (reviewed by Hettich et al., 2013) to describe metabolic functions of a microbial community at a specific time point.

Another method to differentiate the actively growing from the inactive microbial communities is stable isotope probing (SIP). The environmental sample is incubated with substrate enriched with a heavy isotope, which the actively growing and replicating communities will assimilate into their macromolecules (e.g. nucleic acids). The actively growing and inactive can then be separated based on their different densities, by ultra-centrifugation. The actively growing as

well as the inactive groups can then be characterized taxonomically by molecular methods (Radajewski et al., 2000). ^{18}O water SIP in which heavy labelled water is used as a substrate enables the identification of taxa which respond most strongly to changes in soil moisture (Aanderud and Lennon, 2011) without adding additional nutrients or creating a substrate bias (Schwartz et al., 2007, 2014, 2016).

1.4 Plant strategies to resist water limitation

Plants have evolved a range of morphological and physiological strategies as well as beneficial associations with symbiotic and free-living soil microbes, to resist water limitation. The 3 main morphological strategies of plants to resist water limitation include the structure of their root cell wall, the root architecture and the plasticity of the root:shoot ratio. First, unlike microbial cells, plant roots have protective, impermeable layers which prevent water loss through diffusion gradients in periods of drought. Second, due to extensive belowground root networks, plants have a more wide-ranging access to water. Third, during prolonged periods of drought, plants re-allocate nutrients to increase their root:shoot ratio, thus maximizing root water uptake whilst minimizing water loss from shoots (Poorter et al., 2012, Eziz et al., 2017). The main driver of the ratio change appears to depend on nutrient availability status or plant species and may be due to decreased shoot growth (Skinner and Comas, 2010) or increased root growth (Wedderburn et al., 2010). New roots produced during drought have also been reported to commonly be thinner than roots grown under sufficient water availability, which significantly increases the surface area of the root system without a major increase in overall root biomass (Padilla et al., 2013). However, plants have been shown to not only increase their overall root biomass, but to particularly invest in growth of roots in deeper soil layers in response to more erratic water application (Skinner and Comas, 2010, Wedderburn et al., 2010). Upon rewetting, it has been observed that, when sufficient nutrients are available, plants can overcompensate

aboveground biomass production after a period of drought (Hofer et al., 2017). This could be detrimental during the following drought episode as plants with larger biomass are less drought-resistant than less productive plants (Wang et al., 2007). Thus, the predicted shift towards precipitation patterns characterised by repeated cycles of longer dry periods and larger magnitude rain events is likely to significantly alter plant biomass production.

The main physiological strategy of plants to resist water limitation is through the regulation of the stomatal aperture. Stomatal control varies between plant species and some are thus more drought-tolerant than others (Bartlett et al., 2016). However, as closed stomata reduce the rate of photosynthesis, the plant needs to balance water loss with carbon acquisition to sustain physiological function. During periods of reduced photosynthesis, less labile C may be available to the microbes in the rhizosphere by plant exudation. As labile C from plants fuels microbial decomposition of recalcitrant OM, this could lead to less nutrients being made available to the plants by the microbes (Fontaine et al., 2003, 2004).

Finally, plant resistance to water limitation may also be enhanced through associations with symbiotic (mycorrhiza) and free-living (non-mycorrhizal) microbial communities and activities in the rhizosphere. Mycorrhizal networks further extend the scope of the root network and additionally enable access to small soil pores which the larger plant roots may not be able to penetrate (Egerton-Warburton et al., 2004). Mycorrhizal associations have also been shown to increase stomatal conductance in drought-stressed plants (reviewed by Augé et al., 2014). Free-living microbes may enhance plant resistance to drought stress not only by increasing the availability of nutrients through their biogeochemical cycling but also by producing growth-promoting compounds which improve plant water uptake and conductance by mediating stomatal closure (Brunner et al., 2015).

1.5 Changing precipitation patterns, C and N dynamics

As the supply of essential elements such as C and N is finite, it is crucial that they are constantly recycled within the ecosystem. Not only are the terrestrial C and N cycles intricately linked to water availability, the cycles are also tightly coupled to each other. Even though microbes are drivers of biogeochemical cycles, these are fuelled to a large extent by the labile C input of plants (Wardle and van der Putten, 2002). Plant C acquisition in turn is highly dependent on the availability of various nutrients, such as N, for plant growth and physiological function. The predicted intensification of precipitation patterns thus will likely have far-reaching consequences for C and N balances and ecosystem functioning.

1.5.1 C dynamics

The global SOC pool is about 3x larger than the atmosphere CO_2 pool, with largest SOC pools found in cool wet areas such as peat and permafrost whilst much smaller pools are associated with areas of low mean annual precipitation such as arid ecosystems (reviewed by Gougoulias et al., 2014). The exchange of C from the atmosphere to the soil occurs due to C-fixing autotrophs (photosynthesizing plants and photo- or chemoautotrophic microbes), whilst C is returned to the atmosphere through root and microbial respiration (Fig. 5). The balance between microbial decomposition of OM and stabilisation of fresh C input is largely responsible for the regulation of C budgets within the ecosystem (Malik et al., 2016).
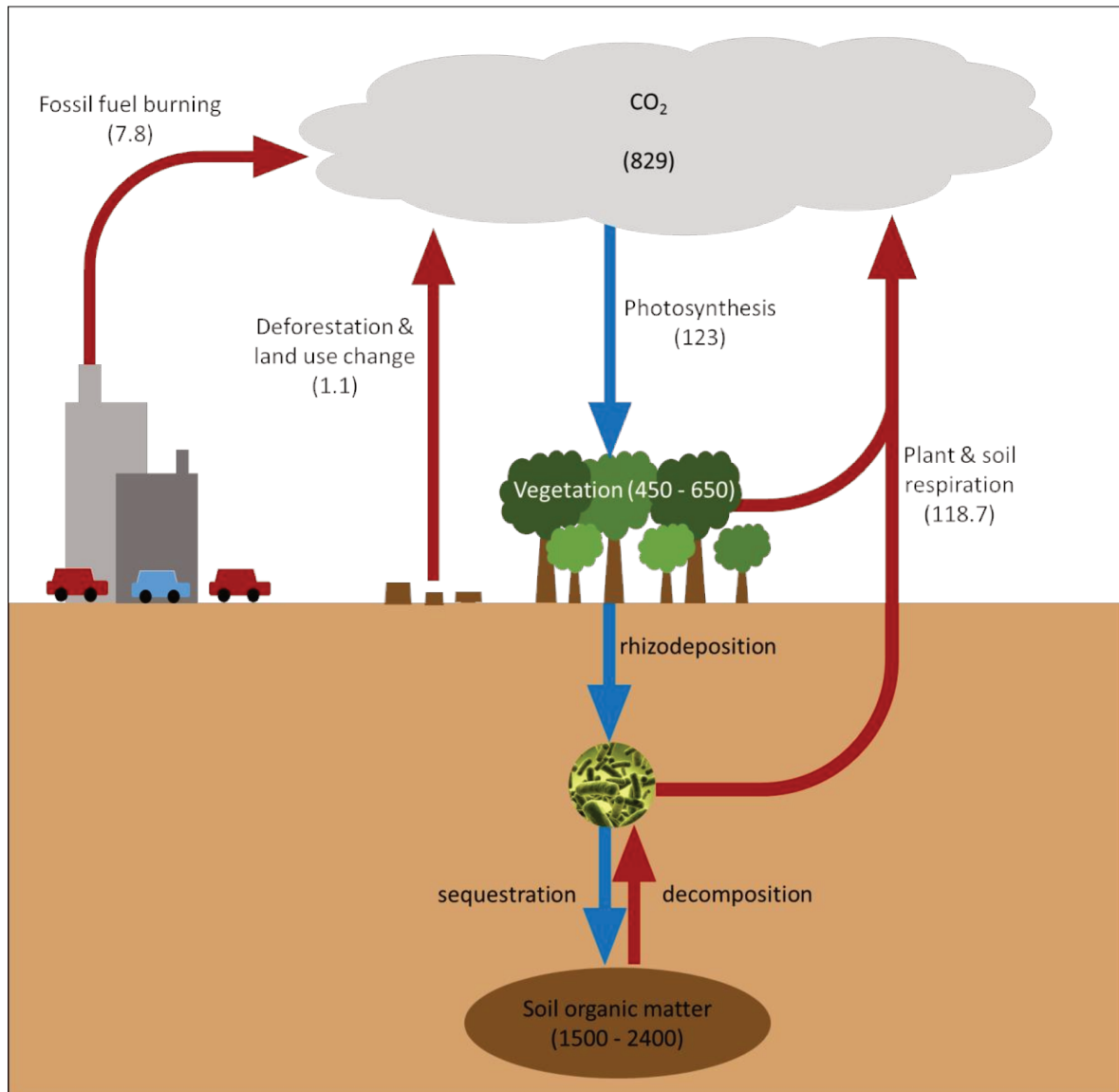
Fig. 5. Simplified schematic of the global terrestrial C cycle. Ecosystem C source fluxes are indicated by red arrows and C sink fluxes with blue arrows. Numbers represent the size of the C pool (SOM, vegetation and atmosphere CO_2) in Pg C and the fluxes in Pg C per year. Values were obtained from chapter 6 of the 5th assessment report of the IPCC 2013 (p. 471). Microbial picture credit: https://www.gettyimages.fr/.

Labile C from plants is assimilated by soil microbes, which may respire the C as CO_2 or partition it for production of more biomass, which will subsequently add to the soil OM pool and has been described as a bank mechanism (Fontaine et al., 2011). Within the soil, organic matter C pools have been divided into 2 categories based on their turnover rates: active pools with a fast turnover time (months) and passive pools with slow turnover time (thousands of years). It was long believed that the molecular structure of the OM was the predominant factor which determined the recalcitrance of the SOC pool. Recent studies however, have brought to the attention that environmental and biological factors may be more instrumental in controlling C turnover in soil (reviewed by Schmidt et al., 2011), leading to current theories that accessibility of the OM and not its molecular structure is the main regulator of decomposition rates (reviewed by Dungait et al., 2012).

Under nutrient-poor conditions, microbes are more likely to decompose SOM to release nutrients, which is amplified when there is a supply of energy-rich C deposit from plant exudation, as recalcitrant SOM decomposition does not yield a great deal of energy (Fontaine et al., 2007). The increased rates of OM decomposition, associated with the input of labile plant derived C, is known as the rhizosphere priming effect (Fontaine et al., 2007, Kuzyakov 2010). It is unclear to what extent the soil microbial community composition impacts the balance between OM decomposition and C sequestration. Indeed, many models predicting the effect of disturbance on ecosystem processes do not consider the soil microbial biomass. C transformations are performed by all soil heterotrophs, which are ubiquitous within the soil. There are however studies which claim that this is an over-simplification of soil processes and thus argue for the inclusion of microbial biomass in ecosystem C models (reviewed by Fontaine et al., 2011). The main aspect of soil microbial community composition which is frequently highlighted as a likely impact on C balance is the fungal to bacterial dominance. Fungal dominance is linked to more stable C balances through their higher C storage potential,

increased OM decomposition potentials and as they are less commonly limited by N. First, fungi have a higher C storage potential as they have a higher C use efficiency (more biomass per unit C used) and form a more recalcitrant necromass (Six et al., 2006, Malik et al., 2016) while bacteria store less of the C they metabolise. Second, fungi play a vital role in the decomposition of the more recalcitrant OM fractions such as lignin (de Boer et al., 2006) and have more far reaching access to secluded OM as they are able to bridge air-filled pores and penetrate solid material with their hyphae (de Boer et al., 2005).

Additionally, aside from a reduction in plant biomass, low N can also lead to a reduction in the photosynthetic rate of plants due to reduced synthesis of the primary CO_2-fixing enzyme, Rubisco in C3 plants (review by Makino 2011). Like for all proteins, N is required for the synthesis of Rubisco, but on top of this, Rubisco has a low rate of catalysis, so a large amount of N needs to be invested. So not only does sufficient N improve plant biomass production and by optimizing photosynthetic rates, enhance the C sink ability of ecosystems, but it has also been suggested that sufficient N may significantly increase the plant's resistance to drought. Indeed, particularly in C3 plants, such as wheat and rice, photosynthetic rates are tightly correlated with leaf N content (Evans 1989).

Currently, at a global scale, the amount of C fixed by ecosystems outweighs the amount of C lost to the atmosphere by respiration, indicating positive net C sequestration on a global scale (reviewed by Gougoulias et al., 2014). However, climate change predictions of not only increased CO_2 and warmer temperature but also shifts in precipitation patterns, may likely impact the terrestrial C cycle (Schimel 2013). Indeed, models predict a shift in terrestrial ecosystems from a net C sink to a net C source within the 21st century (Rayner et al., 2005, Cox et al., 2000).

1.5.2 N dynamics

N is the primary growth limiting nutrient for plants in most terrestrial ecosystems. Plant N assimilation, like microbial N immobilisation, is mostly in the form of inorganic NO_3^- or NH_4^+, made available by the soil microbial N transformations or from fertilizer application. Relatively recently, research on the uptake of organic N by plants has received increased attention and the ability of plants to take up organic N has been well documented in both laboratory as well as field studies (reviewed by Näsholm et al. 2008). However, it is still unclear how much the N from organic sources contributes to overall plant N status. Evidence suggests that inorganic N may be the preferred form of N for plant uptake (Harrison et al., 2007, Ashton et al., 2008). NH_4^+ assimilation by plant roots requires less energy investment than NO_3^-, as it can be incorporated directly into glutamate through the NH_4^+ assimilation pathway. However, NH_4^+ needs to be synthesized into amino acids within the root tissue to prevent accumulation, as NH_4^+ is toxic to plants (Britto et al., 2002). Contrastingly, NO_3^- needs to be reduced first before assimilation, but after assimilation can be directly incorporated into organic compounds in root as well as shoot tissue. Either NH_4^+ or NO_3^- forms can dominate in soils, depending on ecosystem type, but NO_3^- is often more accessible to plants due to its higher mobility. No clear consensus has been reached on the preference of NO_3^- versus NH_4^+ for N uptake by plants, and both plant species as well as environmental factors have been suggested as determinants. Firstly, different plant species may have different preferences for the form of inorganic N and also different levels of tolerance to NH_4^+ toxicity (Britto et al., 2002). Secondly, environmental factors such as water availability and soil pH have been demonstrated to influence plant N uptake (reviewed by Maathuis 2009).

Plants and microbes in terrestrial systems are thus in direct competition for the same inorganic N forms. The intensity of this competition may depend on a variety of factors, among which the most notable are plant morphological traits (including their C:N ratio and its plasticity) and

symbiotic relationships, as well as the C:N ratio of the OM in the soil and the C:N ratio of the soil microbial community.

Different plant species may have different intrinsic N uptake efficiencies and may employ morphological strategies to increase N uptake such as increasing specific root length and fine root production (Cantarel et al., 2014, Moreau et al., 2015). Plants also form symbiotic relationships with soil microbes in which they trade C for other nutrients. One prime example of such symbiosis is the association of N-fixing rhizobia which colonise root nodules of legumes and supply the plant with N in return for C from plant photosynthates. A second example is the wide spread association of plants with mycorrhizal fungi with over 90% of all plant species, forming a mutualistic symbiosis with this heterogenous group of diverse fungal taxa (reviewed by Bonfante and Genre, 2010). Mycorrhizal colonisation of roots can increase plant N uptake through exploration of larger soil volumes by the large hyphal network, the ability of hyphae to access smaller pore spaces than the larger roots and by mineralisation of organic N. Especially arbuscular mycorrhizal fungi (AMF) have been shown to be highly efficient at obtaining nutrients such as N, but as obligate symbionts rely completely on C from their plant host (Gougoulias 2014). Finally, plants can also to some extent regulate N transformations, by releasing inhibitors of nitrification through root exudate, fuelling heterotrophic processes such as denitrification or mineralisation through the input of labile C and affecting the aeration status of the environment through respiration.

The C:N ratio of organic matter and the N demand relative to C of the decomposer community impact the size of the inorganic N pool, the amount of N available to the plant and with this the intensity of the plant microbial competition for N. As the microbes are directly involved in the mineralisation process it is suggested that plants may only have access to the liberated inorganic N forms if they are in excess of the decomposers requirements. When the C:N ratio of the OM is high, in other words very little N relative to C, microbes tend to be more N-limited

and will immobilise the N they mineralise through decomposition. Fresh plant root exudates in fact are associated with higher C:N ratios. The abundant C fuels microbial activity and respiration and microbes are likely to immobilise N from the inorganic N pools to sustain their N requirements associated with this high level of activity. Under these conditions microbes and plants would be in intense competition with each other for N (Fig. 6, towards the right side of the scale). On the other end of the spectrum, decomposition of OM with very low C:N ratio may liberate N in excess of microbial need. Under these conditions, the microbes would be more C-limited and activity levels likely reduced (Månsson et al., 2009). Excess mineralised N may enter the soil inorganic N pool and the plant-microbial competition for N would be much less intense (Fig. 6, towards the left side of the scale). OM with a C:N ratio of less than 12:5 will generally result in net mineralisation of N, whilst a ratio in excess of 30:1 will result in net immobilisation, regardless of the decomposer community composition (reviewed by Hodge et al., 2000). However, not only the C:N ratio of the OM but also the C to N requirements of soil microbes (C:N ratio of decomposers) determines whether net immobilisation or net mineralisation of N occurs. The N relative to C need of microbes may differ substantially between taxa, most notably between fungi and bacteria. Fungi have generally lower N requirements relative to C than bacteria (Hodge et al., 2000). It is important to note the C lost through respiration when considering the C:N requirements of the decomposers.
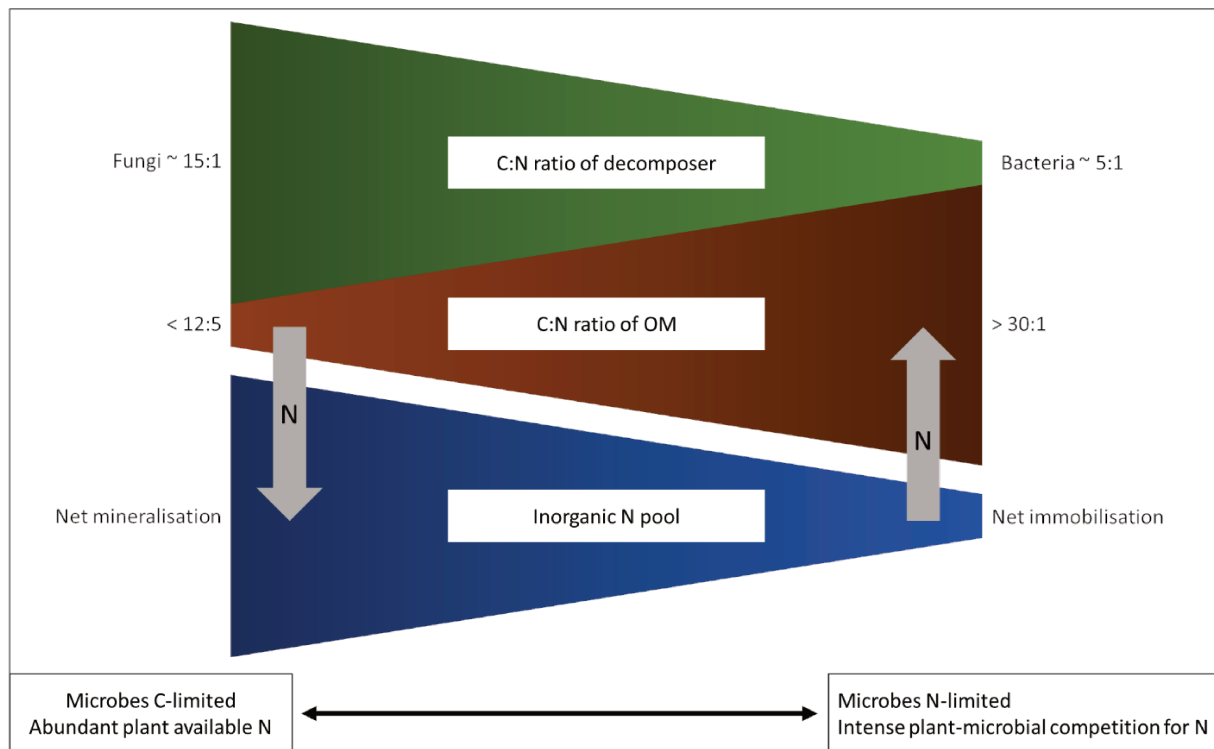
Fig. 6. Simplified schematic demonstrating graphically how the C:N ratio of the decomposer and the C:N ratio of the OM impact the size of the soil inorganic N pool (based on values and figures from Hodge et al. 2000). High C:N ratio of the decomposer (e.g. fungi) with low C:N ratio of the OM may result in the mineralisation of excess N, which may add to the inorganic N pool (net mineralisation, green arrow). Conversely, a high C:N ratio of the decomposer (e.g. bacteria) and low C:N ratio of the OM means the decomposer is likely N-limited and may immobilise N from the soil inorganic N pool (net immobilisation, brown arrow). Conditions on the far left of the graph are likely to result in C-limited microbes and an abundance of plant-available N whilst the conditions on the far right result in N-limited microbes and increased plant-microbial competition for inorganic N pools.

When the competition for N between plants and microbes is intense, studies have shown that both temporal as well as spatial aspects may determine the more competitive player. Soil microbes, with their high affinity for substrate, large surface area to volume ratio and rapid response and growth rates are highlighted as the stronger competitor over short time frames. Microbes immobilise NH_4^+ approximately 5 times and NO_3^- approximately 2 times faster than

plants (Jackson et al. 1989). However, microbial biomass has fast turnover rates, and if there is insufficient C to sustain high activity and growth rates, N is released back into the soil. Microbial N turnover rates are also dependant on the community composition as mycelial structures of fungi and Actinomycetes can recycle N internally, thus having slower N turnover than non-filamentous fungi and bacteria (reviewed by Hodge et al. 2000). Plants, on the other hand, out-compete microbes over longer time scales as they have slower N turnover rates thus retain captured N for extended periods and recycle N internally from senescent tissue. Additionally, disturbance such as cycles of drying and rewetting (or freeze-thawing) may disrupt microbial cells, releasing N which the plants could capture (Clein and Schimel 1994). This N however will be predominantly in organic form.

Changes in precipitation frequency is thus likely to impact not only microbial activity and plant function but also shape the intensity of their competition for resources due to the intricate link between water availability, the C cycle and the N cycle. Furthermore, imbalance in one of these biochemical cycles may like result in an imbalance in the other due to their tight coupling.

## 1.6 Does microbial community composition really matter for ecosystem processes?

1.6.1 Spatial heterogeneity of the soil matrix

The soil matrix as a microbial habitat is extremely heterogeneous with many microsites which may present contrasting environmental conditions, thus harbour contrasting microbial communities and processes within very small spatial scales (Fig. 7). It is made up of a combination of different sized particles (sand, silt, clay), which may be glued together by organic matter (organic polymers, fungal hyphae, plant roots) resulting in aggregates and pore spaces of varying sizes (Ruamps et al., 2011, Six et al., 2004). Soil moisture determines how

connected these pore spaces are and whether the pore is air-filled or water-filled. Larger pores drain of water before smaller pores and as the soil contains a multitude of pore sizes this could lead to very contrasting water-related conditions to occur at small spatial scales (Young et al., 2008). Other than pore size, the hydrophobicity of the aggregate surface as well as the roughness of the surface (Or et al., 2007) may impact water dynamics within the soil matrix. Additionally, the organic matter distribution within this complex 3-dimensional system may be very irregular (Lehmann et al., 2008), which, in addition to the multitude contrasting abiotic parameters, results in isolated hot spots of microbial activity and ecological opportunity for resource specialisation (MacLean, 2005).

The soil microbial communities inhabiting this complex system are extremely diverse and interactions with each other are very dynamic. Microbes not only compete for space and resources but also continuously adapt to fluctuations in environmental conditions (Vos et al., 2013). Microbial interactions, both positive and negative, depend on the habitats being connected, and this is highly dependent on the continuity of water films and thus on soil water saturation. Spatial isolation due to disconnected soil pores commonly occurring in dry soils, has been linked to enhanced microbial diversity by physical sheltering of less competitive species (Zhou et al., 2002, Dechesne et al., 2008) which would not persist in the presence of more competitive species when pores are connected (e.g. wet soils).

The frequency and magnitude of precipitation events thus not only impact the abiotic environmental conditions of the soil matrix by determining aeration status and access to nutrients but also by shaping the extent of biotic interactions at very small spatial scales.
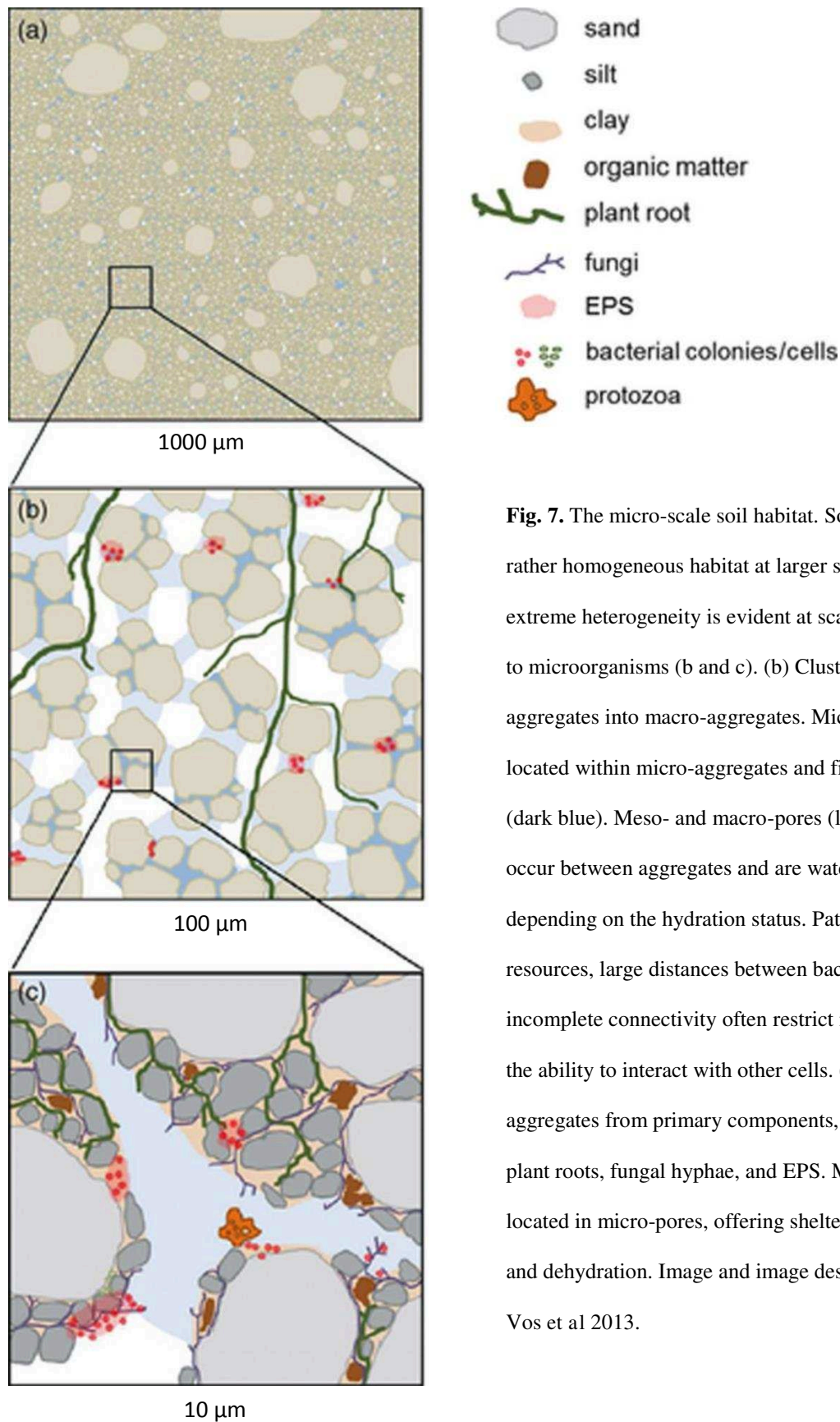
Fig. 7. The micro-scale soil habitat. Soils appear to be a rather homogeneous habitat at larger scales (a), but extreme heterogeneity is evident at scales more relevant to microorganisms (b and c). (b) Clustering of micro-aggregates into macro-aggregates. Micro-pores are mostly located within micro-aggregates and filled with water (dark blue). Meso- and macro-pores (light blue and white) occur between aggregates and are water or air filled, depending on the hydration status. Patchy distribution of resources, large distances between bacterial cells and incomplete connectivity often restrict nutrient access and the ability to interact with other cells. (c) The formation of aggregates from primary components, held together by plant roots, fungal hyphae, and EPS. Many bacteria are located in micro-pores, offering shelter against predators and dehydration. Image and image description source: Vos et al 2013.

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At larger spatial scales, soil microbial communities differ significantly in both their horizontal as well as vertical distribution within the soil matrix. In fact, studies have shown that the vertical distance between communities may be far more influential than the horizontal distance with 10-20 cm of soil depth, leading to more dissimilar communities than communities separated by many kilometres of horizontal space (Eilers, 2012). This is caused by the strong vertical gradient in nutrient availability, water saturation, aeration and temperature of the soil profile (Tecon and Or 2017, Tückmantel et al., 2017).

Root density, and with this root exudation and labile organic C as well as inorganic N concentrations, decrease with depth (Tückmantel et al., 2017). Thus, microbial community composition changes with depth, towards groups which are adapted to the low nutrient conditions (Kramer and Gleixner, 2008). Soil aeration generally decreases with soil depth, as water saturation increases, but this is highly dependent on drying and rewetting conditions (Fig. 8). Shifts in precipitation pattern will likely not impact all soil horizons equally. Short lived, small magnitude rain events may only saturate the surface soils, which are also most prone to water loss through evaporation. So small precipitation pulses result in only transient water availability, which only rapidly responding microbes in top soil levels can take advantage of (Schwinning and Sala, 2004). Microbes in deeper soil horizons generally experience fewer drying-rewetting cycles than microbes in top soil. This shows that on small scales a large amount of heterogeneity exists within soils regarding nutrient availability, abiotic environmental conditions as well as microbial community composition and their functions. However, it remains a question of debate whether shifts in functional potential at these small spatial scales translate into consequences at an ecosystem scale.
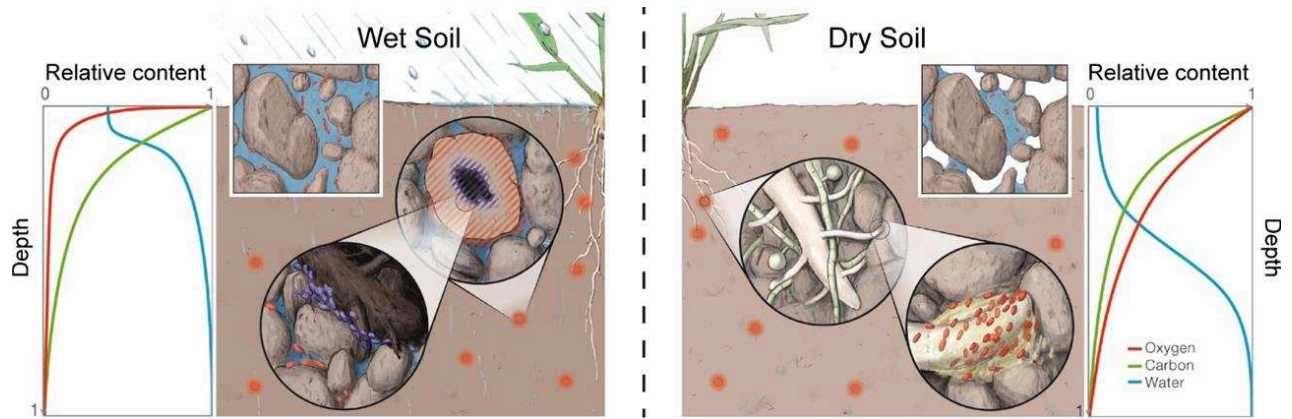
Fig. 8. Microbial hotspots and hydration conditions in soil. Conceptual illustration shows hotspots of microbial activity (orange dots), with on the left anaerobic (purple) and aerobic (red) bacterial populations inside an aggregate, and on the right bacteria colonizing a root hair tip. Squares show water and air configuration in the pore space at the microscale under wet conditions following rainfall or irrigation (left), or under dry conditions after water drainage and evaporation (right). Graphs show macroscopic profiles of oxygen, carbon and water content over soil depth. Oxygen concentration is highest at the soil surface and water saturation maximal when it reaches the water table. Oxygen and water profile change under wet or dry conditions, while carbon profile is unchanged. Figure and figure description from Tecon and Or 2017

1.6.2 Functional redundancy

It is highly debated whether changes in the composition of soil microbial communities can in fact impact ecosystem response to disturbance. One of the key arguments is that microbial communities are inherently resilient to environmental perturbations due to the principle of functional redundancy, especially at the species level (Prosser et al., 2012). This theory argues that the high biodiversity of the microbes found in soil may act as an insurance against environmental fluctuations, where species adapted to sustain their activities under different environmental conditions may perform the same functions (Yachi and Loreau 1999, Lennon and Jones 2011). This insurance hypothesis may be particularly important if environmental

fluctuations become more intense, such as those which are predicted to occur under changing precipitation patterns.

The counter argument for this is that although the argument might hold true for functions which are considered "broad processes", this functional redundancy and insurance hypothesis may not necessarily apply to what is known as "narrow processes" (Schimel and Schaeffer, 2012). A "broad process" is defined as a process which can be performed by a wide range of organisms, so functional redundancy might assure that these processes are likely less impacted by changes in the environmental conditions. Many of the processes involved in C cycling, such as respiration, decomposition and C storage, are "broad processes" as they are essential for the abundantly present heterotrophs found in the soil (Schimel, 2013). "Narrow processes", on the other hand, which are performed by more specialized groups of micro-organisms, are less likely protected by the theory of functional redundancy and thus changes in communities driving these processes is likely to impact ecosystem functioning (Schimel et al., 1996). Examples of "narrow processes" include N-fixation, nitrification, plant-mycorrhizal symbiosis and N_2O emissions (Schimel, 2013).

To determine whether changing precipitation patterns may impact ecosystem functions by shaping microbial community composition, it is thus vital to first determine the structure and diversity of not only the active players but also of the inactive seed pool, and second, to determine whether the functional potential is altered. The inactive seed pool is most likely to supply insight into the genetic potential which can be recruited if conditions change. Higher diversity of the inactive seed pool is linked to a vaster array of functional potential and thus stability of ecosystem processes (Fetzner et al., 2015). By evaluating the impact of precipitation change on particularly the narrow processes and the genetic traits which are required to carry these out, it may be possible to link shifts in the microbial community composition with potential consequences for ecosystem processes.

2. Aims and objectives

The aim of this thesis was to gain further insight into the legacy effect of contrasting precipitation patterns on the response to rewetting of active soil bacterial and fungal communities and on biogeochemical cycles. For this purpose, three independent but complementary experiments were performed on mesocosms exposed to contrasting precipitation input regimes in a controlled environment. The first experiment was performed on soils from cores that were taken in the field and exposed to contrasted precipitation regimes before rewetting, in which the actively growing as well as the inactive bacterial and fungal communities were documented at 48 and 120 hours post rewetting, using ^{18}O water and a SIP approach. In the second experiment, this idea was further explored by including the effects of soil depth and plant-microbial coupling in a plant-soil system with a history of contrasting precipitation patterns in the response of actively growing and inactive bacterial and fungal communities in response to rewetting, also using ^{18}O water and a SIP approach, supplemented by stable isotope labelling and plant ecophysiological measurements. The final experiment investigated the legacy effects of contrasting precipitation patterns under different levels of N availability on the rewetting response of a plant-soil system over time (29 hours). It focused on plant-microbial coupling and plant-microbial competition for N, by documenting the bacterial and fungal response to rewetting over time, using rRNA sequencing, stable isotope labelling, plant ecophysiological and soil biogeochemical approaches.

3. Chapter I

Impact of contrasting moisture regimes on actively growing and inactive microbial communities is sustained up to 5 days after rewetting.

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Short communication

Impact of contrasting moisture regimes on actively growing and inactive microbial communities is sustained up to 5 days after rewetting

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Key words: soil bacteria, soil fungi, heavy water, stable isotope probing, soil rewetting, historical legacies.

Abstract

The predicted shift towards more erratic precipitation patterns will likely impact terrestrial ecosystem processes, since the activity of soil microbes, the drivers of nutrient cycles, is intricately linked to soil moisture. In Mediterranean grassland soils with a history of contrasting precipitation patterns, we investigated the response of the active and inactive bacterial and fungal communities to rewetting using ^{18}O-water DNA stable isotope probing. Our results suggest that soil bacterial and fungal responses to rewetting may be sustained for at least 120 h after the event and that cells which died during the preceding dry period or upon wet-up contribute to fuel the reactivation of active microbes. We also find that precipitation history likely has long-lasting implications for ecosystem stability, as it impacts not only the active microbes but also the inactive microbial seed bank, which represents a large reservoir of functional potential. The increased phylogenetic clustering of bacterial communities under repeated cycles of drying and rewetting suggest that among bacteria, the ability to survive under fluctuating moisture conditions is a phylogenetically more constrained trait whilst strategies to resist desiccation is ubiquitous.

Short communication

Soil microbes are key players in terrestrial ecosystem processes by driving nutrient cycles, C sequestration and trace gas fluxes. Since microbial function is intricately linked with soil moisture (Averill et al., 2016), the predicted shift towards more erratic precipitation patterns with increased periods of water limitation (IPCC 2007), will likely affect these processes. Rewetting a dry soil produces a shift in the composition of the soil microbial community (Placella et al., 2012, Barnard et al., 2013). Not only has it been shown that the microbial response to rewetting is a phylogenetically conserved trait, with certain groups more primed to respond than others (Placella et al., 2012) but also the resistance to desiccation varies among groups (Barnard et al., 2013). Thus to gain a more holistic understanding of the population dynamic during drying and rewetting it is important to recognize the contribution of both the active as well as the inactive microbial communities under a set of environmental conditions. Firstly, microbes which die during the dry period or immediately upon wet-up may likely fuel the growth of the active communities responsible for the burst of CO_2 (Blazewicz et al., 2014). Secondly, the surviving inactive 'seed pool' represents a large reservoir of functional potential in soils which may become active under a different set of environmental conditions which may alter or provide stability to ecosystem processes (Loreau et al., 2001).

In addition to the effect of a rewetting event per se, recent work has shown that soil microbial response to rewetting depends on their precipitation history. The length of the preceding dry period determines the magnitude of the microbial response to rewetting (Barnard et al., 2014) and whether the microbial growth rate upon rewetting is immediate and linear or exponential following a lag phase (Meisner et al., 2015).

The present study investigated the response of the active and inactive bacterial and fungal communities, 48 and 120 hours after rewetting with ^{18}O-water DNA stable isotope probing, in

soils that had been exposed to contrasting precipitation patterns over a 4-month summer dry-down.

In short, intact soil cores from a California grassland where subjected to contrasting water input regimes (no water inputs vs. weekly water inputs) in the greenhouse for 4 months. Three-gram soil samples (dry weight) were taken from the cores and deionised (^{16}O, unlabelled control) water was added to soils from the dry treatment to reach a soil moisture equivalent to the wet treatment. Then 0.61 ml of either deionised water or ^{18}O-water was added to all the soils (final ^{18}O atom % of 67.3). Destructive samples were taken at 48 and 120 h, and DNA was extracted using a modified phenol-chloroform method (see Barnard et al., 2014 for details on experimental setup and DNA extraction). Through isopycnic centrifugation in cesium chloride (1.89 g ml^{-1}), the heavier isotopically enriched DNA (1.735-1.760 g cm^{-3}) was separated from the lighter unenriched DNA (1.670-1.725 g cm^{-3}) in each soil extract. Bacterial and fungal communities were sequenced for the different SIP fractions by Illumina next-generation sequencing of amplicons generated in two steps (Berry et al., 2011) using 16S rRNA (Takahashi et al., 2014) and ITS (modified White et al, 1990) primers respectively (see Engelhardt et al., 2018 for details on SIP and sequencing).

A 3 ml trace gas sample was collected from the headspace of the mesocosms which was then injected into a 10 ml serum vial which had been pre-filled N$_2$ (1 atm). To prevent vacuum formation in the headspace the extracted sample was immediately replaced by injecting 3 ml of synthetic air (20:80 O$_2$:N$_2$). CO$_2$ concentrations were determined using a gas chromatograph (Agilent 6890 series, Agilent Technologies, Wilmington, DE, USA) and soil CO$_2$ efflux rate calculated (see Barnard et al., 2015 for details on trace gas analysis).

Statistical analyses were performed using R 3.1.2 (R Core Team, 2014) on n=3 replicate samples per watering regime and ^{18}O incubation period combination. Data were analyzed by

analysis of variance using a linear mixed-effects model that included precipitation regime, time after rewetting, activity (when relevant, based on communities present in the heavy vs. light DNA fractions) and their interactions as fixed effects variables, and vial as the random effect variable. Bacterial UniFrac distances and fungal Bray-Curtis dissimilarities were used for principal coordinate analysis and analyzed by PERMANOVA (Anderson, 2001). The OTUs that responded significantly to an experimental variable were identified using the linear mixed-effects model described above, then a test to account for false discovery rates (Strimmer, 2008).

The active and inactive bacterial communities differed significantly after rewetting (p=0.006), congruent with recent studies (Barnard et al., 2013, Engelhardt et al., 2018). Furthermore, compositional changes of the active and inactive bacterial communities over time differed significantly (p=0.026). The active bacterial community was only marginally significantly different over time, while the inactive bacterial community differed significantly between 48 and 120 h after rewetting, explaining 22.1% of its variance (p=0.014) (Fig. 1). Bacteria can leave the inactive pool either by resuming activity, thereby incorporating heavy ^{18}O water and becoming part of the active community, or by being consumed, their DNA becoming part of active bacteria or bacterial grazers (note that the members of the inactive community may be alive or dead). Our results indicate little reactivation of slow responding groups from inactive to active state between 48 and 120 h after rewetting, since the composition of the active community did not change significantly over that time. Significantly increased evenness of the inactive (but not of the active) bacterial community (simpson recip, Fig. 2; significant interaction between activity & incubation time; p<0.001) and the absence of significant net new growth in bacterial communities between 48 and 120 h further point towards selective death (or consumption of the dead cell material) of bacterial groups in the inactive community. While we were unable to determine whether the bacteria died during dry-down or upon rewetting, our results are consistent with a contribution of dead bacterial cell material to

bacterial activity generating the CO_2 pulse associated with rewetting soil (Fig. S1) (Blazewicz et al., 2014). We found 2 OTU which increased in relative abundance between 48 and 120 hours post rewetting in the active and the inactive microbial communities, belonging to the phylum of Proteobacteria (class of δ-proteobacteria) and chloroflexi (classes of thermomicrobia and TK10) respectively. This indicates that in our system it was predominantly the δ-proteobacteria, which have been previously described as rapid responders (Placella et al., 2012), which were still growing after 48 hours post rewetting. Some classes of chloroflexi on the other hand, though not actively growing, may be particularly resistant to lysis and predation during desiccation or following wet-up.

In contrast to bacteria, the active and inactive fungal communities did not differ significantly after rewetting. Nevertheless, as with bacteria, active and inactive fungal communities showed a contrasting response to rewetting over time (p=0.042). Only 1 OTU was found to increase significantly in relative abundance between 48 and 120 hours post rewetting in the inactive fungal community, belonging to the Ascomycota phylum (genus of exophilia; class of eurotiomycetes). Not a single OTU differed between 48 and 120 hours post rewetting in the actively growing fungal communities. We detected no other significant changes in fungal communities over time, including no changes in net new growth. Fungi are inherently more resistant than bacteria to drying and rewetting (de Vries and Shade, 2013; Barnard et al., 2013, 2015) due to their extensive hyphal network with which they can access water from distant micro-pores (de Boer et al., 2005). Additionally, they tend to exhibit much slower growth rates than bacteria and thus show a more delayed response to changes in environmental conditions such as rewetting. Previous studies documented little short-term response of the potentially active fungal community to rewetting in the field (Barnard et al., 2013).

Precipitation pattern history, on the other hand, significantly impacted both the bacterial (p=0.002) and the fungal (p<0.001) community composition after rewetting explaining 13.1

and 9.2% of the variance respectively (Fig. 1). Precipitation pattern affected not only the microorganisms that were active upon rewetting but also the functional potential of the bacterial and fungal seed pool.

The wet regime significantly increased bacterial phylogenetic clustering in both the active and the inactive bacterial communities after rewetting (NRI: 9.07±0.74 and 12.52±0.06 under dry and wet regimes, respectively). These results indicate that the physiological ability of bacteria to survive under moist soil conditions without the input of fresh photosynthate is a phylogenetically more constrained trait than resistance to extreme desiccation, which is more or less ubiquitous within the soil microbial community with a range of survival strategies and thresholds (reviewed by Borken and Matzner 2009), in line with the findings of Placella et al (2012). Precipitation pattern significantly affected the relative abundance of many different bacterial OTUs after rewetting (Fig. 3). They were dominated by Proteobacteria (particularly α- and ß-proteobacteria) in soils which were subjected to the dry regime, and by δ-proteobacteria and Actinobacteria in soils which were subjected to the wet regime. Only one fungal OTU after rewetting responded significantly to precipitation pattern. The OTU belonged to the phylum of Mucoromycota and was relatively more abundant in soil which had been subjected to the wet regime.

In conclusion, our study supports the contribution of dead bacterial cells to fuel microbial activity after a rewetting event. Precipitation history likely has long lasting implications for ecosystem stability, as i) it impacts not only the active microbes but also on the inactive microbial seed pool, ii) its effect on the trajectory of the soil bacterial and fungal communities after rewetting is sustained at least up to 120h after the rewetting event.

Acknowledgements

Figures
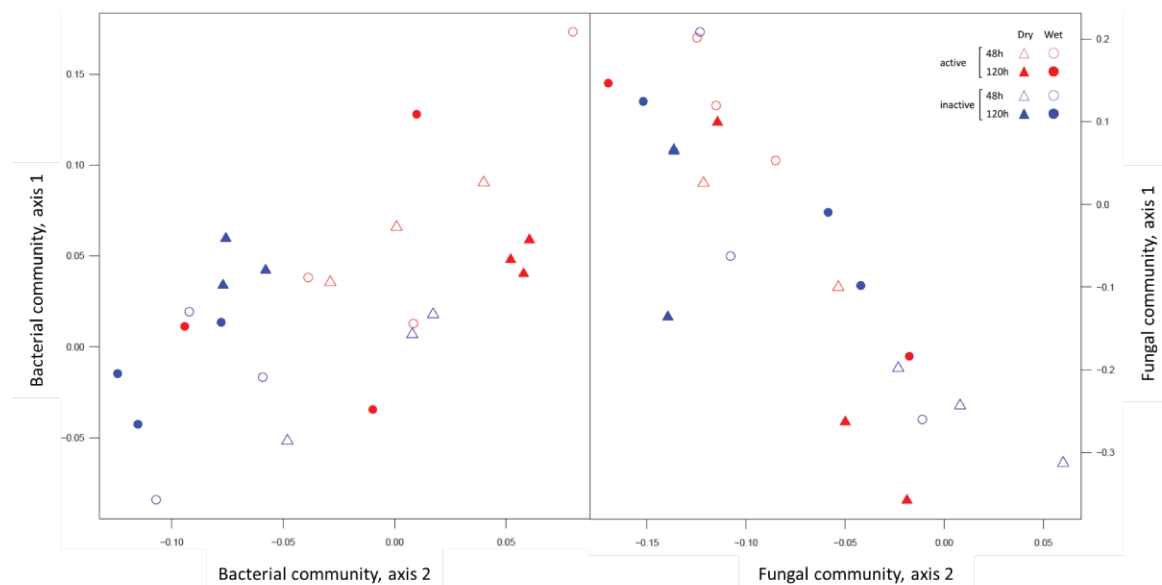
Fig. 1. Principle coordinate analysis (PCoA) of Unifrac pairwise dissimilarity of the relative abundance of 16S based bacterial sequences (left) and Bray-Curtis distance of the relative abundance of ITS based fungal sequences (right) from soils with a history of dry (triangles) or wet (circles) watering regime. Additionally sequences are separated into active (red symbols) and inactive (blue symbols) communities, after 48 (open symbols) and 120 (closed symbols) hours incubation with ^{18}-O water.
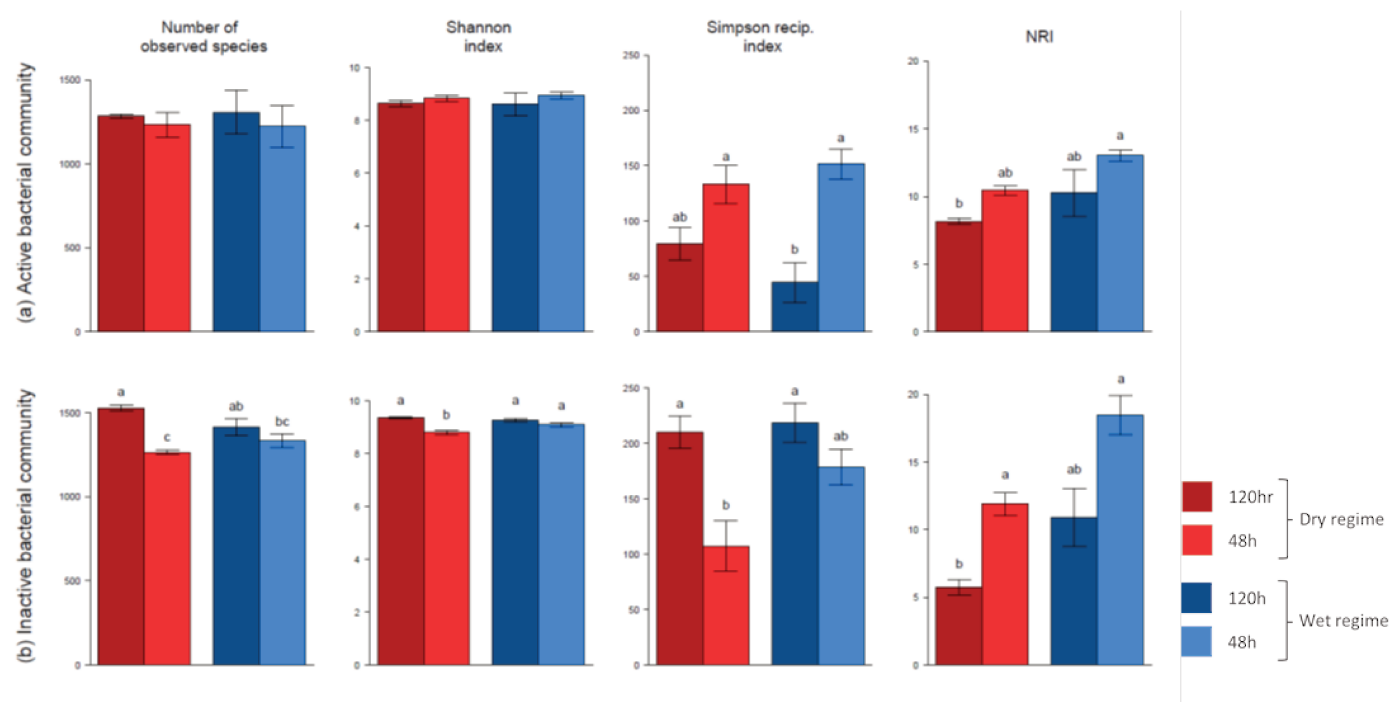
Fig. 2. Diversity indices of active (a) and inactive (b) bacterial communities in soils subjected to either the dry regime (red bar pair) or the wet regime (blue bar pair), after 48 (light shade) and 120 hours (dark shade) incubation with ^{18}O water. Tukey HSD tests were used to compare the means within an index, bars that share a letter are not significantly different. Error bars are based on the mean ± standard error. For treatment significance refer to text.
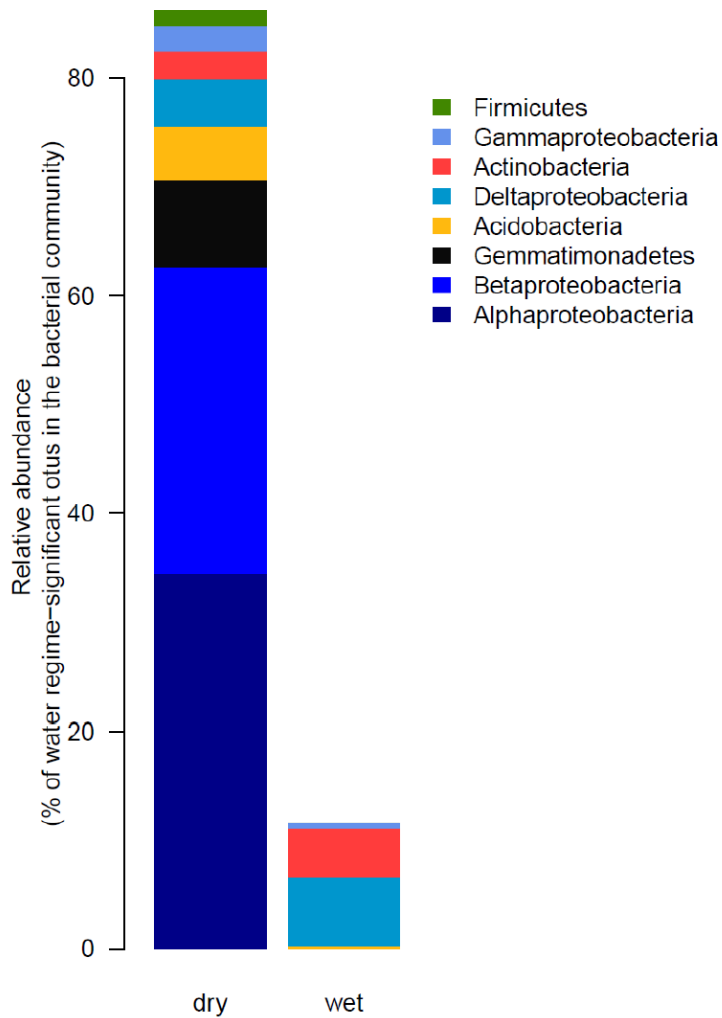
Fig. 3. Relative abundance of OTUs that responded significantly to water history in the total bacterial community. Based on their relative abundance, OTUs clustered by dry and wet regime. All bacterial taxa are at phylum level except Proteobacteria which are shown as representative classes.

References

1. Anderson M (2001). A New Method for Non-Parametric Multivariate Analysis of Variance, Austral Ecology 26(1).
2. Averill C, Waring BG and Hawkes CV (2016) Historical precipitation predictably alters the shape and magnitude of microbial functional response to soil moisture, Global Change Biology 22(5).
3. Barnard RL, Osborne CA, Firestone MK (2013). Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. ISME Journal. 7: 2229–2241
4. Barnard R L, Osborne, CA and Firestone MK (2014). Changing precipitation pattern alters soil microbial community response to wet-up under a Mediterranean-type climate. The ISME journal 9:946-95.
5. Barnard RL, Osborne CA and Firestone MK (2015). Changing precipitation pattern alters soil microbial community response to wet-up under a Mediterranean-type climate, ISME Journal 9(4).
6. Berry D, Mahfoudh KB, Wagner M, Loy A (2011). Barcoded primers used in multiplex amplicon pyrosequencing bias amplification. Appl and Environ Microbiol 77: 7846-7849.
7. Blazewicz SJ and Schwartz E (2011). Dynamics of ^{18}O Incorporation from H_2 ^{18}O into Soil Microbial DNA, Microbial Ecology 61(4).
8. Blazewicz SJ, Schwartz E and Firestone MK (2014). Growth and death of bacteria and fungi underlie rainfall-induced carbon dioxide pulses from seasonally dried soil, Ecology 95(5).
9. 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(4).
10. de Boer W, Folman LB, Summerbell RC, Boddy L (2005). Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiol Rev 29: 795-811.
11. de Vries FT, Shade A (2013). Controls on soil microbial community stability under climate change. Front Microbiol 4: 265.
12. Engelhardt IC, Welty A, Blazewicz SJ, Bru D, Rouard N, Breuil MC, Gessler A, Galiano L, Miranda JC, Spor A and Barnard RL (2018). Depth matters: effects of precipitation regime on soil microbial activity upon rewetting of a plant-soil system, The ISME Journal.
13. IPCC (2007). Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press: Cambridge, UK.
14. Loreau M, Naeem S, Inchausti P, Bengtsson J, Grime JP, Hector A, Hooper DU, Huston MA, Raffaelli D, Schmid B, Tilman D, Wardle DA (2001). Ecology: Biodiversity and ecosystem functioning: Current knowledge and future challenges, Science 294: 804–808.
15. Meisner A, Rousk J and Bååth E (2015). Prolonged drought changes the bacterial growth response to rewetting, Soil Biology and Biochemistry 88.
16. Placella SA, Brodie EL and Firestone MK (2012). Rainfall-induced carbon dioxide pulses result from sequential resuscitation of phylogenetically clustered microbial groups. Proceedings of the National Academy of Sciences of the United States of America, 109(27).

17. R Core Team (2014). R: A language and environment for statistical computing, vol. ISBN 3-900051-07-0: Vienna, Austria.

18. Schwartz E, Hayer M, Hungate BA, Koch BJ, Mchugh TA, Mercurio W, Morrissey EM and Soldanova K (2016). Stable isotope probing with ^{18}O-water to investigate microbial growth and death in environmental samples, Current Opinion in Biotechnology 41.

19. Stolpovsky K, Martinez-Lavanchy P, Heipieper HJ, Van Cappellen P and Thullner M (2011). Incorporating dormancy in dynamic microbial community models, Ecological Modelling 222(17).

20. Strimmer K (2008). fdrtool: a versatile R package for estimating local and tail area-based false discovery rates. Bioinformatics 24: 1461-1462.

21. Takahashi S, Tomita J, Nishioka K, Hisada T, Nishijima M (2014). Development of a prokaryotic universal primer for simultaneous analysis of Bacteria and Archaea using next-generation sequencing. PLoS One 9: e105592.

22. White TJ, Bruns T, Lee S and Taylor JW (1990). PCR Protocols: A Guide to Methods and Applications. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics.

4. Chapter II

Depth matters: Effects of precipitation regime on soil microbial activity upon rewetting of a plant-soil system

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ARTICLE

Depth matters: effects of precipitation regime on soil microbial activity upon rewetting of a plant-soil system

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Abstract
Changes in frequency and amplitude of rain events, that is, precipitation patterns, result in different water conditions with soil depth, and likely affect plant growth and shape plant and soil microbial activity. Here, we used ^{18}O stable isotope probing (SIP) to investigate bacterial and fungal communities that actively grew or not upon rewetting, at three different depths in soil mesocosms previously subjected to frequent or infrequent watering for 12 weeks (equal total water input). Phylogenetic marker genes for bacteria and fungi were sequenced after rewetting, and plant-soil microbial coupling documented by plant ^{13}C-CO_2 labeling. Soil depth, rather than precipitation pattern, was most influential in shaping microbial response to rewetting, and had differential effects on active and inactive bacterial and fungal communities. After rewetting, active bacterial communities were less rich, more even and phylogenetically related than the inactive, and reactivated throughout the soil profile. Active fungal communities after rewetting were less abundant and rich than the inactive. The coupling between plants and soil microbes decreased under infrequent watering in the top soil layer. We suggest that differences in fungal and bacterial abundance and relative activity could result in large effects on subsequent soil biogeochemical cycling.

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Introduction

Water availability is a key regulator of ecosystem functioning, directly controlling plant and soil microbial activity. The predicted large changes in precipitation brought on by climate change include periods of increased water limitation followed by larger magnitude rain events for many parts of the world [1]. Changes in the total amount of precipitation constrain ecosystem functioning, and their effects have been documented in many water input reduction experiments (e.g., [2–8]). However, climate change is predicted to affect not only the amount but also the temporal distribution of rain. Changes in frequency and amplitude of rain events, that is, precipitation patterns, likely shape the activity of plants and soil microorganisms [9–11].

Microbial reactivation upon rewetting is a key moment in ecosystem functioning. Substrate becomes readily available to soil microorganisms, triggering the microbial activity that drives soil biogeochemical cycles. However, not all microbes respond similarly to rewetting events, indicating adaptation in life strategies that may be phylogenetically conserved ecological traits [12, 13]. Furthermore, microbial communities having been exposed to a history of erratic

moisture fluctuations are adapted to these conditions and show smaller changes in response to rewetting events [54].

Within soil microbial communities, bacteria and fungi differ in their resistance to desiccation, as well as in their response to rewetting [14–16]. Fungal populations have been shown to be more resistant to water limitation, likely due to their ability to access water from distant micropores with their extensive hyphal network [17]. Bacteria typically respond faster than fungi to changes in water availability, albeit with a wide range of responses in the bacterial community [12, 13]. Soil microorganisms drive biogeochemical cycles in soil, such that changes in the relative contribution of bacteria and fungi may affect ecosystem functioning. For example, increased fungal:bacterial ratio resulting from dry–wet cycles improved soil nutrient retention [18]. It is therefore crucial to consider both bacterial and fungal responses when evaluating effects of changes in precipitation on the soil microbial community.

Dry–wet cycles are expected to affect soil differentially at different depths, since the top soil layer experiences more fluctuating water conditions, is wet more often, and dries out quicker than the deeper layers, likely shaping microbial community composition and function [19, 20]. Decreased bacterial diversity upon hydration is supported by physical modeling approaches that indicate increased competition when soil rehydration restores connectivity [20–22]. Moreover, as most roots are usually developed in the top soil layers and plants' root activity is expected to respond to precipitation patterns (e.g. [23]), we expect the strongest microbial response in the top soil layers. Under mesic conditions, plants generally remain alive between rain events and their interaction with soil microorganisms may drastically impact how the system responds to precipitation changes. The large amounts of plant carbon allocated to the soil by rhizodeposition is crucial for heterotrophic microorganisms, particularly when considering the carbon costs of microbial water resistance strategies, such as active osmoregulation and exopolysaccharide production (Canarini 2015). The presence of plants can therefore increase microbial resistance and resilience to water stress through sustained C inputs [24, 25]. However, if drought conditions persist, photosynthesis is reduced and becomes less coupled with belowground processes [3]. Despite the intricate link between soil microbes and plants, many drying–rewetting studies focusing on microbial community composition or activity have been performed on systems devoid of live plants, as incubations in soil alone or because plants died during the dry period preceding rewetting.

The present study investigated the response to a rewetting event of bacterial and fungal populations that were actively growing or not (referred to as "active" and "inactive, respectively), at different soil depths in a plant-soil system with a history of contrasting precipitation patterns, that is, its precipitation legacy. Using ^{18}O stable isotope probing (^{18}O-SIP), ^{18}O-labeled water was applied upon rewetting in order to discriminate microbial communities, which are actively growing from those that were not. We hypothesized that (1) rewetting would result in growth of only a small fraction of soil microbes, which closely track soil moisture fluctuations, (2) the microbial community response to rewetting would vary with soil depth, (3) precipitation legacy would affect both plants and microbes, and (4) the precipitation legacy effect would be more pronounced at shallow soil depth.

Materials and methods

Experimental set-up

Mesocosms (56 cm high, 36 cm wide, and 2 cm deep, Fig. S1) were filled (uniform bulk density $1.2\,g\,cm^{-3}$) with sandy soil collected from 0 to 25 cm depth in an ungrazed grassland (Varenne-Saint-Germain, France). Sieving (2 mm mesh) ensured the soil and its associated microbial community was homogeneous throughout the mesocosms. Soil texture was 92% sand, 4% silt, 4% clay, pH was 5.9, cation exchange capacity was $4.0\,cmol\,kg^{-1}$, organic matter was 2.6%, total N 0.12%, and total C 1.51%. Winter wheat (*Triticum aestivum* cv Soissons) was germinated on the same grassland soil, then 72 h after their germination plantlets were transferred to mesocosms (14 plants per mesocosm, 2.5 cm apart) grown under controlled conditions (20/15 °C daytime/nighttime temperature, 18-h photoperiod). After a 2-week establishment period during which all mesocosms were watered daily (ensuring that the plants were properly developed before initiating the experimental treatments), two watering frequency treatments were applied, with the same total amount of water given to each treatment. The high-frequency treatment (i.e., frequent water input) consisted of daily water inputs with the minimum volume of water required to avoid wilting (from 1.4 to 6.3 mm as plant water requirement grew with their size). The low-frequency treatment (infrequent water input) consisted of one input event every 2–3 days for 2 weeks, until the plants were strong enough to survive one input every 5–6 days. The volume equaled the sum total of water delivered over the same period in the high-frequency treatments (from 2.8 to 37.5 mm). The mesocosms were weighed daily to monitor soil water content. Forty mesocosms were used for the experiment. Before wet-up, 10 were used for soil and plant sampling and 10 for ^{13}C-CO_2 labeling (5 replicates per treatment). After wet-up, 10 were used for $H_2{}^{18}O$ labeling and 10 for the unlabeled control (5 replicates per treatment).

Before final wet-up: soil and plant sampling

Three target soil layers were determined at 0–5, 10–15, and 30–35 cm depth, based on the wetting fronts recorded during the experiment. Following a watering event, the 0–5 cm layer was uniformly wet in both treatments, the 10–15 cm layer was uniformly wet in the low-frequency treatment but infrequently wet in the high-frequency treatment, and the 30–35 cm layer was infrequently wet in the low-frequency treatment but uniformly dry in the high-frequency treatment. Twelve weeks after germination (i.e., after 18 low-frequency watering cycles), leaf gas exchange was measured on the youngest, fully developed leaf of three plants per mesocosm (Li-6400 XT portable photosynthesis system, Li-Cor, Lincoln, USA) in five mesocosms per treatment, then all plants were harvested and measured for leaf surface area (Li-3100C, Li-Cor), dry biomass (including root biomass), root length density (WinRHIZO software, Regent Instruments Inc., Canada), and C and N content (NC 2500 elemental analyzer, Carlo Erba, Milan, Italy).

Before final wet-up: plant-soil coupling (^{13}C-CO_2 labeling)

Fourteen weeks after germination, five mesocosms per treatment were labeled for 1.5 h with ^{13}C-CO_2 as follows. An airtight transparent plastic tent was closed around the mesocosms and CO_2 concentration within the tent was allowed to be drawn down by plant photosynthesis to approximately 300 ppm. Fans inside the tent ensured good mixing of air. Labeling was performed by dissolving $CaCO_3$ powder (50% ^{13}C, 50% $^{12}CO_2$) with HCl 1 M and pumping the resulting gas through the tent. $^{12}CO_2$ concentration inside the tent was monitored (Walz GFS 3000 infra-red gas analyzer, Heinz Walz GmbH, Effeltrich, Germany), and averaged 964.2 µmol mol^{-1} (range: 579.3–1215.3 µmol mol^{-1}) over the labeling period. As we added a 50%:50% mixture of $^{12}CO_2$ and $^{13}CO_2$, total CO_2 concentration was approximately twice the measured values. Temperature during labeling period averaged 23.0 °C. After labeling, the tent was removed and the greenhouse flushed with outside air. Repeated measurements of root, leaf, and microbial biomass isotopic signature were made in each mesocosm for 5 days as follows, while precipitation treatments were maintained. Five 4.6 cm wide vertical strips were marked out, each comprising two plant individuals and 2.3 cm away from the next strip. Each day for 5 days, one side of the mesocosms was opened, three soil layers (0–5, 10–15, and 30–35 cm depth) in one random vertical strip per mesocom were sampled and replaced by sand before the mesocosms were closed again. Each layer was subsampled: one subsample was used for microbial biomass and isotopic signature, the other was washed and the roots dried (48 h at 65 °C) and ground for ^{13}C signature measurement. The

youngest fully developed leaf of two plants was taken on the first and last sampling day, dried, and ground for ^{13}C signature measurement. Soil microbial biomass C was determined by chloroform fumigation extraction [26]. One 10 g subsample was fumigated for 24 h with chloroform vapor, whereas another was not. Microbial C was extracted by vigorous shaking in K_2SO_4 0.5 M. Organic C concentration and its isotopic signature were determined by oxidizing extractable carbon to CO_2 [27]. In all, 1 mL of extracted C plus 1 mL of the oxidizing agent (supersaturated potassium persulfate oxidizing solution :100 mL H_2O+ 4.0 g $K_2S_2O_8$+ 200 mL of 85% H_3PO_4) were added to 12 mL vials, then flushed for 5 min with helium to remove atmospheric CO_2. To complete the oxidation, vials were heated to 100° for 1 h. Finally, soil microbial biomass C was calculated as follows ((total C in fumigated soil)–(total C in unfumigated soil))/0.45 [26]. C concentrations and ^{13}C signature were analyzed with a GasBench II system coupled to an isotope ratio mass spectrometer (Delta Plus XL, Thermo Finnigan Mat, Bremen, Germany). Plant biomass ^{13}C signature was measured by carbon isotope analysis (precision of 0.1‰): combustion in an elemental analyzer (EA1110 CHN, Carlo Erba) coupled via a Conflo II interface to an isotope ratio mass spectrometer (Delta S, Finnigan MAT). $^{13}C/^{12}C$ ratio is expressed in δ notation (‰) relative to the Vienna Pee Dee Belemnite (VPDB) standard.

Final wet-up: $H_2{}^{18}O$ application

At the end of the experiment, the short-term response of the active microbial community to a large-scale rain event was assessed with a SIP experiment. Rewetting soil with ^{18}O-labeled water results in the heavy ^{18}O stable isotope being assimilated into the DNA of actively growing and replicating communities, which can then be separated and sequenced [28]. No water was added to the mesocosms for 48 h prior to rewetting. The rewetting SIP was performed in situ, by opening the sides of the mesocosms and applying $H_2{}^{18}O$ (5 ^{18}O-labeled mesocosms, 98.7 atom% ^{18}O, Eurisotop, Saint-Aubin, France) or molecular grade unlabeled H_2O (five control mesocosms) to a 2 cm diameter area in each of the three target soil layers. Molecular grade water was applied to the rest of the soil volume, except a non-watered 4 cm wide buffer zone that was maintained between the labeled and unlabeled water application zones to avoid mixing. To ensure a long enough exposure of the microbial community to the ^{18}O label and compensate plant water uptake, $H_2{}^{18}O$ (and its H_2O counterpart in the control mesocosms) was applied over 5 days as daily 2 ml applications for 2 days then daily 0.5 ml applications for the next 3 days. Following each application, the mesocosms were closed, returned to their original vertical position in the greenhouse, and the plants bagged in clear plastic to

decrease soil moisture loss from evapotranspiration. Six days after initial wet-up, the labeled areas and their control counterparts were sampled and the soil kept at −20 °C prior to SIP processing.

DNA extraction and fractionation

For each sample, DNA was extracted separately from three 0.5 g subsamples (FastDNA kit, MP Biomedicals, Solon, OH, USA), and quantified (Quantus Fluorometer, Promega, Sunnyvale, USA) before pooling the subsamples. Enriched DNA was separated from unenriched DNA through isopycnic centrifugation using a CsCl gradient. In all, 5 µg of extracted DNA was combined with 3.5 ml of CsCl (1.89 g ml^{-1}), 0.3 ml of gradient buffer (200 mM Tris 8.0, 200 mM KCl, 2 mM EDTA), 0.9 ml TE buffer and added to a 4.7 ml centrifuge tube (Beckmann-Coulter, Fullerton, USA) and ultracentrifuged (60,000 rpm—i.e., 149,723 × g at the average radius r(av)-, 18 °C, 115 h).

DNA from a H$_2$^{18}O-labeled soil sample and its natural abundance control were always processed in the same ultracentifuge run. Following centrifugation, each tube was divided into 70 µl fractions and their density determined (AR200 refractometer, Reichert, Depew, USA). DNA was purified by adding 300 µl of molecular grade H$_2$O, 10 µl of glycogen (20 mg ml^{-1}) and 400 µl of isopropanol to each fraction, before overnight incubation at 5 °C. The fractions were centrifuged (13,400 × g, 15 min), the precipitate washed with filter-sterilized 70% ethanol, suspended in 50 µl of TE buffer and kept at −80 °C. DNA concentration in each fraction was quantified by fluorometry (Quant-iT PicoGreen dsDNA assay kit, Invitrogen, Cergy-Pontoise, France). The fractions constituting each sample were binned into four groups based on their density: 1.668 < light ≤ 1.708, 1.708 < mid-light ≤ 1.714, 1.714 < mid-heavy ≤ 1.722, 1.722 < heavy<1.740 g cm^{-3}. The DNA of the active microbial community was defined as the DNA present in the heavy fraction of H$_2$^{18}O sample when no DNA was present in the heavy fraction of the natural abundance control. The inactive microbial community was defined based on the DNA in the light fraction of the H$_2$^{18}O sample. The bacterial and fungal communities were quantified and the DNA in the binned fractions sequenced as described below.

Quantification of the bacterial and fungal communities

The abundance of the soil bacterial and fungal communities was assessed by quantitative polymerase chain reaction (qPCR), using bacterial 16S ribosomal RNA (rRNA) encoding gene primers 341F 5′-CCTACGGGAGGCAG-CAG-3′/534R 5′-ATTACCGCGGCTGCTGGCA-3′ [29]

and fungal internal transcribed spacer (ITS) region primers ITS3 5′-GCATCGATGAAGAACGCAGC-3′/ITS4 5′-TC CTCCGCTTATTGATATGC-3′ [30], respectively. Quantification was based on SYBR Green dye increasing fluorescence intensity during amplification, in a ViiA7 (Life Technologies, Carlsbad, CA, USA). Real-time PCR assays were carried out in triplicate 15 µl reactions containing SYBR green PCR Master Mix (Takyon Low ROX SYBR 2 × MasterMix blue dTTP, Eurogentec, France), 1 µM of each primer, 250 ng of T4 gene 32 (QBiogene, France) and 1 ng of DNA. Standard curves were obtained using serial dilutions of linearized plasmids containing the cloned genes (efficiency: 89–99%). Template-free controls gave negligible values. No inhibition was detected.

Amplicon generation and MiSeq sequencing

Illumina next-generation amplicon sequencing was used to sequence 240 samples. Amplicons were generated in two steps [31]. In the first step, the bacterial 16S rRNA gene V3–V4 hypervariable region was amplified by PCR using the following fusion primers including overhang adapters to allow subsequent addition of multiplexing index sequences [32]: Pro341F (5′-TCGTCGGCAGCGTCAGATGTGTA TAAGAGACAGCCTACGGGRSGCAGCAG-3′) and Pro805R (5′-GTCTCGTGGGCTCGGAGATGTGTATAA-GAGACAG GACTACCAGGGTATCTAAT-3′). PCR was carried out in duplicate 15 µL reactions containing 7.5 µL Phusion High-Fidelity PCR Master Mix (Thermo Scientific), 0.25 µM of each primer, 250 ng T4 gp32 (MPBio) and 1 ng template DNA. Thermal cycling conditions were 98 °C for 3 min followed by 25 cycles of 98 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 10 min. Duplicate first step PCR products were pooled then used as template for the second step PCR. In the second step, PCR amplification added multiplexing index sequences to the overhang adapters using a unique multiplex primer pair combination for each sample, in duplicate 30 µL reactions containing 15 µL Phusion High-Fidelity PCR Master Mix (Thermo Scientific), 1 µM of one forward and one reverse multiplex primer and 6 µL of first step PCR product. Thermal cycling conditions were 98 °C for 3 min, 8 cycles of 98 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, final extension at 72 °C for 10 min. PCR products were pooled, cleaned-up, and purified using AMPure XP beads (Beckman-Coulter), quantified with picogreen (Thermo Scientific), followed by equimolar pooling and gel purification. Sequencing was performed on MiSeq (Illumina, 2 × 250 bp, MiSeq reagent kit v2, 500 cycles). Demultiplexing and trimming of Illumina adaptors and barcodes was done with Illumina MiSeq Reporter software (version 2.5.1.3). Fungal ITS rRNA region was amplified similarly,

using the primers ITS3F (5′-TCGTCGGCAGCGTCA-GATGTGTATAAGAGACAGNNNNGCATCGATGAAG AACGCAGC-3′) and ITS4R (5′-GTCTCGTGGG CTCGGAGATGTGTATAAGAGACAGNNNNTCCT CSSCTTATTGATATGC-3′), modified from White et al. [30], with 30 cycles for the first step PCR and 10 cycles for the second step PCR.

Bioinformatic analysis of the 16S rRNA gene and ITSr RNA region amplicons

Sequences were assembled using PEAR [33]. Further quality checks were conducted using the QIIME pipeline [34] and short sequences were removed (<330 bp for 16S rRNA genes and <230 bp for ITS region). Reference-based and de-novo chimera detection, as well as operational taxonomic unit (OTU) clustering were performed using VSEARCH [35] and the adequate reference databases (Greengenes for 16S, UNITE for ITS region). Identity thresholds were set at 94% for 16S rRNA gene data, based on replicate sequencings of a bacterial mock community containing 40 bacterial species, and 97% for ITS region data for which we did not have a mock community. Representative 16S rRNA genes sequences for each OTU were aligned using PyNAST [36] and a phylogenetic tree constructed using FastTree [37]. Taxonomy was assigned using UCLUST [38] and the latest released Greengenes database (v.05/2013 [39]) for 16S rRNA gene, and BLAST [40] and the UNITE reference database (v.7-08/2016, [41] for ITS region.

Bacterial and fungal α-diversity metrics were calculated in QIIME based on rarefied OTU tables (10,000 sequences per sample for both 16S and ITS region). UniFrac distance matrices [42] and Bray–Curtis dissimilarity matrices were computed for 16S rRNA genes and ITS region, respectively. Net relatedness index (NRI) of the bacterial communities was calculated based on mean phylogenetic distance [43, 44], using a null model of random community phylogenetic relationships (picante package [45]; 999 runs, not abundance weighted).

Statistical analyses

Statistical analyses were performed using R 3.1.2 [46] on n = 5 replicate mesocosms per treatment. Data measured at the overall mesocosm level were assessed by analysis of variance, using precipitation regime as fixed effect variable. The precipitation pattern treatment, applied at the mesocosm level, generated a depth gradient within the mesocosms, therefore this nested design was accounted for in the statistical analysis and allowed the deconvolution of precipitation pattern, depth, and their interaction. Data measured at different soil depths were analyzed by analysis of

variance using a linear mixed-effects model that accounted for the experimental design by including precipitation regime, soil depth, activity (when relevant, based on communities present in heavy vs. light DNA fractions), and their interaction as fixed effects variables and mesocosm as the random effect variable. Bacterial UniFrac distances and fungal Bray–Curtis dissimilarities were used for principal coordinate analysis, and analyzed by non-parametric permutational multivariate analysis of variance [47]. The OTUs responding significantly to experimental treatment were detected using a linear mixed-effects model followed by a test to account for false discovery rates [48]. The OTUs that responded significantly were hierarchically clustered into groups, and the significance of the clustering verified against random clustering.

Results

Precipitation patterns and plant performance

After an initial drop in soil water content, the treatments were stabilized at relatively dry conditions (Fig. 1). The theoretical soil water retention curve for our soil, based on soil texture, bulk density, and horizon [49], shows that our soils dried down close to the theoretical wilting point, and never reached field capacity when watered (Fig. S2). Frequent water input significantly increased live above-ground biomass and decreased dead above-ground biomass, but left root biomass unchanged (Fig. S3). However, root biomass distribution was significantly affected: frequent water inputs
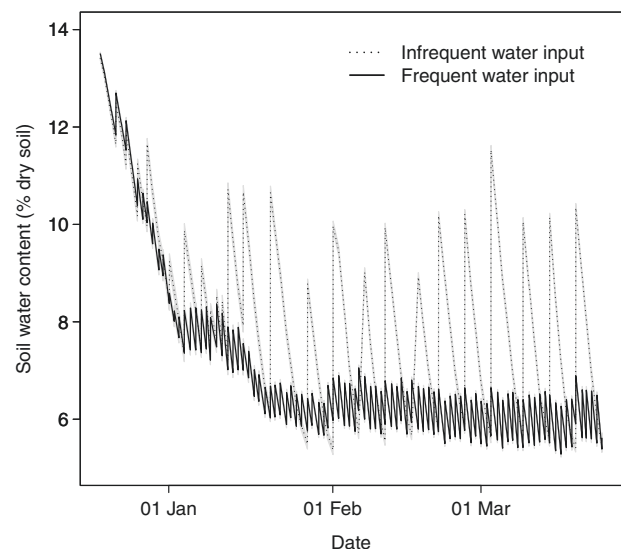
Fig. 1 Dynamics of soil water content in the experimental treatments (infrequent and frequent water input, dotted and full curves, respectively) over the duration of the experiment. Lines and shaded polygons around them indicate mean ± standard error ($n = 5$)
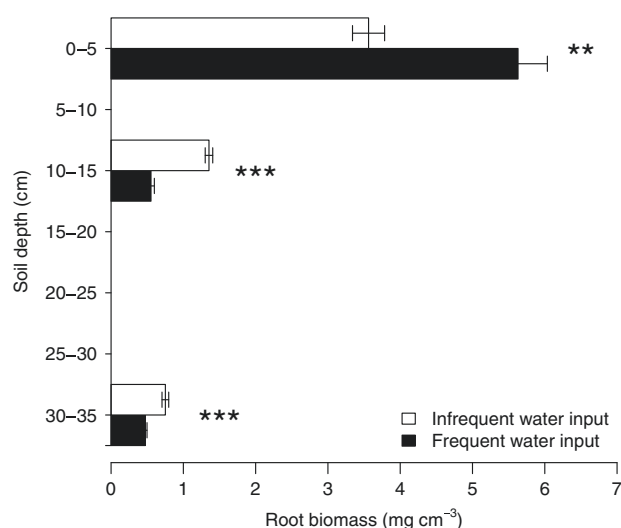
Fig. 2 Root biomass after 12 weeks under contrasting precipitation pattern treatments (open bars: infrequent water input, closed bars: frequent water input), in three soil depth layers (top: 0–5 cm, middle: 10–15 cm, bottom: 30–35 cm). Bars indicate mean ± standard error (n = 5). Significance between treatments in each layer: * $0.05 > p > 0.01$, ** $0.01 > p > 0.001$, *** $0.001 > p$.

significantly increased root biomass in the top soil layer, and decreased it in the middle and bottom soil layers (Fig. 2). Root length density mirrored root biomass pattern (data not shown). We found no significant effect of precipitation regime on stomatal conductance or photosynthetic rate scaled by plant leaf area to account for treatment effect on leaf development, integrated over 4 days at the end of the treatment period (see Fig. S4 for photosynthesis).

Plant-microbial coupling

Bulk root ^{13}C signature was not significantly affected by treatment, soil layer or time (Fig. S5). Soil microbial biomass C significantly decreased with depth ($p = 0.023$; 222.1 ± 37.4, 140.4 ± 15.4, $131.6 \pm 19.8\ \mu g\,C\,g^{-1}$ dry soil in the top, middle, and bottom soil layers, respectively), but was left unchanged by precipitation pattern. Soil microbial biomass ^{13}C signature was significantly higher under frequent water input in the top soil layer, compared with all other treatments and soil layers, and relatively stable over the 5-day measurement period (Fig. S6). The average 5-day microbial biomass ^{13}C signature was significantly related to root biomass under frequent precipitation inputs ($R^2 = 0.80$, $p = 0.011$), but not under infrequent inputs (Fig. S7).

Microbial community abundance, composition, and diversity

Both bacterial 16S rRNA gene and fungal ITS region abundances were significantly higher ($p < 0.001$) in the inactive

($4.8 \times 10^9 \pm 4.6 \times 10^8$ and $6.1 \times 10^8 \pm 7.2 \times 10^7$ copies g^{-1} soil, respectively) than in the active ($9.7 \times 10^7 \pm 2.8 \times 10^7$ and $5.4 \times 10^6 \pm 9.9 \times 10^5$ copies g^{-1} soil, respectively) communities, and was left unchanged by precipitation pattern or depth.

The ^{18}O-SIP allowed us to discriminate the active from the inactive soil microbial community after rewetting, accounting for 61.1% and 9.3% of the variability in bacterial and fungal community composition, respectively (Fig. 3, Table S1). Differences among microbial communities with depth accounted for 4.0% and 14.3% of the overall bacterial and fungal data variability, respectively (Table S1). When considered separately, active and inactive bacterial and fungal communities differed with depth, which explained between 11% and 20% of the variance (Table S2). Precipitation pattern left soil bacterial community composition unchanged, accounted at most for 5% of the variability in overall, active, and inactive fungal community composition (Table S2), and affected overall and inactive fungal community composition differently at different depths (significant precipitation pattern treatment × depth interaction).

The active bacterial and fungal communities after rewetting were significantly less rich than the inactive (Fig. 4), but more even and less phylogenetically diverse for bacteria, whereas their evenness remained unchanged for fungi. Consistently, NRI was significantly higher in the active than in the inactive bacterial community, indicating stronger phylogenetic clustering (12.04 ± 0.38 vs. 8.43 ± 0.33, respectively). We found no significant effect of precipitation pattern on the α-diversity of microbial communities. We detected no significant effects of depth on the α-diversity of the active bacterial community after rewetting. In the inactive bacterial community, all indices decreased significantly with depth, except NRI, which increased (indicating increased phylogenetic clustering) with depth. Evenness-related indexes responded differently to precipitation pattern at different depths, driven by decreased evenness in the top soil layer under infrequent water inputs, which was not detected in the other soil layers (Fig. 4a, Fig. S8). In both the active and inactive fungal communities, evenness significantly increased with soil depth, driven by a large decrease in the top soil layer under frequent water inputs (Fig. 4b, Fig. S9).

Significantly responsive groups

No microbial OTU responded significantly to precipitation pattern. In the active and inactive bacterial and fungal communities, OTUs responded significantly to soil depth, significantly clustering by soil depth into two groups (Fig. S10, S11, S12, S13) comprising the OTUs that were relatively more present in the top and middle soil layers ("top group")
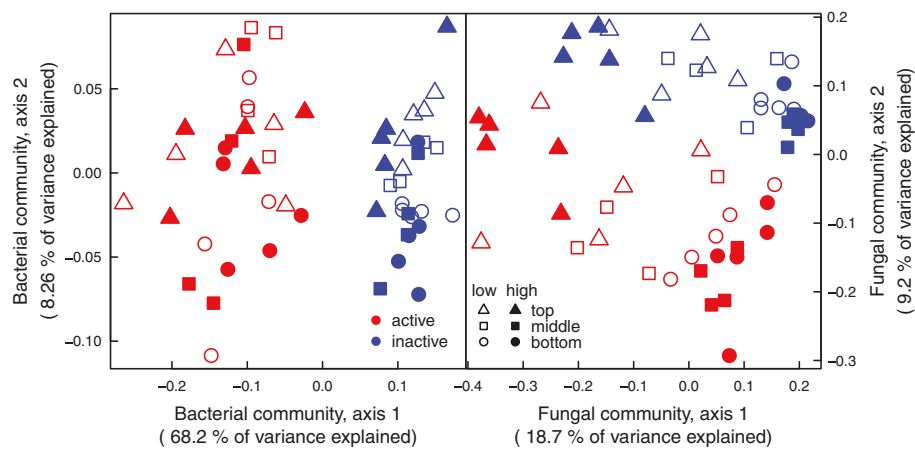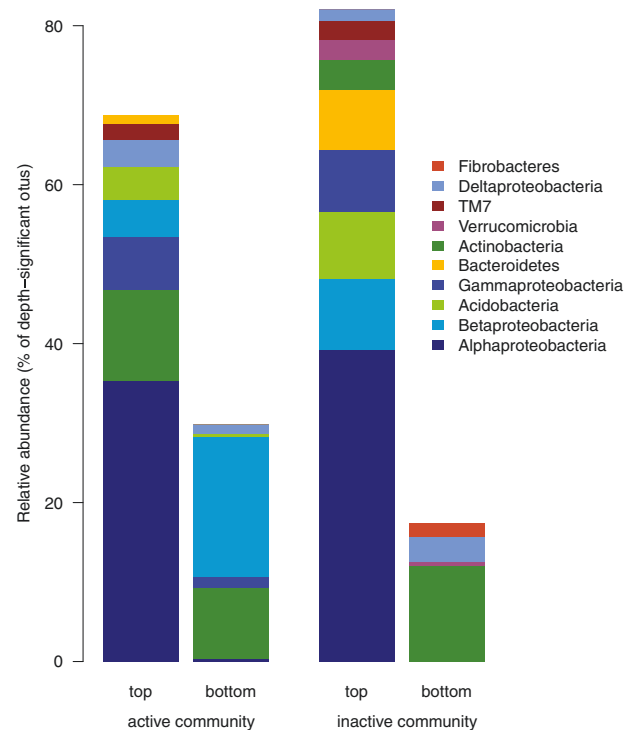
Fig. 3 Principal coordinates analysis (PCoA) of the UniFrac pairwise dissimilarity of the relative abundance of bacterial sequences based on 16S rRNA gene **a** and of the Bray–Curtis distance of the relative abundance of fungal sequences based on ITS rRNA region **b**. The active (red symbols) and the inactive (blue symbols) communities were determined in the infrequent (open symbols) and frequent (closed symbols) water input treatments, in three soil depth layers (top: 0–5 cm, triangles; middle: 10–15 cm, squares, bottom: 30–35 cm, circles)
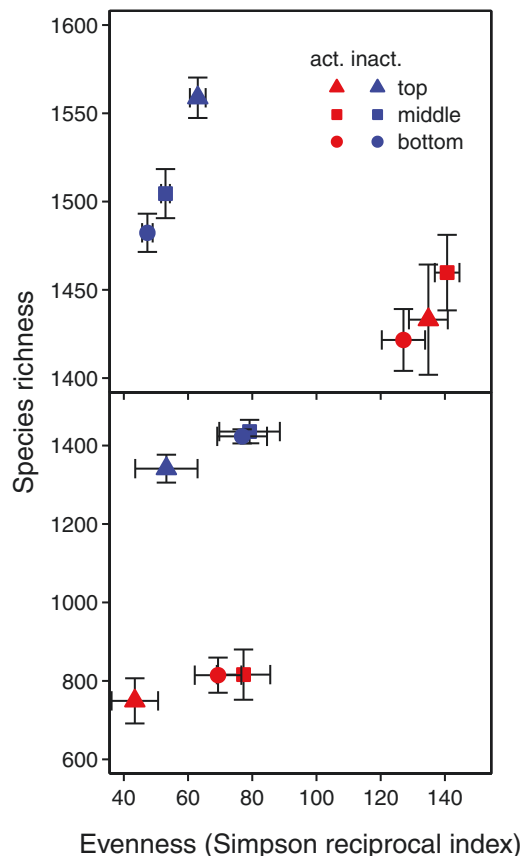

Fig. 4 Bacterial **a** and fungal **b** observed species richness and evenness (determined by the Simpson reciprocal index) in the active (red symbols) and inactive (blue symbols) communities at three soil depths (top: 0–5 cm, triangles; middle: 10–15 cm, squares; bottom: 30–35 cm, circles). Bars indicate mean ± standard error

Fig. 5 Relative abundance of the OTUs that responded significantly to soil depth in the active **a** and inactive **b** bacterial communities (phylum assignment provided, class provided for *Proteobacteria*). Based on their relative abundance, the OTUs clustered by top and bottom groups

or in the bottom and middle soil layers ("bottom group"). The active bacterial OTUs, which responded significantly to depth belonged predominantly to the *Proteobacteria* (mostly

Alphaproteobacteria, driven by *Sphingomonads* in the top group and *Betaproteobacteria* and *Actinobacteria* phyla in the bottom group, Fig. 5a). The depth-significant inactive bacterial OTUs were dominated by *Alphaproteobacteria* and *Actinobacteria* in the top and bottom group, respectively
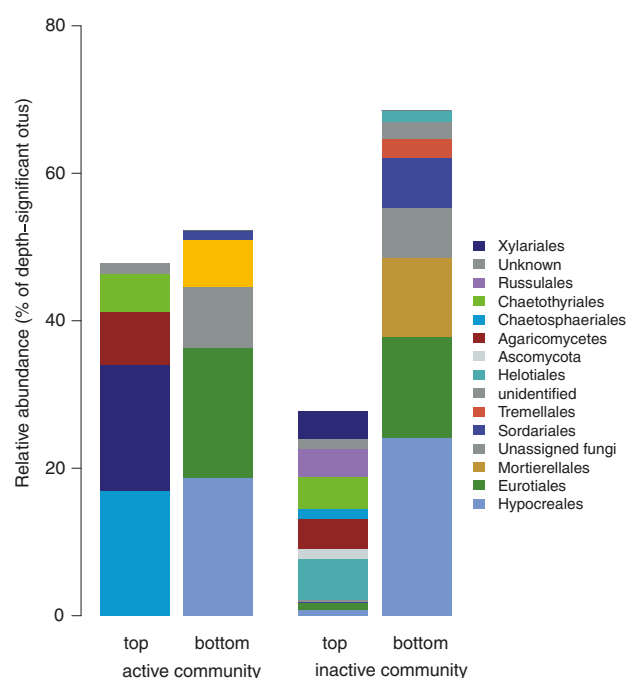
Fig. 6 Relative abundance of the OTUs that responded significantly to soil depth in the active **a** and inactive **b** fungal community (order provided). Based on their relative abundance, the OTUs clustered by top and bottom groups

(Fig. 5b). In the active fungal community, the significant OTUs belonged predominantly to the *Ascomycota* phyla (Fig. 6a): the top group was dominated by *Chaetosphaeriales* and *Xylariales* orders of *Sordariomycetes*, the bottom group by *Hypocreales* (*Sordariomycetes*) and *Eurotiales* (*Eurotiomycetes*). In the inactive fungal community, no taxonomic orders dominated clearly in the top group, which included mostly *Helotiales* and *Chaetothyriales* (*Leothimycetes* and *Eurotiomycetes* classes of *Ascomycota*, respectively), and *Agaricomycetes* (*Agaricomycetes* class of *Basidiomycota*), whereas the bottom group was dominated by *Hypocreales* and *Eurotiales* (*Sordariomycetes* class of *Ascomycota*, Fig. 6b).

Discussion

The root biomass of live plants responded differentially to precipitation pattern, in addition to the expected decreasing root density gradient with depth (Fig. 2). Root distribution response is expected to determine C rhizodeposition, thus affecting microbial community function under dry–wet cycles [9]. Under frequent water inputs, the coupling between plants and soil microbes was most apparent by the top soil layer, where most roots were located. However, under infrequent water inputs, this coupling in the top soil layer broke down, with no additional plant C transferred to microbial biomass despite larger root biomass. These results

are consistent with reports of reduced coupling between plants and soil microbes under drought [3, 50], which can even extend to ulterior drought events [7]. We found no effect of precipitation pattern on the amount of C photosynthesized, and no differences in ^{13}C label in root biomass among treatments, soil depth, or over time, suggesting that the coupling between plants and soil microbes was affected through changes in rhizodeposition or microbial access to rhizodeposits [20, 50–52].

Precipitation pattern effects in our system were limited, both at the community level and the OTU level. Several studies have also found little response of the present bacterial community composition to altered precipitation in the field, even after a year or more of treatment [53–55]. However, altered precipitation has been shown to affect the functional response of soil microbes to subsequent rewetting events [54, 56], suggesting that some response related to microbial activity could be expected. Our results suggest that both soil depth and microbial activity may have to be considered: at the community level, we found a significant effect of precipitation patterns on the soil microbial community that was inactive upon rewetting and located in the 0–5 cm soil layer. These effects were opposite for bacteria and fungi: infrequent precipitation regime decreased bacterial evenness and relatedness but increased fungal evenness. Our results are consistent with reduced bacterial relatedness under drier conditions [57], which prevail in the uppermost soil layer under infrequent water inputs as it dries out first and remains dry for several days. Thus, in our system, precipitation pattern legacy effects upon rewetting had a larger influence on the microbial seedbank than on the active players. This points toward the importance of phylogenetic differences in persistence and mortality as drivers of community change under changing water regimes.

Within a plant-soil system, we were able to characterize the effects of precipitation pattern and soil depth on the active and inactive soil microbial communities. Bacteria responded to rewetting with a large offset in community structure between the active and the inactive community, whereas fungi showed a less contrasted response (Fig. 3). The soil bacterial community tracked soil moisture conditions more closely than the soil fungal community did, which is consistent with the generally higher resistance to dry periods of fungi compared with bacteria [14, 16, 58–60], as well as the more stable properties of fungal-based food webs [18, 61]. The active fungal community was much less abundant (one-tenth) and less rich than the inactive fungal community (Fig. 4), indicating that only a few fungal groups were poised for quick growth upon rewetting, in contrast with the rapid reactivation of many phylogenetically clustered bacterial groups that is consistent with earlier studies [12, 13]. Indeed, the active bacterial community was only moderately less abundant and rich, and more

phylogenetically clustered than the inactive community. Furthermore, the phylogenetic clustering of active bacteria in response to wetting lends support to the idea that phylogeny may have ecological significance [62]. We hypothesize that the life strategy of the active bacteria was based on dynamic adjustment to transient water and nutrient availability whenever water film properties allowed it, whereas the inactive bacterial community relied more on maintaining functionality under drier conditions. In systems that are structured by water availability, such coexisting strategies likely drive the present bacterial communities (i.e., DNA-based), reflecting spatial patterns, whereas the potentially active (i.e., RNA-based communities) or actively growing communities track soil water availability [13, 63].

Soil depth was the main factor shaping the differences among soil bacterial and fungal communities and in our system after rewetting. The inactive bacterial community was more diverse and even in the top soil layer than in the deeper layers (Fig. 4), in line with the expected depth pattern of soil bacterial diversity [64]. In contrast, the inactive fungal community was less diverse and even in the top soil. Due to their filamentous life-form, fungi are less dependent than bacteria on water film continuity to access substrates. Increased fungal evenness and richness is often measured in the top soil [65, 66]. Nevertheless, the strong coupling between plants and soil microbes in the top soil under frequent water inputs may have favored the activity of fungi that are tightly involved with roots, resulting in increased dominance. The dominant depth-significant bacterial OTUs shifted with depth (Fig. 5), from *Alphaproteobacteria* in the top group to *Betaproteobacteria* and *Acidobacteria* in the active and inactive communities (respectively) in the bottom group, suggesting that a change in the identity of the dominant depth-responsive bacteria was involved in the depth effect measured at the community level. This change was clear in the active bacterial community, despite no apparent depth response of its α-diversity. Similarly, despite similar α-diversity patterns in the active and inactive fungal communities, the depth-responsive fungal OTUs in the inactive community were evenly distributed in the top group and dominated by *Hypocreales* (an order that includes many parasitic fungi) in the bottom group, whereas in the active community they shifted with depth (Fig. 6). These shifts in the identity of the dominant species, despite no change in α-diversity, suggests that different functional responses may have occurred at different depths.

Top soil is a dynamic nexus: where most root biomass is, where the legacy of precipitation pattern over time impacts the diversity of inactive fungal and bacterial communities, where plant-soil microbial coupling is tightest. Deeper soil layers show a different picture: different microbial diversity patterns, as well as different microbial groups that are actively growing or not upon rewetting. Our results indicate that the contrasting response between bacteria and fungi transcends soil depth. Changes in fungal:bacterial ratio have often been observed as a consequence of climate change, and are projected to have potential biogeochemical cycling, as well as for food web stability. Here, we extend this view and show that not only bacterial and fungal abundances respond differently to environmental drivers but that their relative activity is also affected. Thus, predicted changes in precipitation patterns may affect the activity patterns of microbial populations, based on their life strategy, which could result in large-scale effects on soil biogeochemical processes, including soil C budgets and nutrient cycling.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. IPCC. Climate change 2007: impacts, adaptation and vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, UK: Cambridge University Press; 2007.

2. Sowerby A, Emmett BA, Tietema A, Beier C. Contrasting effects of repeated summer drought on soil carbon efflux in hydric and mesic heathland soils. Glob Change Biol. 2008;14:2388–404.

3. Ruehr NK, Offerman C, Gessler A, Winkler BJ, Ferrio JP, Buchmann N, et al. Effects of drought on allocation of recent carbon: from beech leaves to soil respiration. New Phytol. 2009;184:950–61.

4. Bloor JMG, Bardgett RD. Stability of above-ground and below-ground processes to extreme drought in model grassland ecosystems: interactions with plant species diversity and soil nitrogen availability. Perspect Plant Ecol Evol Syst. 2012;14:193–204.

5. Hartmann AA, Niklaus PA. Effects of simulated drought and nitrogen fertilizer on plant productivity and nitrous oxide (N_2O) emissions of two pastures. Plant Soil. 2012;361:411–26.

6. Bimüller C, Dannenmann M, Tejedor J, von Lützow M, Buegger F, Meier R, et al. Prolonged summer droughts retard soil N processing and stabilization in organo-mineral fractions. Soil Biol Biochem. 2014;68:241–51.

7. Fuchslueger L, Bahn M, Hasibeder R, Kienzl S, Fritz K, Schmitt M, et al. Drought history affects grassland plant and microbial carbon turnover during and after a subsequent drought event. J Ecol. 2016;104:1453–65.

8. Hoover DL, Knapp AK, Smith MD. The immediate and prolonged effects of climate extremes on soil respiration in a mesic grassland. J Geophys Res Biogeosci. 2016;121:1034–44.

9. Canarini A, Dijkstra FA. Dry-rewetting cycles regulate wheat carbon rhizodeposition, stabilization and nitrogen cycling. Soil Biol Biochem. 2015;81:195–203.

10. Chou WW, Silver WL, Jackson RD, Thompson AW, Allen-Diaz B. The sensitivity of annual grassland carbon cycling to the quantity and timing of rainfall. Glob Change Biol. 2008;14: 1382–94.

11. Nielsen UN, Ball BA. Impacts of altered precipitation regimes on soil communities and biogeochemistry in arid and semi-arid ecosystems. Glob Chang Biol. 2015;21:1407–21.

12. Placella SA, Brodie EL, Firestone MK. Rainfall-induced carbon dioxide pulses result from sequential resuscitation of phylogenetically clustered microbial groups. Proc Natl Acad Sci USA. 2012;109:10931–6.

13. Barnard RL, Osborne CA, Firestone MK. Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. ISME J. 2013;7:2229–41.

14. de Vries FT, Shade A. Controls on soil microbial community stability under climate change. Front Microbiol. 2013;4:265.

15. Landesman WJ, Dighton J. Shifts in microbial biomass and the bacteria: fungi ratio occur under field conditions within 3 h after rainfall. Microb Ecol. 2011;62:228–36.

16. Barnard RL, Osborne CA, Firestone MK. Changing precipitation pattern alters soil microbial community response to wet-up under a Mediterranean-type climate. ISME J. 2015;9:947–57.

17. de Boer W, Folman LB, Summerbell RC, Boddy L. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiol Rev. 2005;29:795–811.

18. Gordon H, Haygarth PM, Bardgett RD. Drying and rewetting effects on soil microbial community composition and nutrient leaching. Soil Biol Biochem. 2008;40:302–11.

19. Ebrahimi A, Or D. Hydration and diffusion processes shape microbial community organization and function in model soil aggregates. Water Resour Res. 2015;51:9804–27.

20. Tecon R, Or D. Bacterial flagellar motility on hydrated rough surfaces controlled by aqueous film thickness and connectedness. Sci Rep. 2016;6:19409.

21. Dechesne A, Wang G, Gülez G, Or D, Smets BF. Hydration-controlled bacterial motility and dispersal on surfaces. Proc Natl Acad Sci USA. 2010;107:14369–72.

22. Wang G, Or D. Hydration dynamics promote bacterial coexistence on rough surfaces. ISME J. 2013;7:395–404.

23. Volkmann TH, Haberer K, Gessler A, Weiler M. High-resolution isotope measurements resolve rapid ecohydrological dynamics at the soil-plant interface. New Phytol. 2016;210:839–49.

24. Hasibeder R, Fuchslueger L, Richter A, Bahn M. Summer drought alters carbon allocation to roots and root respiration in mountain grassland. New Phytol. 2015;205:1117–27.

25. von Rein I, Gessler A, Premke K, Keitel C, Ulrich A, Kayler ZE. Forest understory plant and soil microbial response to an experimentally induced drought and heat-pulse event: the importance of maintaining the continuum. Glob Chang Biol. 2016a;22:2861–74.

26. Vance ED, Brookes PC, Jenkinson DS. An extraction method for measuring soil microbial biomass C. Soil Biol Biochem. 1987;19:703–7.

27. Lang SQ, Bernasconi SM, Früh-Green GL. Stable isotope analysis of organic carbon in small (μg C) samples and dissolved organic matter using a GasBench preparation device. Rapid Commun Mass Spectrom. 2012;6:9–16.

28. Blazewicz SJ, Schwartz E. Dynamics of ^{18}O incorporation from $H_2^{18}O$ into soil microbial DNA. Microb Ecol. 2011;61:911–6.

29. Muyzer G, de Waal EC, Uitierlinden AG. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol. 1993;59:695–700.

30. White TJ, Bruns T, Lee S, Taylor JW. PCR protocols: a guide to methods and applications. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. New York: Academic Press Inc.; 1990. p. 315–22.

31. Berry D, Ben Mahfoudh K, Wagner M, Loy A. Barcoded primers used in multiplex amplicon pyrosequencing bias amplification. Appl Environ Microbiol. 2011;77:7846–9.

32. Takahashi S, Tomita J, Nishioka K, Hisada T, Nishijima M. Development of a prokaryotic universal primer for simultaneous analysis of Bacteria and Archaea using next-generation sequencing. PLoS ONE. 2014;9:e105592.

33. Zhang J, Kobert K, Flouri T, Stamatakis A. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics. 2014;30:614–20.

34. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010b;7:335–6.

35. Rognes T, Flouri T, Nichols B, Quince C, Mahe F. VSEARCH: a versatile open source tool for metagenomics. PeerJ. 2016;4:e2584.

36. Caporaso JG, Bittinger K, Bushman FD, DeSantis TZ, Andersen GL, Knight R. PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics. 2010a;26:266–7.

37. Price MN, Dehal PS, Arkin AP. FastTree: computing large minimum-evolution trees with profiles instead of a distance matrix. Mol Biol Evol. 2009;26:1641–50.

38. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010;26:2460–1.

39. McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, et al. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. ISME J. 2012;6:610–8.

40. Altschul SF, Gish W, Webb M, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215:403-10.

41. Abarenkov K, Nilsson RH, Larsson KH, Alexander IJ, Eberhardt U, Erland S, et al. The UNITE database for molecular identification of fungi – recent updates and future perspectives. New Phytologist. 2010;186:281–5.

42. Lozupone C, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol. 2005;71:8228–35.

43. Webb CO. Exploring the phylogenetic structure of ecological communities: an example for rain forest trees. Am Nat. 2000;156:145–55.

44. Webb CO, Ackerly DD, M MA, Donoghue MJ. Phylogenies and community ecology. Annu Rev Ecol Syst. 2002;33:475–505.

45. Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, et al. Picante: R tools for integrating phylogenies and ecology. Bioinformatics. 2010;26:1463–4.

46. R Core Team (2014). R: a language and environment for statistical computing. Vol. ISBN 3-900051-07-0: Vienna, Austria.

47. Anderson MJ. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 2001;26:32–46.

48. Strimmer K. fdrtool: a versatile R package for estimating local and tail area-based false discovery rates. Bioinformatics. 2008;24: 1461–2.

49. Al Majou H, Bruand A, Duval O, Le Bas C, Vautier A. Prediction of soil water retention properties after stratification by combining texture, bulk density and the type of horizon. Soil Use Manag. 2008;24:383–91.

50. Fuchslueger L, Bahn M, Fritz K, Hasibeder R, Richter A. Experimental drought reduces the transfer of recently fixed plant carbon to soil microbes and alters the bacterial community composition in a mountain meadow. New Phytol. 2014;201:916–27.

51. Moyano FE, Manzoni S, Chenu C. Responses of soil heterotrophic respiration to moisture availability: an exploration of processes and models. Soil Biol Biochem. 2013;59:72–85.

52. von Rein I, Kayler ZE, Premke K, Gessler A. Desiccation of sediments affects assimilate transport within aquatic plants and carbon transfer to microorganisms. Plant Biol (Stuttg). 2016b;18: 947–61.

53. Cruz-Martinez K, Suttle KB, Brodie EL, Power ME, Andersen GL, Banfield JF. Despite strong seasonal responses, soil microbial consortia are more resilient to long-term changes in rainfall than overlying grassland. ISME J. 2009;3:738–44.

54. Evans SE, Wallenstein MD. Soil microbial community response to drying and rewetting stress: does historical precipitation regime matter? Biogeochemistry. 2012;109:101–16.

55. Slaughter LC, Weintraub MN, McCulley RL. Seasonal effects stronger than three-year climate manipulation on grassland soil microbial community. Soil Sci Soc Am J. 2015;79:1352.

56. Zeglin LH, Bottomley PJ, Jumpponen A, Rice CW, Arango M, Lindsley A, et al. Altered precipitation regime affects the function and composition of soil microbial communities on multiple time scales. Ecology. 2013;94:2334–45.

57. Taketani RG, Lanconi MD, Kavamura VN, Durrer A, Andreote FD, Melo IS. Dry season constrains bacterial phylogenetic diversity in a semi-arid rhizosphere system. Microb Ecol. 2017;73:153–61.

58. Jumpponen A, Jones KL. Tallgrass prairie soil fungal communities are resilient to climate change. Fungal Ecol. 2014;10:44–57.

59. Bapiri A, Bååth E, Rousk J. Drying–rewetting cycles affect fungal and bacterial growth differently in an arable soil. Microb Ecol. 2010;60:419–28.

60. Yuste JC, Penuelas J, Estiarte M, Garcia-Mas J, Mattana S, Ogaya R, et al. Drought-resistant fungi control soil organic matter decomposition and its response to temperature. Glob Change Biol. 2011;17:1475–86.

61. de Vries FT, Liiri ME, Bjornlund L, Bowker MA, Christensen S, Setala HM, et al. Land use alters the resistance and resilience of soil food webs to drought. Nat Clim Change. 2012;2:276–80.

62. Morrissey EM, Mau RL, Schwartz E, Caporaso JG, Dijkstra P, van Gestel N, et al. Phylogenetic organization of bacterial activity. ISME J. 2016;10:2336–40.

63. Angel R, Pasternak Z, Soares MI, Conrad R, Gillor O. Active and total prokaryotic communities in dryland soils. FEMS Microbiol Ecol. 2013;86:130–8.

64. Eilers KG, Debenport S, Anderson S, Fierer N. Digging deeper to find unique microbial communities: the strong effect of depth on the structure of bacterial and archaeal communities in soil. Soil Biol Biochem. 2012;50:58–65.

65. Jumpponen A, Jones KL, Blair J. Vertical distribution of fungal communities in tallgrass prairie soil. Mycologia. 2010;102: 1027–41.

66. Vargas-Gastelum L, Romero-Olivares AL, Escalante AE, Rocha-Olivares A, Brizuela C, Riquelme M. Impact of seasonal changes on fungal diversity of a semi-arid ecosystem revealed by 454 pyrosequencing. FEMS Microbiol Ecol. 2015;91:fiv044.

5. Chapter III

Contrasting water and N-availability patterns have a legacy effect on plant-microbial response to rewetting and biogeochemical cycling

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Contrasting water and N-availability patterns have a legacy effect on plant-microbial response to rewetting and biogeochemical cycling

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Keywords

Abstract

Shifts in the frequency and magnitude of rain events (precipitation patterns) associated with climate change may negatively impact ecosystem N and C cycling through its effect on plant morphological and physiological strategies as well as soil bacterial and fungal activity. The objective of this study was to determine how precipitation history shapes the microbial community response to rewetting as well as the plant-microbial competition for N and how the N status of the system may modulate the effect of precipitation patterns. First we describe how a history (12 weeks) of contrasting precipitation and N input sets the scene for the final rewetting event through its impact on plant biomass production, microbial communities (potentially active and seed banks) and N cycling within the system. Second we demonstrate how this legacy effect shapes the potentially active bacterial and fungal response to rewetting over a 29 hour period using 16S and 18S total RNA sequencing. Plant microbial coupling and plant-microbial competition for N over the time post rewetting were documented using ^{13}C-CO_2 and ^{15}N- NH_4^+ labeling respectively. Despite contrasting effect of precipitation and N input history on plant physiology, fungal:bacterial ratio, microbial community composition and C availability to the microbes, this did not alter the timing of the potentially active bacterial and fungal response to rewetting. Regardless of precipitation or N input history, potentially active bacteria responded with a small shift in community composition within 1 hour of rewetting but did not change further for the remaining 28 hours analyzed. Contrastingly, the potentially active fungi did not respond to rewetting within the 29 hour time period post rewetting. Immediately after rewetting microbes outcompete plants for N but over time plant competitiveness increases regardless of precipitation or N input history. However, we did find that a history of favorable conditions for the plant increases its overall competitiveness for N over microbes. Soil CO_2 efflux upon rewetting was higher from systems with a history of frequent precipitation input and was not modulated by N availability. A short N_2O flux

immediately post rewetting was captured from soils with a combined history of frequent precipitation and high N input only. Our evidence suggests that the predicted shift toward more extreme fluctuations in soil moisture may have negative implications for ecosystem functioning due to altered N dynamics between plants and soil microbes and reduced soil C sequestration potential.

Introduction

Water availability affects both plant and soil microbial activity and growth and is thus a key regulator of nutrient cycling and ecosystem functioning. Predicted shifts in frequency and magnitude of rain events may thus have important consequences for ecosystem-level functioning (Huntington et al., 2006, IPCC 2007, Knapp et al., 2008, Gobiet et al., 2014). Anthropogenic input of reactive nitrogen (N) into terrestrial systems has increased massively (Fowler et al., 2015), shifting the N-cycling balance of many ecosystems and promoting emission of radiatively active N_2O (Galloway et al., 2004, Philippot et al., 2011) and eutrophication of water through leaching (Galloway et al., 2004). The interactive effects of changes in water and N availability on ecosystem functioning is frequently highlighted but is yet to be fully understood (Wang et al., 2015, Zhang et al., 2015).

Plants have morphological and physiological strategies to cope with fluctuating water availability which may impact N cycling processes through the quantity and quality of rhizodeposits (Paterson et al., 2003, Ruiz-Ruenda et al., 2009), their impact on soil structure, the aeration of the soil matrix through root respiration and competition for resources such as N. Morphological strategies of plants include the increased allocation of carbon and nutrients towards production of root biomass during prolonged dry periods to maximize potential access to water (Poorter et al., 2012, Eziz et al., 2017). The re-allocation of nutrients towards root

biomass during drought commonly occurs at the expense of aboveground biomass but it has been shown that plants may overcompensate for this once abundant water is available (Hofer et al., 2016), which may be detrimental in subsequent droughts (Wang et al., 2007). Overall, more intense variability in water availability has been shown to reduce net above ground biomass yield (Grant et al., 2014). Physiological strategies of plants to cope with water fluctuations is predominantly due to stomatal control of leaves. During dry periods plants may close their leaf stomata to limit water loss but this results in a reduced rate of photosynthesis and thus the amount of C the plants may acquire and allocate below ground (Ruehr et al., 2009, Hasibeder et al., 2014, Canarini and Dijkstra, 2015, Fuchslueger et al., 2016). This indicates a potential decrease in labile C from photosynthate availability for heterotrophic microbes, a time when microbial demand for C is high (Hasibeder et al., 2014; von Rein et al., 2016), potentially turning an ecosystem from being a net C sink to a C source (Reichstein et al., 2013).

N availability may modulate the effect of precipitation on plant morphological and physiological strategies. The morphological strategy of increased root: shoot ratio in response to drought may be due to either decreased shoot growth (Skinner and Comas, 2010) or increased root growth (Wedderburn et al., 2010) depending on availability of nutrients such as inorganic N. Low N-availability may also further exacerbate the effect of drought on the physiological strategies of plants as rubisco, a leaf protein which is essential for C assimilation, is strongly affected by N availability (Shangguan et al., 2000, DaMatta et al., 2002, Wang et al., 2015). Studies suggest that plants are thus better able to cope with water fluctuations when sufficient N is available (Saneoka et al., 2004).

The activity of soil microbes is governed both by water availability as well as plant physiological functioning. Cycles of drying and rewetting result in contrasting environments for soil microbes regarding aeration status, osmotic pressure, motility and access to nutrients. Microbes have semi- permeable membranes, live in water films (bacteria) or in contact with

water films (fungi) and may have varying intrinsic resistance to fluctuations in water potential. First, during periods of drought, microbes actively invest significant amounts of C and N into the accumulation of intracellular osmolytes to prevent cellular dehydration (Bonaterra et al., 2005, Boot et al., 2013, Canarini and Dijkstra, 2015). The thresholds for resistance to dehydration may vary extensively within the diverse microbial communities (Landesman and Dighton, 2011; de Vries and Shade, 2013; Barnard et al., 2015). Second, heightened resistance to drying has been shown in fungi and actinomyces bacteria which due to their extensive hyphal network have more extensive access to water in distant micropores (Gordon et al., 2008; de Vries et al., 2012) and for Gram-positive bacteria whose thick peptidoglycan cell wall offers resistance (Schimel et al., 2007, Manzoni and Katul, 2014, Fuchslueger et al., 2016). However, it is not only the level of resistance to drying but also the response to rewetting and community resilience which determines the microbial water-related life-strategies. Upon rewetting, microbes need to actively pump out or metabolise the intracellular osmolytes to avoid cellular lysis. Microbes which are able to respond quickly, when conditions become more favorable, can take advantage of an abundance of accumulated, readily available and accessible nutrients and outgrow slower responding organisms (Barnard et al., 2013, de Vries et al., 2013, Griffiths and Philippot, 2013). Quick responding and fast growing microbes commonly include the often more drought-sensitive Gram-negative bacteria (Steenwerth et al., 2005). Persistent shifts in precipitation frequency may thus alter soil microbial communities toward those which are better able to withstand and thrive under the new environmental conditions (Owens et al., 2012; Placella et al., 2012; Sistla and Schimel, 2012), leading to changes in the dominant groups which may carry different biogeochemical abilities (Evans and Wallenstein, 2012), thus impacting ecosystem functioning (Lennon and Jones, 2011, Wallenstein and Hall, 2012). Additionally, communities with a history of exposure to more extreme moisture fluctuations

are adapted to these and remain relatively unchanged in response to rewetting (Fierer et al., 2003; Evans and Wallenstein, 2012).

Both plants and soil microbes require N for growth and thus compete for its inorganic forms such as nitrate (NO_3^-) and ammonium (NH_4^+) and to a lesser extent small organic molecules such as amino acids. Soil ammonia oxidisers and nitrifiers oxidize NH_4^+ sequentially to NO_3^- under aerobic conditions, the latter being the substrate for denitrification, an alternate respiratory pathway under anaerobic conditions. Denitrification leads to the release of inert di-nitrogen (N_2) or the potent greenhouse gas nitrous oxide (N_2O) back into the atmosphere. Soil N cycling is thus tightly coupled to water availability, both directly by influencing microbial activity and indirectly by determining the aeration status of the soil environment.

The objectives of this study were to determine how precipitation history shapes the microbial community response to rewetting as well as the plant-microbial competition for N and how the N status of the system may modulate the impact of precipitation patterns. First, we focused on how contrasting precipitation and N input histories for 12 weeks could set a potentially contrasting scene for a rewetting response. Treatment effects were determined on plant performance, microbial communities (present and potentially active bacterial and fungal communities), soil N transformations (potential nitrification and denitrification as well as the abundance of selected N cycling genes) and N pools (plant biomass, microbial biomass, inorganic soil N pools). Second, we evaluated how the plant-soil systems responded to a rewetting event over a 29 hour time period, monitoring the response of the potentially active bacterial and fungal communities, tracing the allocation of C and N between microbes and plants using stable isotope labelling (^{13}C-CO_2 and ^{15}N- NH_4^+) and relating them to soil CO_2 and N_2O efflux rates.

Materials and Methods

Experimental set-up

Sandy natural grassland soil (79.4% sand, 7.7% silt and 12.8% clay) with 15.8 g kg^{-1} organic carbon and 1.5 g kg^{-1} organic nitrogen and a cation exchange capacity of 7.33 cmol+ kg^{-1} was used to fill 4 L mesocosms (average 16 cm diameter) to a uniform bulk density of 1.2 g cm^{-3}. A PVC cup (6.5 cm diameter, 10 cm high) was inserted (1 cm deep) in the center of each mesocosm for CO_2 and N_2O efflux rate measurements. Winter wheat (Triticum aestivum cv Soissons) seeds were germinated in the same soil and after 4 days, 8 seedlings were transplanted equidistant from each other around the PVC cup of each mesocosm. After a plant establishment period (1 week) in which all mesocosms received daily precipitation input, two contrasting precipitation input regimes combined with two levels of N input (total of four experimental treatments), were applied for a total of 12 weeks. All mesocosms received the same total amount of water for the duration of the experimental treatments. The frequent precipitation treatment received a daily input of the minimum volume of water (2.8 – 7 mm d^{-1}) required to prevent plant wilting. The infrequent precipitation treatment received water inputs every 4-5 days. The volume of water received by the infrequent precipitation treatment was the accumulated equivalent received by the frequent treatment over the same period (up to 35mm). Soil moisture was monitored in three mesocosms per treatment (EC-5 sensors, Decagon Devices Inc., Pullman, WA, USA) at 5 and 18 cm depth. N input was in the form of NO_3^- with high N treatment receiving 140 mg of N per L and the low N treatment receiving 70 mg of N per L.

Photosynthesis rate

Leaf gas exchange rates were measured daily between 10:00 and 12:00, for 5 days during the 8-9th week of treatment (12th cycle) using a portable photosynthesis system (Li-6400 XT, Li-Cor, Lincoln, NE, USA). In the frequent precipitation treatment, leaf gas exchange was measured after the daily water input. In the infrequent precipitation treatment the mesocosms received their water input just prior to the first leaf gas measurement but did not receive any further water inputs within the 5 day period. Measurements were taken from the youngest, fully developed leaf of 5 mesocosms per treatment, for 5 minutes each.

Pre-wet: plant and soil sampling

After 12 weeks of treatment (corresponding to 16 infrequent watering cycles), before watering of the frequent treatment, when all mesocosms had roughly the same soil moisture content, 5 mesocosms per treatment were harvested for pre-wet as well as isotopic natural abundance baseline measurements. Plants were harvested and measured for leaf surface area (Li-3100C, Li-Cor), dry biomass (live and dead leaves, stems and roots) and root length density (WinRHIZO software, Regent Instruments Inc., Canada). Additionally, plant C and N content as well as ^{13}C and ^{15}N signature was measured for each of the plant components (dead aboveground, live aboveground and belowground biomass) by combustion in an elemental analyzer (NC 2500 elemental analyzer, Carlo Erba, Milan, Italy) coupled with an isotope ratio mass spectrometer (Delta S, Finnigan MAT, Bremen, Germany). Measurements were calibrated using commercial standards with known $\delta^{13}C$ and $\delta^{15}N$ values, resulting in a precision of 0.1‰. Six soil cores (10 cm deep, 2.4 cm diameter) were collected and homogenized. Soil samples were frozen in liquid N and kept at -80°C before DNA and RNA analysis (see below). The remaining soil was sieved (2 mm mesh) and stored at 4°C before

measuring potential nitrification and denitrification rates, soil inorganic N content, as well as microbial biomass C and N.

^{13}C labelling

Prior to wet-up, plants were labeled with ^{13}C-CO_2 as follows. An airtight transparent plastic tent was installed around the mesocosms. $^{12}CO_2$ and ^{13}C-CO_2 concentration in the tent were monitored using a cavity ring-down spectroscopy analyzer (G2201-i, Picarro, Santa Clara, CA, USA). The CO_2 was scrubbed down to 200 µmol mol^{-1}, then labelling was performed by injecting ^{13}C-CO_2 while fans ensured air circulation within the tent. $^{12}CO_2$ and $^{13}CO_2$ concentrations averaged 401 and 476 µmol mol^{-1} (maximum: 658 and 1387 µmol mol^{-1}), respectively.

Wet-up: ^{15}N labeling, plant, soil, trace gas exchange rates

After ^{13}C labeling, all mesocosms were watered with $^{15}NH_4Cl$ (0.56g $^{15}NH_4Cl$ m^{-2}, 98 atom % ^{15}N) amounting to a 12 mm rainfall. Soil CO_2 and N_2O gas exchange rates were measured at 1.4, 3.2, 6.1, 10.6, 17.6 and 28.6 hours post rewetting. Briefly, 3 consecutive gas samples were collected through a rubber septum at 15 minute intervals from the headspace of each PVC cup, and injected into pre-evacuated vials (Labco, Lampeter, Wales,UK). N_2O, CO_2 and CH_4 concentrations in the gas samples was measured using a gas chromatograph equipped with a flame ionization and an electron capture detector (Agilent 6890 Technologies Inc., Santa Clara, CA, USA). Gas exchange rate was calculated from the increased concentration in the collar headspace over time. Plant and soil samples were taken and measured as described above (pre-wet section), at 1.0, 2.2, 3.3, 4.8, 7.8, 11.8, 19.4 and 29.2 hours after rewetting.

Soil extractable inorganic N pools, nitrification and denitrification potentials

Soil nitrate and ammonium were extracted from 10 g fresh samples in 50 mL KCl (1M) by shaking for 1 hour (80 rpm) at room temperature. Extractable nitrate and ammonium concentration were quantified in the supernatant by colorimetry (BPC global 240 photometer, Rome, Italy).

Potential nitrification and denitrification assays were performed on the pre-wet samples only as these are unlikely to change in the time frame of this experiment. Potential nitrification activity (PNA) measurement was based on the ISO 15685 protocol. In short, 1.4 mM $(NH_4)_2SO_4$ and 500 mM of $NaClO_4$ were added to 10 g fresh soil. The rate of NH_4^+ was then estimated by measuring nitrite accumulation in solution after 2, 4 and 6 h using a colorimetric assay (Kandeler et al., 1995) quantified on a spectrophotometer (DU 800, Beckman Coulter, Brea, CA, USA).

Denitrification end-product ratio, potential denitrification activity as well as potential nitrous oxide production were measured by acetylene inhibition (Yoshinari et al., 1977). For each sample, 2 subsamples of 10g fresh soil were amended with 20 mL distilled water before adding a final concentration of 3mM KNO_3, 1.5 mM succinate, 1 mM glucose and 3 mM acetate. One of each of the pairs of subsamples was then further amended with 0.1 atm partial pressure acetylene before 30 min incubation (25°C, shaking 175 rpm). Finally, gas samples were collected from the head space every 30 min for 150 min (Pell et al., 1996). The N_2O concentration in each of the gas samples was measured using a gas chromatograph (TraceGCUltra, ThermoScientific) equipped with an EC detector. The denitrification end-product ratio was determined by dividing the potential rate of N_2O production by the potential denitrification activity $[rN_2O/r(N_2O+N_2)]$.

Microbial biomass C and N

Soil microbial biomass C and N were determined 1 and 29 h post rewet by chloroform fumigation extraction (Vance et al 1987). Subsamples of 10 g fresh soil were extracted in 50 mL 0.03 M K_2SO_4 and organic C and N contents determined using an automated analyzer (DimaTOC 2000, Dimatec, Essen, Germany). Microbial constituents are rendered extractable by fumigation, and microbial C and N calculated by difference in extractable C or N between fumigated and un-fumigated soils, accounting for the extraction efficiency $k_{EC}=0.45$ for C (Vance et al., 1987) and $k_{EN}=0.54$ for N (Brookes et al., 1985). The microbial biomass extracts of all time points were then lyophilized and used to measure microbial ^{13}C and ^{15}N signature by combustion in an elemental analyzer (EA-110, Carlo Erba, Milano, Italy) coupled with an isotope ratio mass spectrometer (Delta-S Finnigan MAT, Bremen, Germany) by a variable open split interface (ConFlo II, Finnigan MAT, Bremen, Germany). The abundance of ^{13}C and ^{15}N were then expressed in δ notation (‰) relative to the Vienna Pee Dee Belemnite (VPDB) and atmospheric air standards, respectively.

Nucleic acid extraction and reverse transcription

Total RNA was extracted from 2 g of soil using the RNA PowerSoil Total RNA Isolation Kit (MO BIO Laboratories Inc. Carlsbad, CA) and total genomic DNA was co-extracted but eluted separately, using the RNA PowerSoil DNA Elution Accessory Kit (MO BIO Laboratories Inc. Carlsbad, CA). Genomic DNA contamination in the total RNA extracts was removed using DNase Max Kit (MO BIO Laboratories Inc. Carlsbad, CA) following which the quality and quantity of extracted RNA and DNA were measured using a NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Scientific, Waltham, MA, USA). Finally, a target template of 100

ng of total RNA was reverse transcribed to cDNA using iScript cDNA Synthesis Kit (BIO-RAD, Hercules, CA, USA).

Amplicon generation and MiSeq sequencing

cDNA from all timepoints and DNA from the prewet time point were sequenced using Illumina MiSeq sequencing (Microsynth AG, Balgach, Switzerland). Amplicons were generated using a two-step polymerase chain reaction protocol. In the first PCR step, fusion primers 341F (5'-CCTACGGGRSGCAGCAG-3') and 805R (5'-GACTACCAGGGTATCTAAT-3') (Takahashi et al., 2014) were used to amplify the V3-V4 hypervariable region of the bacterial 16S rRNA gene and fusion primers FF390 (5'-CGATAACGAACGAGACCT-3') and FR1 (5'-SNCCATTCAATCGGTANT -3') (Vainio and Hantula 2000) were used to amplify the V7-V8 hypervariable region of the fungal 18S rRNA gene. Overhang adaptors were TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG (forward) and GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG (reverse).

PCR was carried out in duplicate 15µL reactions containing 7.5 µL Phusion High-Fidelity PCR Master Mix (Thermo Scientific, Waltham, MA, USA), 0.25 µM of each primer, 250 ng T4 gp32 (MPBio, Santa Ana, CA, USA) and 1 ng template cDNA or DNA. Thermal cycling conditions were 98°C for 3 min followed by 25 cycles (for 16S) or 30 cycles (for 18S) of 98°C for 30 s, 55°C for 30 s and 72°C for 30 s, with a final extension at 72°C for 10 min. Duplicate first step PCR products were pooled and then used as template for the second step PCR.

Multiplexing index-sequences were added to the overhang adapters by performing a second PCR amplification using a unique multiplex primer pair (Eurogentec, Liège, Belgium) combination for each sample. For 16S the reaction was carried out in duplicate 30 µL volumes containing 15 µL Phusion High-Fidelity PCR Master Mix (Thermo Scientific), 6 µL of first

step PCR product and 1 µM (for 16S) or 0.167 µM (for 18S) of a forward and a reverse multiplex primer. Thermal cycling conditions were 98°C for 3 min followed by 8 cycles (for 16S) or 10 cycles (for 18S) of 98°C for 30 s, 55°C for 30 s and 72°C for 30 s, with a final extension at 72°C for 10 min. Duplicate second step PCR products were pooled and then visualized in 2% agarose gel to verify amplification and size of amplicons (around 630 bp for bacterial 16S amplicon and around 550 bp for the fungal 18S amplicon).

PCR products were pooled, cleaned-up and purified using AMPure XP beads (Beckman-Coulter). Next they were quantified with picogreen (Thermo Scientific), followed by equimolar pooling and purification in a gel. Sequencing was performed on MiSeq (Illumina, 2x250 bp, MiSeq reagent kit v2, 500 cycles) and demultiplexing as well as trimming of Illumina adaptors and barcodes was done with Illumina MiSeq Reporter software (version 2.5.1.3).

Quantification of bacterial and fungal communities

Abundance of bacterial and fungal communities as well as potential nitrifying and denitrifying communities were assessed by quantitative polymerase chain reaction (qPCR). The bacterial and fungal abundance was quantified by targeting the 16S (Takahashi et al., 2014) and 18S (Vainio and Hantula 2000) ribosomal RNA genes respectively.

The denitrifier communities were assessed by quantifying nirK and nirS genes as markers for for N_2O producers (Henry et al., 2004, Kandeler et al., 2006) and nosZ1 and nosZ2genes as markers for N_2O reducers (Jones et al., 2013). The nitrifier communities were assessed by quantifying the amoA gene abundance as a marker for bacterial (AOB) and archaeal (AOA) ammonia oxidation (Bru et al., 2011). Quantification was based on the increasing intensity in fluorescence of the SYBR Green dye during amplification in a Step One Plus (Life Technologies, Carlsbad, CA, USA). QPCR reactions were carried out in triplicate 15 µl

reactions containing 7.5 µl SYBR green PCR Master Mix (Takyon Low ROX SYBR 2x MasterMix blue dTTP, Eurogentec, France), 1 µM of each primer , 250 ng of T4 gene 32 (QBiogene, Illkrich, France) and 1ng of DNA. Standard curves were obtained from serial dilutions of linearized plasmids containing the cloned genes. No inhibition was detected and template-free controls resulted in negligible amplifications.

Bioinformatics analysis

Sequences were assembled using PEAR (Zhang *et al.,* 2014) and the QIIME pipeline (Caporaso *et al.,* 2010b) was used to conduct quality checks. Short sequences (<350bp) were removed for both 16S and 18S. OTU clustering was performed using VSEARCH (Rognes *et al.,* 2016) including reference-based as well as *de-novo* chimera detection, using Greengenes and Silva reference databases for 16S and 18S respectively. The identity threshold was set at 94% for 16S data, based on replicate sequencings of a bacterial mock community containing 40 bacterial species. For the 18S data a threshold of 97% was set as we did not have a mock community. Sequence alignment for each OTU was performed using PyNAST (Caporaso *et al.,* 2010a) and a phylogenetic tree was constructed using FastTree (Price *et al.,* 2009). Taxonomy for 16S data were assigned using UCLUST (Edgar, 2010) with the latest version of the Greengenes database (v.05/2013, McDonald et al 2012). Taxonomy for 18S data were assigned using BLAST (Altschul *et al.,* 1990) and the latest version of the Silva reference database. Bacterial and fungal α-diversity metrics were calculated based on rarefied OTU tables (9 000 sequences per sample for 16S and 10 000 sequences per sample for 18S) and UniFrac distance matrices (Lozupone and Knight, 2005) created.

Statistical analyses

Statistical analyses were performed using R 3.1.2 (R Core Team, 2014) on n=5 replicate mesocosms per treatment. Data were assessed by analysis of variance, using precipitation and N-input regime and time post rewetting as fixed effect variable. Bacterial and fungal UniFrac distances were analyzed by non-parametric permutational multivariate analysis of variance (Anderson, 2001). The OTUs responding significantly to experimental treatment were detected using the ANOVA model described above, followed by a test to account for false discovery rates (Strimmer, 2008). The OTUs that responded significantly to precipitation input history, N input history, or re-wetting over time were hierarchically clustered into groups, and the significance of the clustering verified against random clustering.

Results

Treatment history and plant performance

Following an initial plant establishing period, contrasting soil moisture fluctuations of frequent and infrequent precipitation input were recorded at 5cm and 18cm soil depth (Fig. 1). Initially all systems experienced a net decrease in soil moisture until leveling out for the remainder of the treatment period. Plants with frequent precipitation inputs had 35.4% higher (p=0.043) average rates of photosynthesis over a 5 day watering cycle than plants with infrequent precipitation inputs (77 ± 5 µmol CO_2 m^{-2} soil s^{-1} under frequent vs. 57.2 ± 4.6 µmol CO_2 m^{-2} soil s^{-1} under infrequent precipitation inputs) (Fig. S1). Both precipitation and N input shaped the plant biomass production (Fig. 2). Infrequent precipitation inputs significant reduced root and live aboveground biomass (-62% and -39%, respectively, p<0.001 for both), and significantly increased dead leaf biomass (p<0.001). Infrequent precipitation thus led to a shift in the root:shoot ratio in favor of shoots. High N application significantly decreased root and

dead leaf biomass (P<0.001 for both), regardless of precipitation input level. High N application significantly reduced live aboveground biomass under infrequent but not frequent precipitation inputs (precipitation $\times$ N input interaction, p=0.002).

N transformations and N pools

Infrequent precipitation inputs significantly stimulated potential nitrification (P<0.001) (Fig. 3a), potential denitrification (P<0.001) as well as potential N_2O production (P=0.034) (Fig. 3b). The denitrification gaseous end-product ratio on the other hand was not significantly affected by precipitation or N input history (Fig. 3c). The abundance of molecular marker genes for N transformations (Fig. S2) confirmed this observation, with a significant increase in the abundance of archeal ammonium oxidizing amoA genes (AOA), of genes necessary for the reduction of nitrite to nitric oxide (nirK, nirS) and of genes responsible for the reduction of nitrous oxide to di-nitrogen (nosZ1 and nosZ2). Bacterial amoA gene (AOB) abundance was left unaffected by precipitation and N input and was about 44.7 times less abundant than archaeal amoA genes in all systems. Both precipitation and N input significantly impacted the inorganic N pools whilst the organic N pools were shaped by N input only (Fig. 4). Infrequent precipitation input significantly decreased soil NH_4^+ and NO_3^- pool sizes (P<0.001 for both). Regardless of watering history, high N application significantly increased NO_3^- pool size (P<0.001), but significant affected NH_4^+ pools only under infrequent precipitation inputs (precipitation input $\times$ N input, P<0.001). Irrespective of precipitation input, high N input significantly reduced microbial biomass N. Contrastingly, high N input significantly increased plant N uptake (p<0.001), particularly in systems which were additionally subjected to infrequent precipitation inputs (precipitation $\times$ N interaction, P=0.013).

Present microbial community composition and diversity

Regardless of precipitation or N input history, bacterial community size, estimated by bacterial 16S gene abundance, was larger than fungal community size, estimated by fungal 18S gene abundance (Fig. 5). Infrequent precipitation input significantly increased (p=0.033) bacterial 16S gene abundance but did not significantly impact fungal 18S gene abundance. This led to a shift in the bacterial: fungal ratio from 18.1 times more bacterial 16S than fungal 18S copies under frequent precipitation inputs to 29.7 times more bacterial 16S than fungal 18S copies under infrequent precipitation inputs. Whilst fungi were not significantly impacted by the precipitation input treatment, we found a small, marginally significant trend (p=0.072) towards lower 18S abundance under high N input.

Contrasting precipitation inputs led to large, significant differences in the present bacterial (Fig. S3a) and present fungal (Fig. S3b) community composition, explaining 33% (p<0.001) and 35% (p<0.001) of their variance respectively. N input had a smaller but significant impact on the present bacterial community composition (8.3% variance, p=0.036) but did not result in a significant shift in the present fungal communities. Infrequent precipitation inputs resulted in richer (Chao1 P=0.007; observed species P<0.001; Shannon P=0.041), more phylogenetically diverse (PD tree p<0.001) and less phylogenetically clustered (NRI p<0.001) present bacteria (Fig. S4a) but less rich (marginal Chao1 p=0.073; Observed species p<0.001), more phylogenetically diverse (PD tree p<0.001) and less phylogenetically clustered (NRI p<0.001) but more even (Shannon p<0.001; Equitability p<0.001; Simpson reciprocal p<0.001; log dominance p<0.001) present fungal communities (Fig. S4c). N input had no significant effect on the richness, evenness or relatedness of the present bacterial or fungal communities. High N input led to increased phylogenetic clustering (NRI p=0.005) in present fungal communities regardless of precipitation input but led to increased phylogenetic clustering (NRI precipitation x N input P=0.013) in present bacterial communities under frequent precipitation input only.

Potentially active microbial community composition and diversity

Precipitation inputs significantly shaped potentially active fungal (Fig. S3d) and bacterial (Fig. S3b) communities, explaining 35% and 24% (P<0.001) of the variance, respectively (p<0.001 for both). N input had a large, significant influence on the potentially active fungal communities explaining 21% of the variance (P=0.005) but did not result in a significant shift in the potentially active bacterial communities. Infrequent precipitation inputs resulted in more phylogenetically diverse (P=0.031) and less phylogenetically clustered (NRI P=0.001) potentially active bacteria communities, whilst richness and evenness were not significantly affected (Fig. S4c). The richness, evenness and phylogenetic diversity of potentially active fungal communities was unaffected by precipitation inputs but infrequent precipitation input led to significantly less phylogenetic clustering (NRI P<0.001).

N input had no significant effect on potentially active bacterial community richness, evenness or phylogenetic diversity (Fig. S4b) but low N input resulted in significantly more even (Shannon P=0.013; Equitability p=0.010; Simpson reciprocal p=0.008; dominance p=0.015), more phylogenetically diverse (p=0.033) and less phylogenetically clustered (NRI p=0.009) potentially active fungal communities (Fig. S4d).

Significantly responsive groups

The OTUs that responded significantly to precipitation input in the present bacterial community were dominated by α-Proteobacteria (mainly Rhizobiales) and Actinobacteria under frequent precipitation inputs, and by α-Proteobacteria (mainly Sphingomonadales) and Acidobacteria under infrequent precipitation inputs (Fig. 6a). In the potentially active bacterial communities, the OTUs that responded significantly to precipitation input were dominated by α-Proteobacteria (Rhizobiales and Caulobacteriales) under frequent precipitation inputs and

Acidobacteria (Solibacterales) under infrequent precipitation inputs (Fig. 6b). In the present bacterial community, the OTUs that responded significantly to N input were dominated by Bacteroidetes (Cytophagales) and to a lesser extent α-Proteobacteria under high N application, and Bacteroidetes (Sphingobacteriales) and Acidobacteria under low N application. We detected no OTUs in the potentially active bacterial community that responded significantly to N input.

In the present fungal communities, the OTUs that responded significantly to precipitation input were clearly dominated by the orders Hypocreales (Ascomycota phylum) and to a lesser extent, Chytriales (Chytridiomycota phylum) under frequent precipitation inputs, whilst there was no clear dominance of any OTU under infrequent precipitation inputs (Fig. 7a). Hypocreales and Glomerales dominated the OTUs that responded significantly to precipitation input in the potentially active fungal communities under frequent and infrequent precipitation inputs, respectively (Fig. 7b). We detected no OTUs in the present fungal community that responded significantly to N input. Leotiomycetes and Glomerales dominated the OTUs that responded significantly to N input in the potentially active fungal communities under high and low N application levels, respectively.

Plant-microbial coupling

After ^{13}C-CO_2 labelling which took place before the rewetting event, plants acquired significantly higher amounts of ^{13}C tracer relative to baseline values, in all treatment combinations ($p < 0.001$, Fig. 8). However, significantly more label was taken up by plants which had a history of combined frequent precipitation and high N input (precipitation x N input $p = 0.028$) than any other of the treatment combinations. Regardless of precipitation or N

input, the ^{13}C signal in roots was significantly higher than baseline from 1 hour post rewetting (all p <0.001). However, the plants with a history of frequent precipitation and high N input, which had acquired significantly more ^{13}C during the dry period, allocated significantly more of the labelled C to roots between 7.8 and 29.2 hours upon rewetting (time x precipitation x N input p<0.001). This resulted in significantly more excess ^{13}C in microbial biomass under frequent precipitation and high N input, from 12hours after labeling.

Trace gas efflux upon rewetting

Increased soil CO_2 efflux rates upon rewetting were in all treatment combinations (Fig. 9a). However, precipitation input significantly impacted the magnitude of the flux (p<0.001), with a history of frequent precipitation input resulting in a flux of CO_2 upon rewetting that was 3-5 times higher (p<0.001) than the that from systems with a history of infrequent precipitation at all measured time points. Additionally, systems with contrasting precipitation input history had different response of CO_2 pulse over time post rewetting (Precipitation x time p<0.001). The flux of CO_2 from systems with a history of frequent precipitation decreased sequentially at each time point. From systems with a history of infrequent precipitation, the overall much lower CO_2 pulse remained constant for up to 10.6 hours post rewetting, before significantly decreasing only at 17.6 and 28.6 hours post rewetting. We detected a short (<3h) pulse of soil N_2O efflux upon rewetting from soils with a history of frequent water and high N input (Fig. 9b). All other treatment combinations did not result in a significant flux of N_2O between 1.6-28.6 hours post rewetting.

Microbial response to rewetting

Rewetting resulted in a small but significant shift in the potentially active bacterial community composition (1.2% variance explained, p=0.021), with no significant change in community richness, evenness, phylogenetic diversity or net relatedness index (NRI). The shift in potentially active bacterial communities was predominantly driven by an increase in the relative abundance of potentially active OTUs following rewetting, mainly Bacteroidetes but also to a lesser extent α- and β-Proteobacteria, Gemmatimonadetes and Verrucomicrobia. Rewetting did not result in a significant shift in the potentially active fungal community composition. There was no significant difference in diversity or NRI of the potentially active fungi between pre and post-wet but potentially active fungal communities did show a fluctuating increase in richness (Chao1 p<0.001; Observed species p=0.001; Shannon p=0.004), evenness (Equitability only p=0.029) and relatedness (PD tree p=0.005) over time following rewetting. Fungal NRI did not significantly change over time post rewetting. No OTU responded significantly to rewetting in the potentially active fungal communities.

Overall post-wet potentially active bacterial (Fig. 10a) and fungal (Fig. 10b) communities, were significantly shaped by both precipitation (17.6% variance, p<0.001 for bacteria and 36.4% variance, p<0.001 for fungi) and N (35% variance, p<0.001 for bacteria and 10.4% variance, p<0.001 for fungi) input history. Infrequent precipitation inputs resulted in richer (Chao1 p<0.001; Observed species p<0.001; Shannon p=0.016) but less even (Simpson reciprocal p=0.004; log dominance p=0.045), more phylogenetically diverse (PD whole tree p<0.001) and less phylogenetically clustered (NRI p<0.001) post-wet potentially active bacterial communities. N input did not significantly impact richness, evenness or phylogenetic diversity of potentially active post-wet bacterial communities. Infrequent precipitation input resulted in

overall richer (Chao1 p<0.001; Observed species p<0.001) and less phylogenetically clustered post-wet potentially active fungal communities. Low N input lead to more even (Shannon p<0.001; equitability p<0.001; Simpson reciprocal p=0.004; log dominance p=0.008) and more phylogenetically diverse (PD tree p=0.0005) and less phylogenetically clustered (NRI p<0.001) post-wet potentially active fungal communities.

Plant-microbial competition for N

Both plant roots and soil microbes took up the ^{15}N-labelled ammonium within 1 hour of rewetting, irrespective of precipitation or N input. Plant ^{15}N label uptake increased over the 29h of measurements (Fig. S5). In contrast, we did not detect a significant net change in microbial ^{15}N immobilization during this timeframe. Plant-microbial competition for ^{15}N followed a consistent pattern over time post-rewetting in all treatment combinations (Fig. 11). After an initial significant decrease in plant: microbial ^{15}N uptake ratio at 1-hour post-rewetting, indicating that microbes outcompete plants for the ^{15}N, plant N assimilation gradually increased relative to microbial N immobilization between 2.2 and 19.4 hours post re-wetting as the competitive edge gradually moved in favor of plants. Finally, plant: microbial ^{15}N uptake ratio is significantly in favor of plants 29 hours post rewetting. Frequent precipitation inputs significantly shifted this pattern in favor of plants (p=0.047) over the measurement timeframe, while we detected no effect of N input history.

Discussion

Legacy effects

A history of contrasting precipitation patterns shaped (1) plant physiology (biomass production and photosynthesis rate), (2) soil microbial communities (both present and metabolically active communities) and (3) soil N cycling (pool sizes and transformation rates), setting contrasting scenes for the plant-soil response to rewetting.

1. Plant physiology

Changes in plant biomass production, in response to precipitation and N input regimes, can influence ecosystem N budgets either directly, due to biomass-related plant N uptake, or indirectly, due to the contrasting environments they create for the soil microbes which drive N transformations. In resource-limited environments, the general theory is that plants allocate resources preferentially to the structures which may increase acquisition of the limited resource (Bloom et al., 1985). In concurrence with previous findings (see meta-analysis by He and Dijkstra 2014) we found that water availability more than N availability shaped plant biomass production. Infrequent precipitation input lead to significantly smaller plants than frequent precipitation input. Root biomass reduction was more pronounced than shoot biomass reduction, indicating a decreased root:shoot ratio which is contrary to what has been previously shown in plants subjected to prolonged dry periods (Poorter et al., 2012, Wang et al., 2015, Xie et al., 2016) but has been shown as a response to more severe cycles of drying and rewetting (Zhu et al., 2013). High N application exacerbated the effect of infrequent precipitation with a further reduction in root biomass with no observable benefit to shoot biomass production or photosynthesis rate. Under infrequent precipitation input, smaller root biomass coupled with reduced plant C acquisition resulted in reduced rhizodeposition of recent photoassimilates, with likely negative consequences for soil microbial activity and biogeochemical cycling.

2. Soil microbial communities

Precipitation as well as N input shape soil biochemical cycling due to their impact on (1) the fungal:bacterial ratio, (2) the composition and diversity of bacterial and fungal seedbanks and potentially active communities and (3) the present and potentially active OTUs.

2.1. Abundance

Our experiment showed that infrequent precipitation impacted bacterial but not fungal abundance, which may progressively lead to increased bacterial dominance over fungi. This could potentially have important consequences for ecosystem services such as C sequestration (Six et al., 2006, Bardgett et al., 2014, Malik et al., 2016) and N retention (Gordon et al., 2008, de Vries et al., 2011, de Vries et al., 2012), both of which are enhanced under increased fungal dominance. Additionally, the trend we found towards decreased fungal abundance under high N application rates has been well documented (Bardgett et al., 1999, Högberg et al., 2003, deVries et al., 2006, Demoling et al., 2008). This could suggest a potential for an even greater shift towards bacterial dominance when infrequent precipitation patterns are combined with high N input, highlighting the need for further investigation of the interactive effect of precipitation and N input on soil fungal:bacterial ratio.

2.2. Composition and diversity

Precipitation regime shaped the composition of the present and potentially active bacterial and fungal communities, but had differential effects on their diversity. Infrequent precipitation input regime increased species richness as well as phylogenetic diversity in the bacterial seed bank, which was confirmed by a decreased clustering in the NRI. Infrequent precipitation also increased phylogenetic diversity with decreased clustering in the NRI but decreased species richness in the fungal seed bank. Richness and phylogenetic

diversity of the microbial seed bank is associated with resistance to fluctuations in environmental conditions (Yachi and Loreau, 1999), which suggests that, in our system, bacterial communities may mobilize species from a more extensive pool to cope with a broader water-related environmental envelope. Though more phylogenetically diverse, the increased richness was not observed for the fungal seed bank, likely due to their high inherent resistance to moisture fluctuations. Similarly, infrequent precipitation decreased phylogenetic clustering of both active bacterial and fungal communities, likely due to the larger seed-pool to choose from, but had only a limited impact on their richness.

N-input history impacted the fungal communities more than the bacterial communities. N input history contributed to shaping the composition of the total present bacterial communities but did not influence the potentially active bacterial communities. Contrastingly, in fungal communities, N input did not impact the structure of the present community but contributed to determine the structure of the potentially active fungal communities, where high N application led to a loss of evenness and phylogenetic diversity confirmed by the observed increased phylogenetic clustering. These results are consistent with the relatively lower N requirements of fungi compared to bacteria and with the vulnerability of fungal food webs to high inorganic N inputs, due to loss of fungal diversity.

2.3. Significantly responsive groups

More extreme fluctuations in environmental conditions experienced in the infrequent precipitation input treatment likely selected for different communities which are potentially active at different times. For both bacteria and fungi, we found that OTUs which responded significantly to precipitation input regimes mainly belonged to similar groups in present and potentially active communities under frequent precipitation input (i.e. α-

Proteobacteria for bacteria, hypocreales and chytridiales for fungi) but showed different main phyla under infrequent precipitation input (i.e. the co-dominance of α-proteobacteria and acidobacteria is taken over by acidobacteria, no clear dominant present fungal group, but mainly active glomerales). The microbes which are active under the frequent precipitation regime are relatively constant whilst under the more extreme fluctuations in environmental conditions under infrequent precipitation input, taxa with different life strategies may be active at different times.

3. N cycling

Infrequent precipitation input consistently impacted soil N cycling in our system. Cycles of more extensive drying and more complete saturation led to increased nitrifying and denitrifying enzyme activity as well as reduced inorganic N pools. Overall, our results indicate enhanced cycling of N in the soil under infrequent precipitation input, likely generated by alternating aeration conditions for aerobic nitrification and anaerobic denitrification respectively (Shrestha et al., 2014). Plant and soil microbial N pools on the other hand may be more buffered in regard to cycles of drying and rewetting (Morillas et al., 2013) and in our system were more strongly affected by N availability. High N input history increased plant N pools whilst low N input history lead to increased microbial biomass N pools. This suggests that N availability may be the key factor determining the outcome of plant and microbial competition for N over the 12 week treatment period.

Our results show a clear legacy effect of precipitation patterns on key drivers of soil biogeochemical cycling, which sets the scene for potentially contrasting rewetting responses of the plant-soil system.

Plant-microbial coupling

Under a history of frequent precipitation and high N input, we were able to trace the transfer of recent photoassimilates from plants to microbes, i.e. plant-microbial coupling. However, under both infrequent precipitation and low N input in the other treatments, we observed a breakdown of this plant-microbial coupling. Drought can significantly reduce plant photosynthetic rates and the transfer of recent photoassimilates to soil microorganisms, thereby hampering plant-microbial coupling (Ruehr et al., 2009), which may have occurred during the extended dry periods under the infrequent precipitation input. Low N input left plant photosynthetic rates in week 8-9 unchanged, and had only limited impacts on plant biomass and active microbial communities. Yet by week 12, plants under frequent precipitation and low N inputs were unable to acquire as much C through photosynthesis as their high N input counterparts. Thus, we hypothesize that the breakdown in plant-microbial coupling under low N input occurred only towards the end of the treatment period. This reduction in the flow of C from above- to belowground may have consequences for (1) microbial resistance to desiccation and (2) potential C sequestration of the ecosystem.

1. Microbial resistance to desiccation

Access to labile C greatly increases microbial resistance to desiccation (review by Berard et al., 2015). First, microbes accumulate intracellular osmolytes to prevent water loss and dehydration, which tend to be rich in C and N (reviewed by Borken and Matzner 2009). Particularly fungi, who predominantly accumulate C-rich sugar alcohols such as polyols (Csonka 1989, Boot et al., 2013) may benefit from increased resistance to desiccation when they have access to fresh C rich plant exudates. Second, some bacteria and fungi produce a

polysaccharide based mucilage, which provides protection against dehydration and predation but requires large amounts of C and energy (Schimel et al., 2007).

2. Potential C sequestration

A decrease in plant-microbial coupling indicates a loss of C sequestration potential and may thus lead to the ecosystem becoming a net C source rather than a net C sink. Fresh, easily decomposable photoassimilates from plants fuel microbial activity or are incorporated into microbial biomass and is eventually stored as soil organic matter (contributing to the ecosystem C sink) and, under nutrient limited conditions, may fuel microbial decomposition of soil organic matter (contributing to the ecosystem C source). The balance between C storage and C losses is tightly linked to the quantity and the quality of as well as the microbial access to labile C exudate of plant roots.

Our findings also suggest that a large fraction of C fixed by photosynthesis may not in fact be stored but lost from the system through the significantly higher and more persistent soil CO_2 efflux from systems with a history of frequent precipitation input. The significantly larger root biomass and enhanced C availability to microbes in systems with frequent precipitation input history likely contribute to the large biotic CO_2 flux through increased root and microbial respiration respectively. However, we found that N availability did not impact the CO_2 efflux in our systems, despite the reduced C acquisition towards the end of the experimental period under low N input. Root biomass under frequent water and low N input was as large as under frequent water and high N input and may have contributed to the CO2 efflux through respiration. We nevertheless hypothesize that a large amount of the CO_2 efflux from the soil

may have been from microbial respiration but originated from organic sources other than recent photoassimilate.

Microbial response to rewetting over time

The composition of the potentially active bacterial and fungal communities after rewetting was still shaped by precipitation history, as the pre-wet communities were. Both the bacterial and fungal response to rewetting was stable over time. The response to rewetting of potentially active bacteria occurred within the first hour, and was sustained over the next 28 hours with no significant change in the richness or phylogenetic clustering of the communities over time. Our study thus suggests that rewetting studies that focused on the short term response of soil microorganisms (Fierer et al., 2003, Iovieno and Bååth, 2008, Placella et al., 2012, Barnard et al., 2013) have likely adequately captured at least the bacterial component of the response. We were unable to detect a significant change in fungal community composition within the first 29 h after rewetting. However, the richness, relatedness and evenness of the potentially active fungal community gradually increased over time, suggesting that some fungi were becoming metabolically active, but the response was not fast or large enough to result in a significant shift in the community structure.

Plant-microbial competition for N over time post rewetting

Plant-microbial competition for N followed an expected overall pattern over time post-rewetting in all treatment combinations: soil microbes, with their overall fast response rates and high substrate affinity, had the competitive edge immediately after rewetting, whilst plant competitiveness increased gradually over time, likely due to microbial turnover (Schimel and Bennet, 2004). However, a history of favorable conditions for plants increased their overall

competitiveness for N over microbes. Even though precipitation legacy does not impact the general pattern of plant-microbial competition for N, under frequent precipitation input the larger, more productive plants (based on biomass and photosynthesis rate) had an increased competitive edge over microbes at all time points analyzed within our 29 hour time frame. Yet, even though microbes with a history of frequent precipitation input are less competitive for N over plants and showed slower N-cycling transformations than microbes with a history of infrequent precipitation input, a short lived N_2O flux, immediately post rewetting was measured only in systems with frequent precipitation and high N availability. This suggests an increased loss of N from systems with combined frequent precipitation and high N input to the atmosphere, where N_2O contributes to the progression of global warming.

Conclusion

While N availability only had limited effects on our systems, contrasting drying-rewetting cycles not only shaped microbial community structure and richness, but also impacted bacterial and fungal components differently. The fungal:bacterial ratio appears highlighted as playing a pivotal role in soil biogeochemical cycling and food web stability. Our evidence suggests that more extreme fluctuations in soil moisture may lead to decreased plant-microbial coupling and alter N dynamics between plants and soil microbes suggesting reduced soil C sequestration potential and imbalances in ecosystem N and C cycling, with negative implications for ecosystem functioning under predicted future climate conditions.
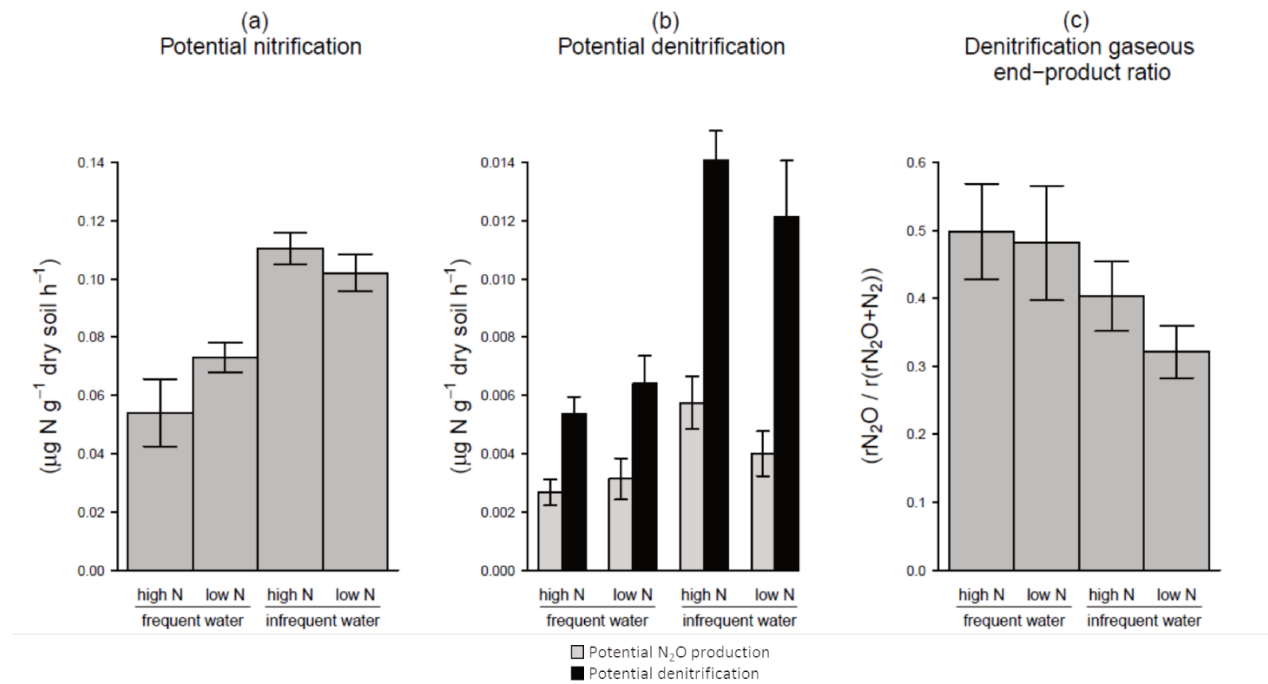
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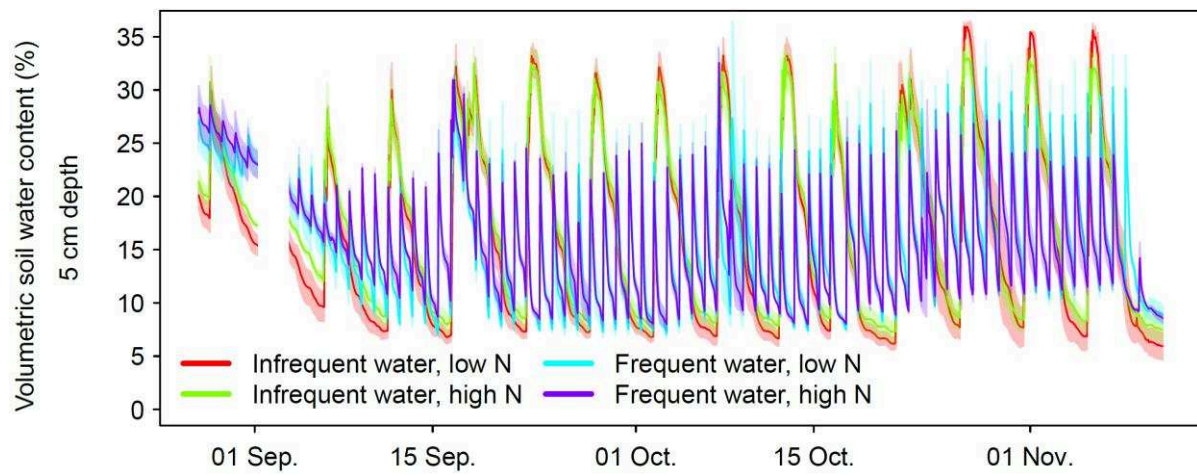
Fig. 1. Sensor data for volumetric soil water content at 5 cm soil depth, for each of the precipitation and N input combinations over the entire experimental period. Lines and shaded polygons around them indicate mean ± standard error (n=3).
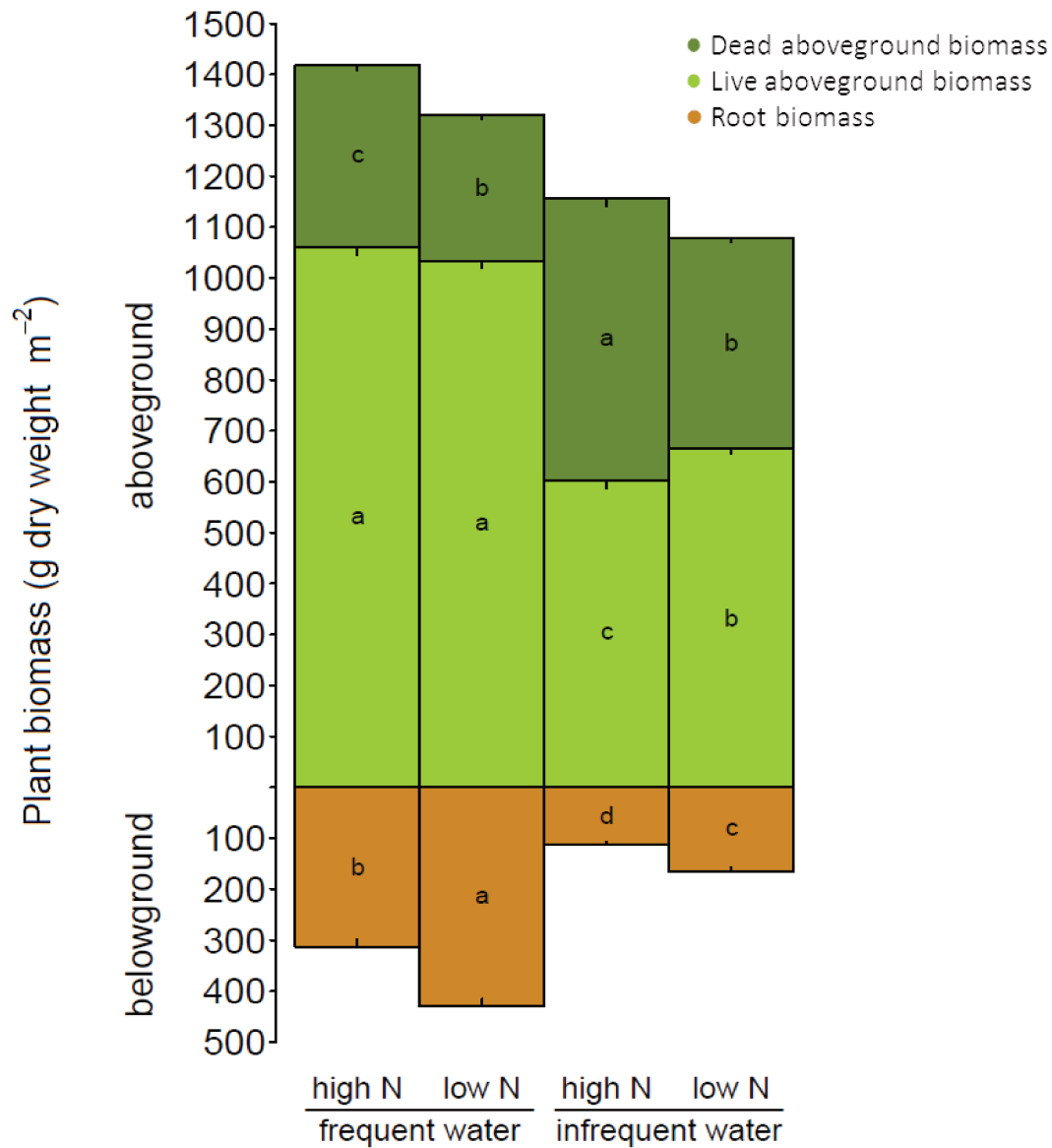
Fig. 2. Plant biomass in g dry weight per m² soil surface, after 12 weeks under contrasting precipitation and N input, separated into dead aboveground (dark green), live aboveground (light green) and below ground biomass (orange). Bars indicate mean - standard error (n=5).

(a)
Potential nitrification

(b)
Potential denitrification

(c)
Denitrification gaseous end−product ratio

Fig. 3(a-c). N transformation potentials for each of the precipitation and N input combinations, shown as nitrification rate (grey bars in Fig. 3a), denitrification and N_2O production rates (black and grey bars respectively in Fig. 3b) and the ratio between the gaseous end-products (N_2O or N_2O plus N_2). Bars indicate mean ± standard error (n=5).
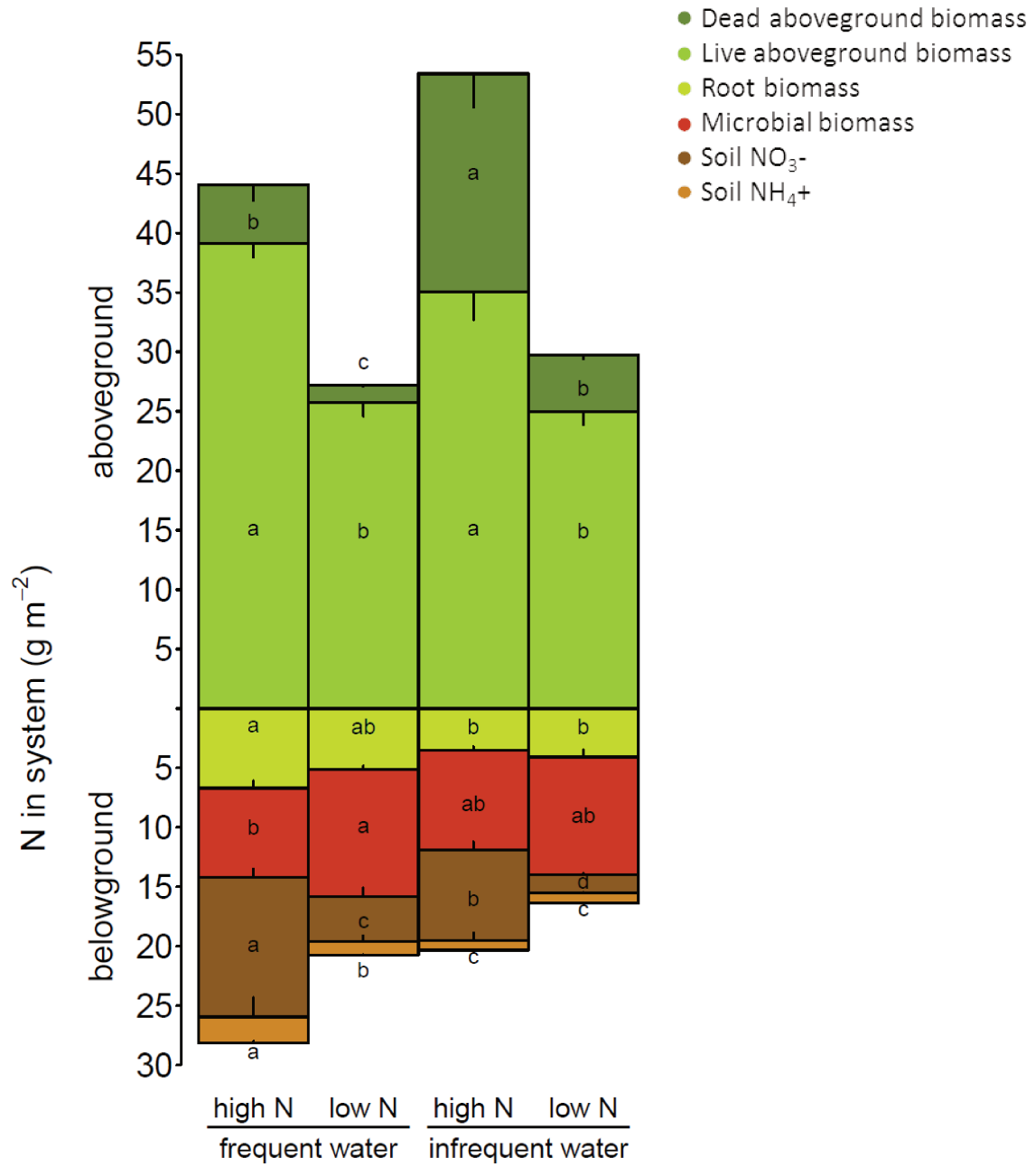
Fig. 4. Organic (plant and microbial biomass) and inorganic (soil NH_4^+ and NO_3^-) N pools for each of the precipitation and N input histories in g N per m^{-2} soil surface, for the entire depth of the mesocosm (~19 cm). Bars indicate mean - standard error (n=5).
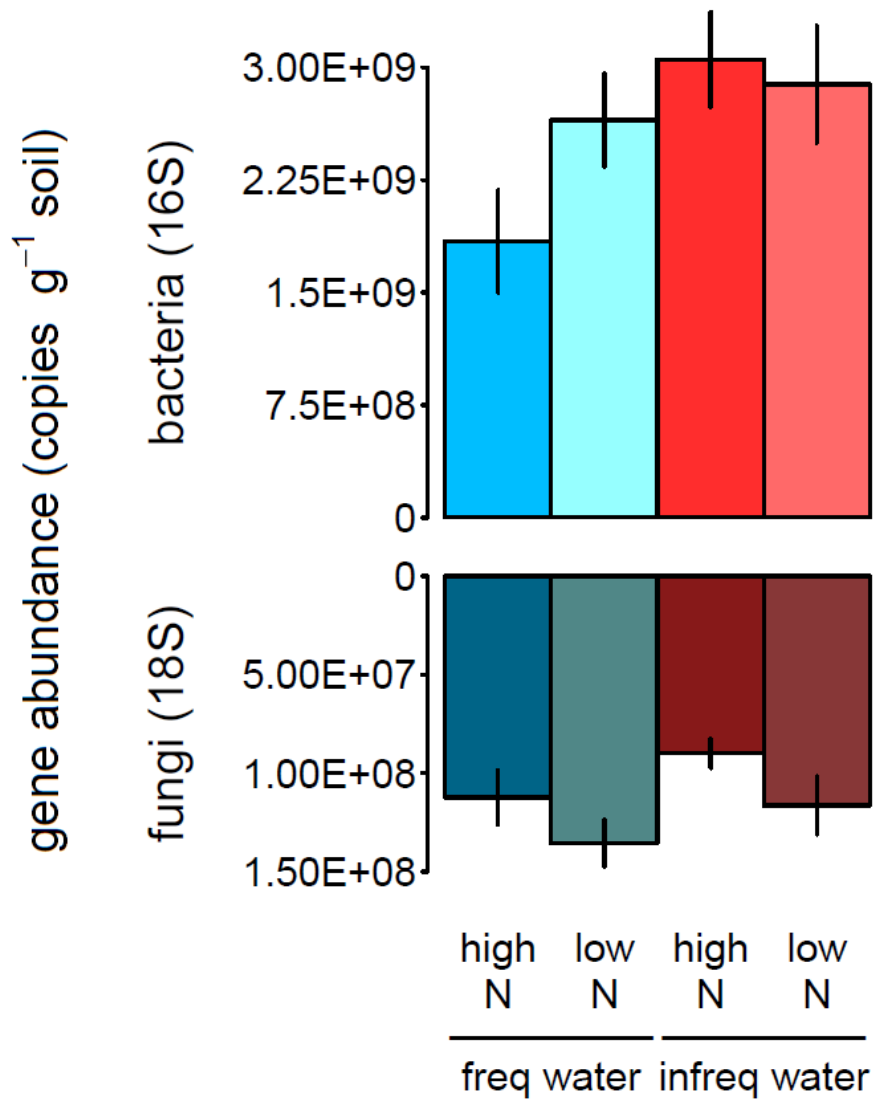
Fig. 5. Bacterial 16S RNA (top part of graph) and fungal 18S RNA (bottom part of graph) gene abundance in number of copies per g of dry soil, for each of the precipitation and N input histories. Note the fungal scale is almost 1 magnitude lower than that of the bacterial. Bars indicate mean ± standard error (n=5).
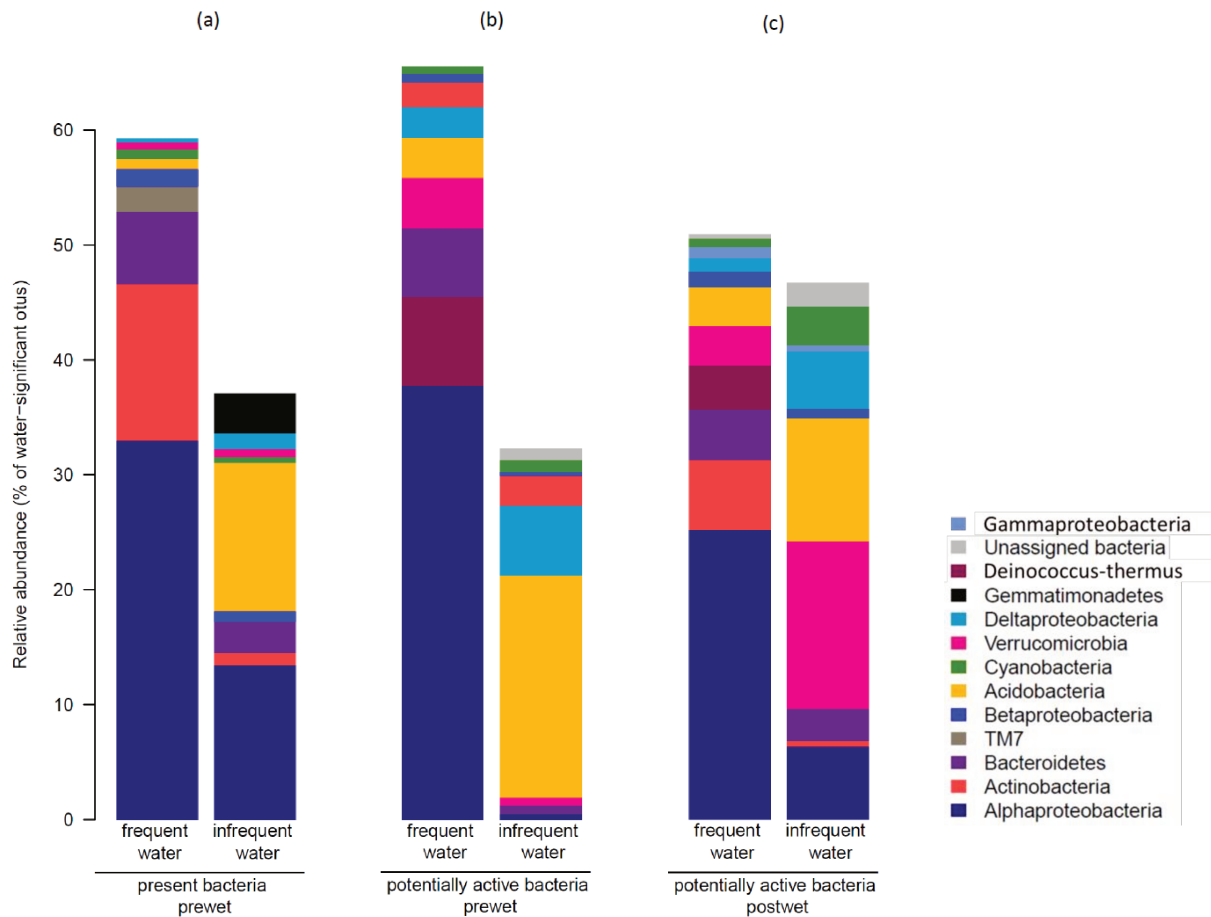
Fig. 6(a-c). Relative abundance of the OTUs that responded significantly to precipitation input in the present (a) and potentially active (b) bacterial communities before the final rewetting event, and the potentially active bacterial communities after the rewetting event (c). All phylum assignment is provided except for Proteobacteria for which the representative classes are shown (different shades of blue).
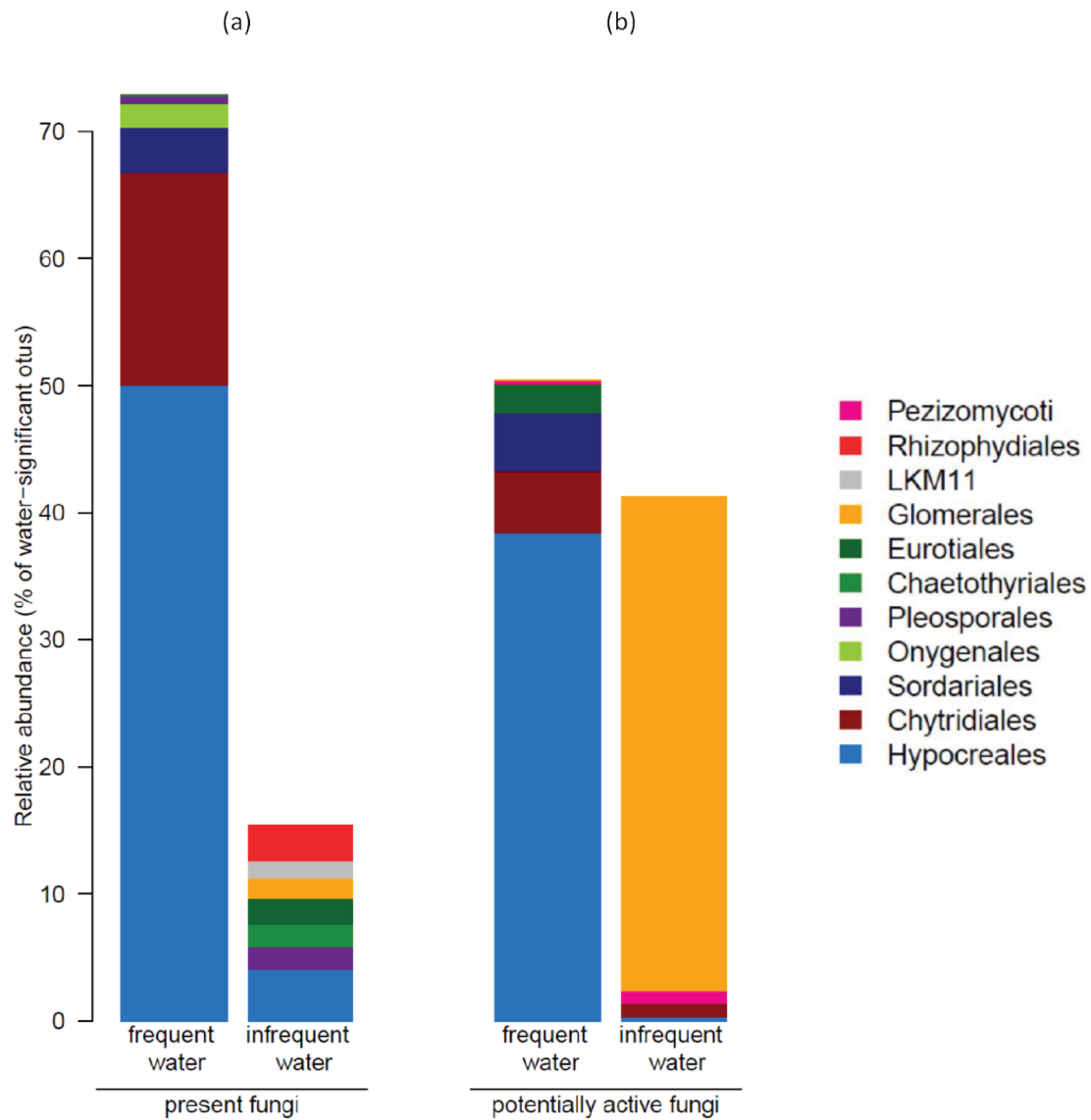
Fig. 7(a-b). Relative abundance of the OTUs that responded significantly to precipitation input in the present (a) and potentially active (b) fungal communities before the final rewetting event. All order assignment is provided.
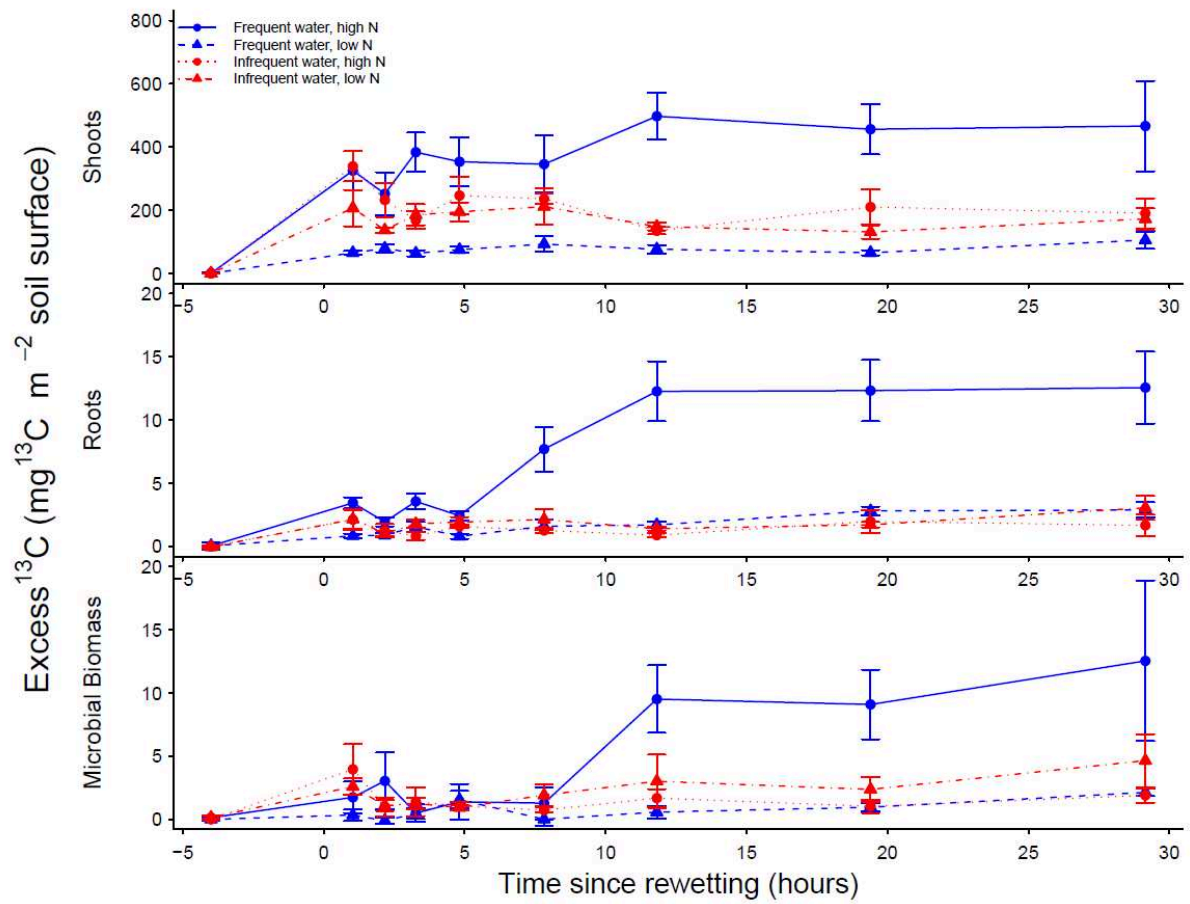
Fig. 8. Pre-wet ^{13}C tracer assimilation by plant shoots (top), post wet allocation of ^{13}C tracer to plant roots (middle) and post-wet assimilation of ^{13}C tracer into microbial biomass (bottom).
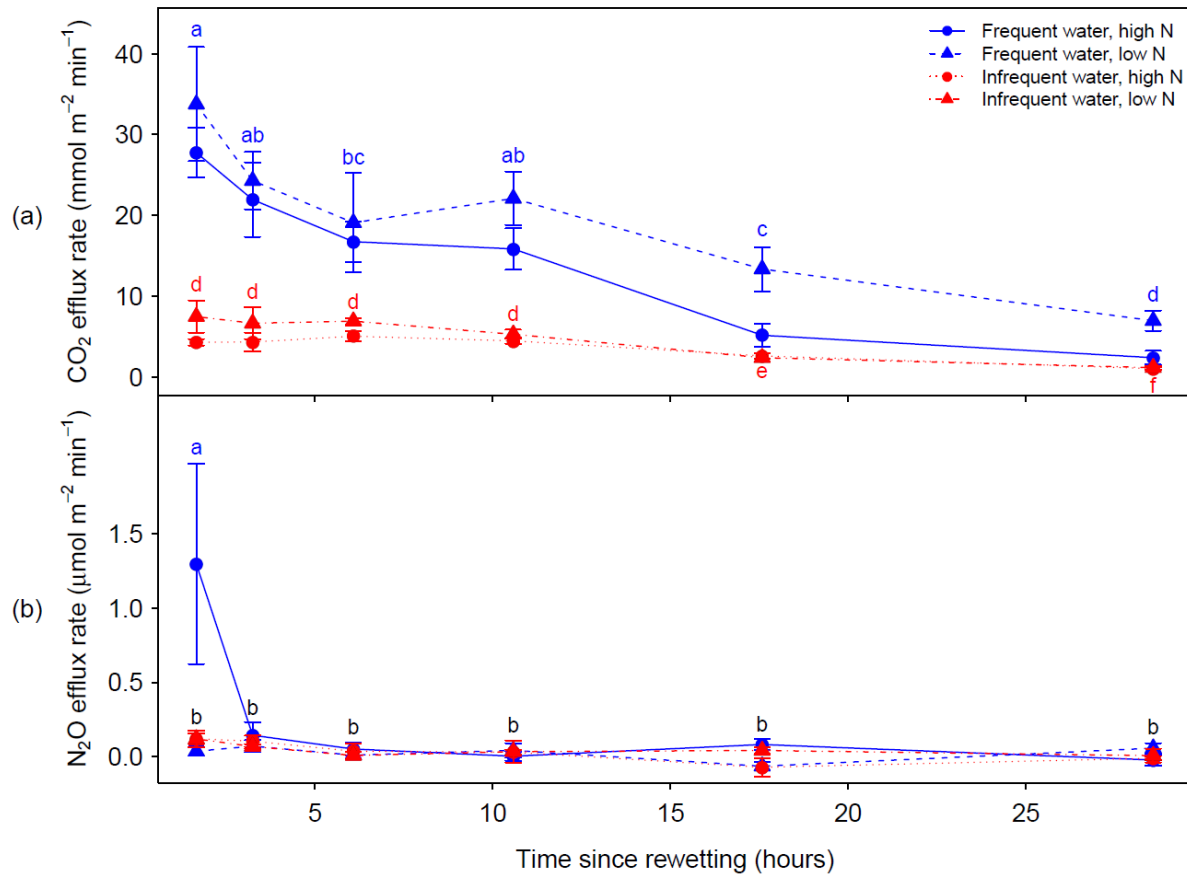
Fig. 9(a-b). Trace gas soil efflux rates over time (in hours) post rewetting for each of the precipitation (blue or red color) and N (solid, dotted lines) input histories. For CO_2 efflux (a), letters indicate significantly different groups for frequent (blue letters) and infrequent (red letters) precipitation histories. For N_2O efflux (b) all groups were not significantly different from each other (black letters) except for the first time point in systems with a combined history of frequent precipitation and high N input (blue letter). Bars indicate mean ± standard error (n=5).
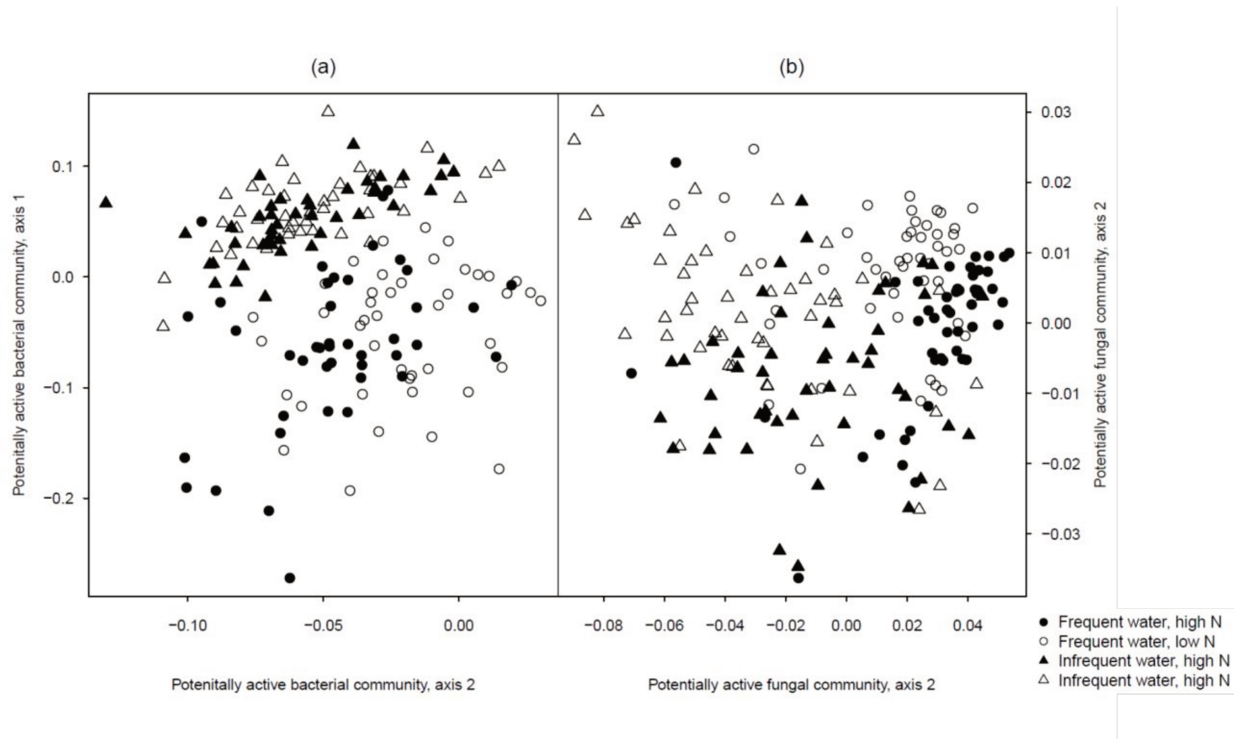
Fig. 10(a-b). Principal coordinates analysis (PCoA) of the UniFrac pairwise dissimilarity of the relative abundance of bacterial 16S RNA sequences (a) and fungal 18S RNA sequences (b) of potentially active communities after the final rewetting event.
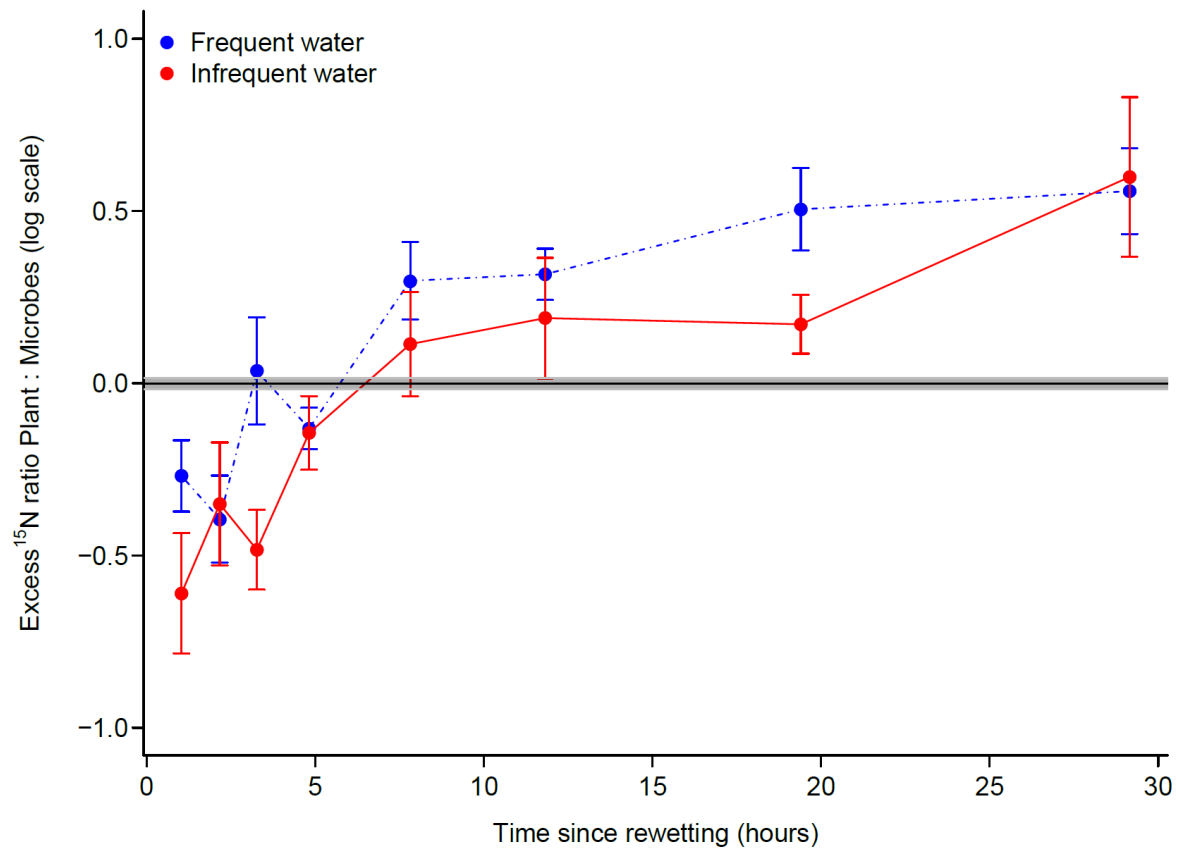
Fig. 11. Log transformed ratio between excess ^{15}N signal in plant (above and belowground) and microbial biomass for frequent (blue, dotted line) and infrequent (red, solid line) precipitation input. The black line at y=0 indicates the mean baseline ratio with the grey polygon showing the mean error. Bars indicate mean ± standard error (n=5).

Acknowledgements

References

1. Altschul SF, Gish W, Webb M, Myers EW, Lipman DJ (1990). Basic local alignment search tool. Journal of Molecular Biology: 403-410.
2. Anderson M (2001). A New Method for Non-Parametric Multivariate Analysis of Variance, Austral Ecology 26(1).
3. Bardgett RD, Mawdsley JL, Edwards S, Hobbs PJ, Rodwell JS and Davies WJ (1999). Plant species and nitrogen effects on soil biological properties of temperate upland grasslands, Functional Ecology 13:650-660
4. Bardgett RD, Van Der Putten WH (2014). Belowground biodiversity and ecosystem functioning, Nature. 515: 505–511
5. Barnard RL, Osborne CA, Firestone MK (2013). Responses of soil bacterial and fungal communities to extreme desiccation and rewetting, ISME Journal 11: 2229-2241.
6. Barnard RL, Osborne CA and Firestone MK (2015). Changing precipitation pattern alters soil microbial community response to wet-up under a Mediterranean-type climate, ISME Journal 9: 946-957.
7. Berard A, Ben Sassi M, Kaisermann A and Renault P (2015). Soil microbial community responses to heat wave components: Drought and high temperature, Climate Research 66: 243-264.
8. Bloom AJ, Chapin FS and Mooney HA (1985). Plants-an Economic Analogy, Annual Review of Ecology and Systematics 16:363-392.
9. Bonaterra A, Camps J and Montesinos E (2005). Osmotically induced trehalose and glycine betaine accumulation improves tolerance to desiccation, survival and efficacy of the postharvest biocontrol agent Pantoea agglomerans EPS125, FEMS Microbiology Letters 250: 1-8.
10. Boot CM, Schaeffer SM and Schimel JP (2013). Static osmolyte concentrations in microbial biomass during seasonal drought in a California grassland, Soil Biology and Biochemistry 57: 356-361.
11. Borken W, Matzner E (2009). Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils, Global Change Biology 15: 808-824.
12. Brookes PC, Landman A, Pruden G, Jenkinson DS (1985). Chloroform Fumigation and the Release of Soil Nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil, Soil Biology and Biochemistry, 17: 837–842.
13. Bru D, Ramette A, Saby NPA, Dequiedt S, Ranjard L, Jolivet C, Arrouays D, Philippot L (2011). Determinants of the distribution of nitrogen-cycling microbial communities at the landscape scale. ISME Journal 5: 532-542.
14. Canarini A, Dijkstra FA (2015). Dry-rewetting cycles regulate wheat carbon rhizodeposition, stabilization and nitrogen cycling, Soil Biology and Biochemistry 81: 195-203.
15. Caporaso JG, Bittinger K, Bushman FD, DeSantis TZ, Andersen GL, Knight R (2010a). PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics 26: 266-267.
16. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK et al (2010b). QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7: 335-336.

17. Csonka LN (1989). Physiological and genetic responses of bacteria to osmotic stress. Microbiological reviews 53: 121-147.
18. DaMatta FM, Loos RA, Silva EA, Loureiro ME and Ducatti C (2002). Effects of soil water deficit and nitrogen nutrition on water relations and photosynthesis of pot-grown Coffea canephora Pierre, Trees - Structure and Function 16: 555-558.
19. de Boer W, Folman LB, Summerbell RC and Boddy L (2005). Living in a fungal world: Impact of fungi on soil bacterial niche development, FEMS Microbiology Reviews 29: 795-811.
20. de Vries FT, Hoffland E, van Eekeren N, Brussaard L and Bloem J (2006). Fungal/bacterial ratios in grasslands with contrasting nitrogen management, Soil Biology and Biochemistry 38: 2092-2103.
21. de Vries FT, van Groenigen JW, Hoffland E and Bloem J (2011). Nitrogen losses from two grassland soils with different fungal biomass, Soil Biology and Biochemistry 43: 997-1005.
22. de Vries FT, Liiri ME, Bjørnlund L, Setälä HM, Christensen S and Bardgett RD (2012). Legacy effects of drought on plant growth and the soil food web, Oecologia 170: 821-833.
23. de Vries FT and Shade A (2013). Controls on soil microbial community stability under climate change, Frontiers in Microbiology 4: 1-16.
24. Demoling F, Ola Nilsson L and Bååth E (2008). Bacterial and fungal response to nitrogen fertilization in three coniferous forest soils, Soil Biology and Biochemistry 40: 370-379.
25. de Vries FT, Thebault E, Liiri M, Birkhofer K, Tsiafouli MA, Bjornlund L, Bracht Jorgensen H, Brady MV, Christensen S, de Ruiter PC, d'Hertefeldt T, Frouz J, Hedlund K, Hemerik L, Hol WHG, Hotes S, Mortimer SR, Setala H, Sgardelis SP, Uteseny K, van der Putten WH, Wolters V and Bardgett RD (2013). Soil food web properties explain ecosystem services across European land use systems, Proceedings of the National Academy of Sciences 110: 14296-14301.
26. Edgar RC (2010). Search and clustering orders of magnitude faster than BLAST, Bioinformatics 26: 2460-2461.
27. Evans SE and Wallenstein MD (2012). Soil microbial community response to drying and rewetting stress: Does historical precipitation regime matter? Biogeochemistry 109: 101-116.
28. Eziz A, Yan Z, Tian D, Han W, Tang Z, Fang J (2017). Drought effect on plant biomass allocation: A meta-analysis, Ecology and Evolution 7: 11002–11010.
29. Fierer N, Allen AS, Schimel JP, Holden PA (2003). Controls on microbial CO_2 production: a comparison of surface and subsurface soil horizons, Global Change Biology 9: 1322-1332.
30. Fowler D, Steadman CE, Stevenson D, Coyle M, Rees RM, Skiba UM, Sutton MA, Cape JN, Dore AJ, Vieno M, Simpson D, Zaehle S, Stocker BD, Rinaldi M, Facchini MC, Flechard CR, Nemitz E, Twigg M, Erisman JW, Butterbach-Bahl K, Galloway JN (2015). Effects of global change during the 21st century on the nitrogen cycle, Atmospheric Chemistry and Physics 15: 13849–13893.
31. Fuchslueger L, Bahn M, Hasibeder R, Kienzl S, Fritz K, Schmitt M, Watzka M, Richter A (2016). Drought history affects grassland plant and microbial carbon turnover during and after a subsequent drought event, Journal of Ecology 104: 1453–1465.

32. Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, Karl DM, Michaels AF, Porter JH, Townsend AR, Vo CJ (2004). Nitrogen cycles: past, present and future, Biogeochemistry 70: 153-226.

33. Gobiet A, Kotlarski S, Beniston M, Heinrich G, Rajczak J, Stoffel M (2014). 21st century climate change in the European Alps-A review, Science of the Total Environment 493: 1138–1151.

34. Gordon H, Haygarth PM, Bardgett RD (2008). Drying and rewetting effects on soil microbial community composition and nutrient leaching, Soil Biology and Biochemistry, 40: 302–311.

35. Grant K, Kreyling J, Dienstbach LFH, Beierkuhnlein C, Jentsch A (2014). Water stress due to increased intra-annual precipitation variability reduced forage yield but raised forage quality of a temperate grassland, Agriculture, Ecosystems and Environment 186: 11–22.

36. Griffiths BS, Philippot L (2013). Insights into the resistance and resilience of the soil microbial community, FEMS Microbiology Reviews 37: 112–129.

37. Hasibeder R, Fuchslueger L, Richter A, Bahn M (2014). Summer drought alters carbon allocation to roots and root respiration in mountain grassland Summer drought alters carbon allocation to roots and root respiration in mountain grassland, New Phytologist 205: 1117–1127

38. He M, Dijkstra FA (2014). Drought effect on plant nitrogen and phosphorus: a meta-analysis, New Phytologist 204: 924–931.

39. Henry S, Baudoin E, López-Gutiérrez JC, Martin-Laurent F, Brauman A, Philippot L (2004). Quantification of denitrifying bacteria in soils by nirK gene targeted real-time PCR. Journal of Microbiological Methods 59: 327–335.

40. Hofer D, Suter M, Haughey E, Finn JA, Hoekstra NJ, Buchmann N, Lüscher A (2016). Yield of temperate forage grassland species is either largely resistant or resilient to experimental summer drought, Journal of Applied Ecology 53: 1023–1034.

41. Högberg MN, Bååth E, Nordgren A, Arnebrant K, Högberg P (2003). Contrasting effects of nitrogen availability on plant carbon supply to mycorrhizal fungi and saprotrophs - A hypothesis based on field observations in boreal forest, New Phytologist 160: 225–238.

42. Huntington TG (2006). Evidence for intensification of the global water cycle: Review and synthesis, Journal of Hydrology 319: 83–95.

43. Iovieno P, Bååth E (2008). Effect of drying and rewetting on bacterial growth rates in soil, FEMS Microbiology Ecology 65: 400–407.

44. IPCC (2007). Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press: Cambridge, UK.

45. Jones CM, Graf DRH, Bru D, Philippot L, Hallin S (2013). The unaccounted yet abundant nitrous oxide-reducing microbial community: a potential nitrous oxide sink. ISME Journal 7: 417e426.

46. Kandeler, E (1995). Nitrate reductase activity. Schinner F, Öhlinger R, Kandeler E, Margesin R (ed.), Methods in soil biology.

47. Kandeler E, Deiglmayr K, Tscherko D, Bru D, Philippot L (2006). Abundance of narG, nirS, nirK, and nosZ genes of denitrifying bacteria during primary successions of a glacier foreland. Applied Environmental Microbiology 72:5957–5962.

48. Knapp AK, Beier C, Briske DD, Classen AT, Luo Y, Reichstein M, Smith MD, Smith SD, Bell JE, Fay PA, Heisler JL, Leavitt SW, Sherry R, Smith B, Weng E (2008). Consequences of More Extreme Precipitation Regimes for Terrestrial Ecosystems, BioScience 58: 811-821.

49. Landesman WJ, Dighton J (2011). Shifts in Microbial Biomass and the Bacteria: Fungi Ratio Occur Under Field Conditions Within 3 h After Rainfall, Microbial Ecology 62: 228–236.

50. Lozupone C, Knight R (2005). UniFrac: a new phylogenetic method for comparing microbial communities. Applied Environmental Microbiology 71: 8228-8235.

51. Lennon JT, Jones SE (2011). Microbial seed banks: The ecological and evolutionary implications of dormancy, Nature Reviews Microbiology 9: 119–130.

52. Malik AA, Chowdhury S, Schlager V, Oliver A, Puissant J, Vazquez PGM, Jehmlich N, von Bergen M, Griffiths RI, Gleixner G (2016). Soil fungal:bacterial ratios are linked to altered carbon cycling, Frontiers in Microbiology 7: 1-11.

53. Manzoni S, Katul G (2014). Invariant soil water potential at zero microbial respiration explained by hydrological discontinuity in dry soils, Geophysical Research Letters 41: 7151–7158.

54. McDonald D, Price MN, Goodrich J, Nawrocki EP, Desantis TZ, Probst A, Andersen GL, Knight R, Hugenholtz P (2012). An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea, ISME Journal 6: 610–618.

55. Morillas L, Portillo-Estrada M, Gallardo A (2013). Wetting and drying events determine soil N pools in two Mediterranean ecosystems, Applied Soil Ecology 72: 160–170.

56. Owens H, LaFantasie J, Adler PB (2012). Mycorrhization rates of two grasses following alterations in moisture inputs in a southern mixed grass prairie, Applied Soil Ecology 60: 56–60.

57. Paterson E (2003) 'Importance of rhizodeposition in the coupling of plant and microbial productivity, European Journal of Soil Science 54: 741–750.

58. Pell M, Stenberg B, Stenstrom J, Torstensson L (1996). Potential denitrification activity assay in soil - With or without chloramphenicol? Soil Biology & Biochemistry 28: 393-398.

59. Philippot L, Andert J, Jones CM, Bru D, Hallin S (2011). Importance of denitrifiers lacking the genes encoding the nitrous oxide reductase for N_2O emissions from soil, Global Change Biology 17: 1497–1504.

60. Placella SA, Brodie EL, Firestone MK (2012). Rainfall-induced carbon dioxide pulses result from sequential resuscitation of phylogenetically clustered microbial groups, Proceedings of the National Academy of Sciences 109: 10931–10936.

61. Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L (2012). Tansley review: Biomass allocation to leaves, stems and roots : meta-analyses of interspecific variation and environmental control, New Phytologist 193: 30–50.

62. Price MN, Dehal PS, Arkin AP (2009). Fasttree: Computing large minimum evolution trees with profiles instead of a distance matrix, Molecular Biology and Evolution, 26: 1641–1650.

63. Reichstein M, Bahn M, Ciais P, Frank D, Mahecha MD, Seneviratne SI, Zscheischler J, Beer C, Buchmann N, Frank DC, Papale D, Rammig A, Smith P, Thonicke K, Van der Velde M, Vicca S, Walz A, Wattenbach M (2013). Climate extremes and the carbon cycle, Nature 500: 287–295.

64. Rognes T, Flouri T, Nichols B, Quince C, Mahé F (2016). VSEARCH: a versatile open source tool for metagenomics, PeerJ, 4: e2584.

65. Ruehr NK, Offermann CA, Gessler A, Winkler JB, Ferrio JP, Buchmann N, Barnard RL (2009). Drought effects on allocation of recent carbon: From beech leaves to soil CO_2 efflux, New Phytologist 184: 950–961.

66. Ruiz-Rueda O, Hallin S, Bañeras L (2009). Structure and function of denitrifying and nitrifying bacterial communities in relation to the plant species in a constructed wetland, FEMS Microbiology Ecology 67: 308–319.

67. Saneoka H, Moghaieb REA, Premachandra GS, Fujita K (2004). Nitrogen nutrition and water stress effects on cell membrane stability and leaf water relations in Agrostis palustris Huds, Environmental and Experimental Botany 52: 131–138.

68. Schimel JP, Bennet J (2004). Concepts & Synthesis, Ecology 85: 591–602.

69. Schimel JP, Balser TC, Wallenstein M (2007). Microbial Stress-Response Physiology and Its Implications for Ecosystem Function, Ecology 88: 1386–1394.

70. Shangguan Z, Shao M, Dyckmans J (2000). Effects of nitrogen nutrition and water deficit on net photosynthetic rate and chlorophyll fluorescence in winter wheat, Journal of Plant Physiology 156: 46–51.

71. Shrestha J, Niklaus PA, Pasquale N, Huber B, Barnard RL, Frossard E, Schleppi P (2014). Flood pulses control soil nitrogen cycling in a dynamic river floodplain, Geoderma 228–229: 14–24.

72. Sistla SA, Schimel JP (2012). Stoichiometric flexibility as a regulator of carbon and nutrient cycling in terrestrial ecosystems under change, New Phytologist, 196: 68–78.

73. Six J, Frey SD, Thiet RK, Batten KM (2006). Bacterial and Fungal Contributions to Carbon Sequestration in Agroecosystems, Soil Science Society of America Journal 70: 555-569.

74. Skinner RH, Comas LH (2010). Root distribution of temperate forage species subjected to water and nitrogen stress, Crop Science 50: 2178–2185.

75. Steenwerth KL, Jackson LE, Calderón FJ, Scow KM, Rolston DE (2005). Response of microbial community composition and activity in agricultural and grassland soils after a simulated rainfall, Soil Biology and Biochemistry 37: 2249–2262.

76. Strimmer K (2008). fdrtool: A versatile R package for estimating local and tail area-based false discovery rates, Bioinformatics 24: 1461–1462.

77. Takahashi S, Tomita J, Nishioka K, Hisada T, Nishijima M (2014). Development of a prokaryotic universal primer for simultaneous analysis of Bacteria and Archaea using next-generation sequencing, PLoS ONE 9: e105592.

78. Vance ED, Brookes PC, Jenkinson DS (1987). An Extraction Method for Measuring Microbial Biomass C, Soil Biology and Biochemistry 19: 703–707.

79. Vainio EJ, Hantula J (2000). Direct analysis of wood-inhabiting fungi using denaturing gradient gel electrophoresis of amplified ribosomal DNA. Mycological Research 104: 927–936.

80. von Rein I, Gessler A, Premke K, Keitel C, Ulrich A, Kayler ZE (2016). Forest understory plant and soil microbial response to an experimentally induced drought and heat-pulse event: the importance of maintaining the continuum, Global Change Biology 22: 2861–2874.

81. Wallenstein MD, Hall EK (2012). A trait-based framework for predicting when and where microbial adaptation to climate change will affect ecosystem functioning, Biogeochemistry 109: 35–47.

82. Wang Y, Yu S, Wang J (2007). Biomass-dependent susceptibility to drought in experimental grassland communities, Ecology Letters 10: 401–410.

83. Wang Y, Jiang Q, Yang Z, Sun W, Wang D (2015). Effects of water and nitrogen addition on ecosystem carbon exchange in a meadow steppe, PLoS ONE 10: 1–16.

84. Wedderburn ME, Crush JR, Pengelly WJ, Walcroft JL (2010). Root growth patterns of perennial ryegrasses under well-watered and drought conditions, New Zealand Journal of Agricultural Research 53: 377–388.

85. Xie XF, Hu YK, Pan X, Liu FH, Song YB, Dong M (2016). Biomass Allocation of Stoloniferous and Rhizomatous Plant in Response to Resource Availability: A Phylogenetic Meta-Analysis, Frontiers in Plant Science 7: 1–11.

86. Yoshinari T, Hynes R, Knowles R (1977). Acetylene inhibition of nitrous oxide reduction and measurement of denitrification and nitrogen fixation in soil, Soil Biology and Biochemistry 9: 177–183.

87. Zhang J, Kobert K, Flouri T, Stamatakis A (2014). PEAR: A fast and accurate Illumina Paired-End reAd mergeR, Bioinformatics 30: 614–620.

88. Zhang X, Tan Y, Li A, Ren T, Chen S, Wang L, Huang J (2015). Water and nitrogen availability co-control ecosystem CO_2 exchange in a semiarid temperate steppe, Scientific Reports 5: 1–10.

89. Zhu B, Cheng W (2013). Impacts of drying-wetting cycles on rhizosphere respiration and soil organic matter decomposition, Soil Biology and Biochemistry 63: 89–96.

6. General discussion

6.1 Before rewetting: Precipitation regimes sets contrasting scenes for rewetting response

In all three experiments presented here, precipitation regime served to set a scene for a large rewetting event. The precipitation regimes were most contrasted in Chapter 1 since the experiment was originally designed to document microbial reactivation upon rewetting a Mediterranean soil after the dry summer. Plants were thus not included in the picture. In chapters 2 and 3, the experiments targeted more mesic systems that included live plants, in mesocosms that were exposed to contrasting precipitation regimes under controlled conditions. The precipitation regimes and treatment length were very similar in these 2 experiments. In short, one treatment consisted of daily water inputs, amounting to the minimum amount of water necessary to prevent plant wilting and the other treatment received the accumulated amount over 5-6 days (infrequent precipitation input). The contrasting precipitation regimes set the scene for the rewetting response by shaping plant morphological and physiological water-related strategies (chapters 2 and 3), soil microbial communities (chapter 1, 2 and 3) and system N transformations and budgets (chapter 3).

6.1.1 Plant morphological and physiological water-related strategies

Towards the end of the treatment period, the plants in chapter 3 showed decreased C acquisition through photosynthesis over a 5-day infrequent precipitation input cycle, whilst precipitation regime did not significantly impact the C acquisition through photosynthesis (over a 5-day infrequent precipitation input cycle) in chapter 2. In both experiments infrequent precipitation

input led to smaller total plant and above-ground plant biomass (Grant et al., 2014). Infrequent precipitation resulted in reduced overall root biomass in chapter 3 and modified the distribution of roots at different soil depths in chapter 2.

Aboveground and belowground processes are typically intensely coupled by the transfer of recent plant C to microbes, which is estimated to account for roughly half of the CO_2 emitted from soils (Högberg and Read, 2006). The amount of C that is transferred from plants to microbes through rhizodeposition is positively related to root biomass and can significantly influence microbial growth and activity (Canarini and Dijkstra, 2015). In both chapter 2 and chapter 3, tracking ^{13}C-labelled CO_2 from assimilation by photosynthesis to incorporation into microbial biomass showed that the higher root biomass measured generally corresponded to increased immobilisation of plant-derived C by soil microbes. However, we highlighted the following 2 conditions under which we found no correlation between increased microbial assimilation of plant-derived C and larger root biomass.

(1) In chapter 2 we observed reduced plant-microbial coupling in the top soil layer under infrequent precipitation input. Under this precipitation regime, despite root biomass being significantly higher in the top soil layer than the deeper soil layers, we did not observe the expected larger C assimilation by microbes. As neither photosynthesis nor the allocation of C from shoots to roots was affected by precipitation regime in this study, we deduct that the decrease in plant-microbial coupling was likely due to reduced C rhizodeposition or reduced immobilisation of C by the soil microbes. A potential reason for the latter hypothesis, despite potentially large amounts of exudated C available, is that the microbes had reduced access to exudates because of the diffusional limitations associated with very dry soils (Schimel and Bennet, 2004, Or et al., 2007).

(2) In chapter 3 we found a reduction of plant-microbial coupling in systems with frequent precipitation and low N availability. Both frequent precipitation as well as low N application treatments enhanced root biomass production, yet microbial immobilization of C was reduced in this treatment. However, in this case we found evidence for severely limited C assimilation by the plant and thus suggest this is the main cause for the reduced flow of C from plant to microbes. Studies have shown that high levels of N can improve plant photosynthesis, especially under water-stressed conditions (Saneoka et al., 2004). In our system, the lower N application treatment may thus have resulted in insufficient N for the plant to sustain optimal rates of photosynthesis whilst maintaining its relatively larger biomass under water-limited conditions.

6.1.2 Soil microbial communities

Microbial communities and activities in soil are tightly linked to both soil moisture and plant function (root respiration, C availability and competition for N). Thus, we expected to find significant shifts in the bacterial and fungal communities in response to contrasting precipitation regime, directly due to the contrasting soil moisture fluctuations but also indirectly due to the effects of precipitation regime on plant morphological and physiological traits. Contrasting precipitation regimes led to significantly different bacterial and fungal community compositions in all 3 chapters. The changes most likely to impact ecosystem process included (1) a shift in fungal:bacterial ratio, (2) shifts in dominant OTUs and (3) the impact of precipitation patterns on the microbial seed banks in the surface soils. These 3 mechanisms are discussed below.

(1) Infrequent precipitation input shifts fungal:bacterial ratio in favour of bacterial dominance. In chapter 3, which was performed on soils from the top 10 cm of soil, infrequent precipitation

application led to an increase in the present bacterial but did not significantly impact present fungal abundance. We thus showed that on top of the expectation that fungi are more resistant to cycles of drying and rewetting than bacteria (Gordon et al., 2008), their abundance is unaffected by the length of the dry period and magnitude of the rewetting event in our experiment. However, under infrequent precipitation input, increased bacterial abundance while fungal abundance remained unchanged suggests a progressive increase in bacterial dominance over fungi. As fungal-rich microbial communities are linked to enhanced stability of soil food webs, increased bacterial dominance could indicate enhanced N losses (de Vries et al., 2012, Gordon et al., 2008) as well as reduced C sequestration potential (Six et al., 2006, Malik et al., 2016) of the systems.

(2) Infrequent precipitation input resulted in more contrasting dominant precipitation significant OTU between the present and potentially active communities of both bacteria and fungi in chapter 3. The larger fluctuations in soil water potential (from wilting point to near water holding capacity) experienced in the infrequent treatment likely promoted the activity of more contrasted communities at different times of the dry-wet cycles. The findings from chapter 3 were based on communities from the top 10 cm of the soil profile of plant-soil systems respectively.

(3) The findings from chapter 2 elaborate how the precipitation regimes may impact the bacterial and fungal seed banks throughout the soil profile. The microbial seedbanks referred to here are the communities which are not actively growing upon rewetting and thus did not incorporate the heavy ^{18}O label. Any shifts in this inactive community composition would have occurred before the final rewetting. Even though we cannot differentiate between inactive microbes and relic DNA, it is likely that the relic DNA makes up only a small fraction and we thus consider this fraction to include predominantly seedbanks. Both the bacterial and fungal inactive seed banks showed a strong depth gradient, characterised by decreased evenness. The

inactive bacterial communities additionally decreased in richness but increased in phylogenetic relatedness down the soil profile. Decreased root density and thus lower labile C supply with depth leads to increased need for resource specialisation for microbial growth, even when considering a soil profile of only 35 cm. There was variation in how far down the soil profile the watering front moved, based on the precipitation regime (i.e. The volume of water applied), resulting in contrasting soil moisture fluctuations between different depth and different treatments. Yet precipitation history only impacted the microbial seed banks in the surface soils where it had contrasting effects on bacterial and fungal evenness. Infrequent precipitation input resulted in a loss of bacterial evenness but enhanced fungal evenness, confirming the contrasting water-related strategies of bacteria and fungi. The top soil is where root density was greatest and where soils are most exposed to moisture fluctuations (reviewed by Tecon and Or, 2017). Surface soils experience an increase in soil moisture even with small precipitation volume inputs and dry out faster due to evaporation as well as root water uptake.

We thus demonstrate that a history of contrasting precipitation patterns results in shifts in the active as well as the inactive microbial communities and affect bacterial and fungal communities differently, which may likely impact biogeochemical cycling in the systems.

6.1.3 N transformations, N pools and how N availability modulates the effect of precipitation regime

Soil microbes drive biogeochemical cycling, which is tightly modulated by their numerous interactions with plants. In chapter 3 we evaluated the impacts of precipitation regime on N transformations and N pools within experimental plant-soil systems and found that infrequent precipitation input enhanced soil N cycling. The cycles of more extensive drying and more complete saturation likely resulted in alternating optimal soil aeration conditions for aerobic

nitrification and anaerobic denitrification processes, respectively, resulting in smaller soil inorganic N pool sizes. We found limited impacts of precipitation regime on organic N pools (i.e. plant biomass and microbial biomass) suggesting that they may be more buffered to cycles of drying and rewetting than inorganic N pools (Morillas et al., 2013).

In addition to the observed impacts of precipitation regime on N transformations and N pools, we found that N availability in turn may modulate the impact of precipitation regime. In chapter 3 we added contrasting N input as a treatment in order to evaluate the interactive effects of precipitation regime and N availability. Out findings show that high N availability may modulate the effect of precipitation regime by (1) further reducing plant root biomass production, (2) further increasing bacterial dominance over fungi and (3) altering the relative amounts of N captured by plants and microbes. These 3 modes of action are discussed below.

(1) High N application may further exacerbate the root biomass reduction observed under infrequent precipitation regime. In resource-limited environments, the general theory is that plants allocate resources preferentially to the structures which may increase acquisition of the limiting resource, such as investing in larger root biomass to improve water or N uptake (Bloom et al., 1985). In our systems we found indeed that plants with a history of low N application had larger root biomass.

(2) Not only did high N input further exacerbate the increased bacterial dominance over fungi which was observed under infrequent precipitation input, but high N input also impacted different groups in the bacterial and fungal communities. N input shaped the total present bacterial communities but did not impact potentially active bacterial communities, suggesting a large impact on the seed bank but not on the active bacterial players. Contrastingly though, N input did not affect the overall present fungal communities but did determine the structure

of the potentially active communities, where, in accordance with previous studies, high N application led to a loss of evenness and phylogenetic diversity (Freedman et al., 2015).

(3) N availability had contrasting effects on the relative distribution of N within the organic N pools (microbial biomass versus plant biomass). In our system, high N input levels increased plant biomass N pools whilst low N input history increased microbial biomass N pools. Thus, even though organic N pools showed resistance to precipitation regime, inorganic N input can potentially alter the dynamics between plants and microbes in their competition for N.

In our system, precipitation patterns determined microbial N transformation rates and with this the size of the soil inorganic N pools. N availability in turn may modulate the effects of precipitation regime by shaping plant morphology and organic N pools. This indicates potentially contrasting N dynamics within our systems which may likely influence their response to rewetting.

6.2 Rewetting response of the plant-soil system: Legacy of precipitation regime

Exposure of our soil-only and plant-soil systems to a history of contrasting precipitation regimes set contrasting scenes by influencing plant biomass production, microbial access to fresh plant-derived C, soil bacterial and fungal communities and N budgets. We evaluated how these contrasting scenes may govern the response of our systems to a large rewetting event by evaluating the flux of CO_2 emitted from soils upon rewetting, the active bacterial and fungal responses over time and at different soil depths, and the plant-microbial competition for N.

6.2.1 Soil CO₂ efflux upon rewetting

In chapter 1 and 3, we linked the microbial response to the pulse of CO_2 released from the soils upon rewetting, a phenomenon termed the Birch effect (Birch, 1958). In chapter 3 we found that in the presence of live plants, frequent precipitation input resulted in a larger soil CO_2 emissions upon rewetting than infrequent precipitation input, which was not related to N input history. In our systems, we showed that despite observing a decrease in C flux from plants to microbes in systems under frequent precipitation and low N, N application did not significantly impact the magnitude or persistence of the CO_2 pulse upon rewetting. Evidence suggests that the microbes in these systems might be respiring significant amounts of C from sources other than fresh photosynthate or that larger root biomass in these systems is contributing to the flux of respiration. With the results from chapter 1, we add that, in the absence of plants i.e. the absence of a supply of labile C, the pulse of CO_2 upon rewetting is likely to be fuelled by dead microbial cell material, in line with previous findings (Blazewicz et al., 2014). Shifts in the balance between C sequestration and C loss from the system could drastically alter C budgets and may also likely have consequences for N-cycling within the system.

6.2.2 Microbial response to rewetting: Temporal and depth aspects

Bacteria and fungi have different water-related strategies (see Barnard et al. 2013 for contrasting water-related strategies within the bacterial domain), and this led to contrasting responses to rewetting in all 3 chapters. Bacteria showed an overall rapid and sustained response to rewetting, which was not modulated by precipitation or N input history and was consistent across the soil profile. The bacterial response to rewetting was driven by a relatively small group of phylogenetically clustered taxa which were primed to respond to the flush of nutrients upon rewetting. In chapter 3 we showed that regardless of precipitation or N input history, the bacteria which became potentially active within 1 hour after rewetting remained so

for up to at least 29 hours in our system with no further significant changes within this time frame. The response to rewetting was driven predominantly by a relative increase in the abundance of Bacteroidetes, a phylum of Gram-negative, non-sporeforming bacteria which are widely distributed in the soil, particularly in the root rhizosphere (Shi and Marschner, 2014). They have been demonstrated to be highly capable organic matter degraders which rapidly increase in abundance in response to organic matter input (reviewed by Thomas et al., 2011) but decrease during desiccation (Pohlon et al., 2013). Findings in chapter 1 show that this bacterial response to rewetting may even be sustained for as long as 5 days. In this study, we found that the bacterial communities which were active 48 hours after rewetting remained unchanged in structure or diversity up to 120 hours. With the findings from chapter 2, we add that the bacterial response to rewetting was also consistent throughout the soil profile (up to 35 cm depth), regardless of precipitation history, suggesting that the bacterial response to rewetting is a well-conserved trait.

Fungi, on the other hand, show a much more delayed response to rewetting than bacteria, regardless of precipitation or N input legacy. In chapter 3 we found a gradual increase in richness, relatedness and evenness of the potentially active fungi over time, but even at 29 hours post-rewetting the fungal community structure was not significantly different from pre-wet. This was further emphasised by our findings in chapter 1, which showed that the active fungal communities upon rewetting were only marginally different from the seed bank and showed no significant shifts in structure for up to 120 hours. Studies have frequently highlighted that fungi are inherently more resistant to drying and may thus not be as primed to respond to a rewetting event (Landesman and Dighton, 2011, Barnard et al., 2015). In addition to the fact that fungi might be less water-deprived than bacteria (de Boer et al., 2005), they also exhibit much slower response and growth rates (de Vries and Shade, 2013). With the findings from chapter 2, we add that the active fungal response to rewetting (unlike bacteria) is not

consistent throughout the soil profile. The active fungi showed a decrease in evenness with soil depth across all treatments. Fungi have a higher requirement for C (relative to N) than bacteria (Hodge et al., 2000) and C supply by roots may thus not only impact the inactive seed pool but also the active responders. The active fungal response to rewetting is much smaller than and not as conserved as the bacterial response. The ability to respond to increased moisture is inherent to fungi and is more likely shaped by the access to labile C rather than by water availability.

6.2.3 Plant-microbial competition for N

Our results showed that the timing of plant-microbial competition for N upon rewetting was not impacted by precipitation history, but a history of frequent precipitation input resulted in overall enhanced competitiveness of plants. In chapter 3, we also elaborated on plant microbial interactions upon rewetting by including plant microbial competition for N over a 29-hour time period. We found that immediately after rewetting, microbes had the competitive edge over plants, likely due to their fast response rates and high affinity for substrate (Schimel and Bennet, 2004). However, it has been suggested that the competitiveness of the plants improves gradually over time as microbial biomass turns over more rapidly than plants who are able retain their assimilated N for longer (Schimel and Bennet, 2004, Hodge et al., 2000, Kuzyakov et al., 2013). This timing aspect of the dynamic competition for N between plants and microbes was observed in our systems, regardless of precipitation or N availability history. A history of favourable conditions for plants, however, may increase their overall competitiveness for N over microbes. In our system, the larger plants associated with frequent precipitation history were overall more competitive for N at all time points. This indicates that a shift in precipitation patterns could alter the dynamics between plants and microbes in their competition for N, with potentially contrasting consequences for plant biomass production and microbial N cycling.

Finally, we also found that even though the N cycle was enhanced under infrequent precipitation regime, upon rewetting a short (<1 hour post rewetting) pulse of N_2O was emitted from systems which had a combined history of frequent precipitation and high N input. Possible explanations include that the N_2O pulse from the fast cycling infrequent treatment was immediate and transient and may thus not have been captured in our 1-29 hour time frame.

7. Conclusion and perspectives

Predicted shifts in precipitation regimes, characterized by longer dry periods and rain events of larger magnitude, will likely have negative impacts on ecosystem services, particularly in systems which are frequently water-constrained, such as dry and seasonally dry climate zones.

This work shed light onto how precipitation patterns may impact the amount of CO_2 fixed by plants, the potential access soil microbes may have to recent photoassimilates and how much of the C is released back into the atmosphere upon rewetting. Contrary to expectations, we found that in our plant-soil systems, neither the length of the preceding dry period nor the C acquisition potential of plants had any significant impact on the magnitude or persistence of the CO_2 flux emitted by rewet soil. In the presence of plants, we hypothesise that increased root respiration linked to larger root biomass may be a significant contributor to the soil CO_2 efflux pulse upon rewetting, whilst dead microbial cells may have been its major source in soil-only systems. Further research is needed to determine the sources of the contrasting magnitude of soil CO_2 efflux and whether the predicted shifts in water availability may significantly alter the stabilisation of C into SOM. Understanding the impact of changing climate on SOC stabilisation is crucial for understanding long-term C budgets of the ecosystem.

Our findings highlight that a pattern of infrequent, larger magnitude rain events may stimulate microbial N cycling within the soil, resulting in smaller inorganic N pools. On the other hand, frequent, smaller magnitude events enhance the competitiveness of plants for inorganic N over soil microbes upon rewetting.

Further, our findings strengthen the existing theory of contrasting water-related strategies between bacteria and fungi and highlight the potential consequences this may have for soil food web stability. We add to this that whilst long term (about 12 weeks) exposure to contrasting precipitation regimes resulted in significantly different bacterial and fungal communities, the

short term (up to 120 hours) active response to rewetting was relatively well conserved. This suggests that the microbial response to rewetting may have a high level of resistance to disturbance. However, a loss of diversity in the bacterial and fungal inactive seed banks suggests that the communities may lose some functional potential with long-term exposure, which could lead to changes in ecosystem processes under different environmental conditions. Our findings suggest that changes in precipitation pattern impact soil microbes most in the surface soils whilst deeper soil horizons appeared to be more buffered. Thus, we highlight the importance of considering the whole soil profile when determining the impact of disturbances such as changing precipitation patterns on ecosystem processes.

Linking microbial community composition and ecosystem processes is a major challenge in microbial ecology and with the work presented here we add to the growing body of literature. There are several avenues of future research that could significantly advance our understanding:

 (1) Trait-based approaches

The aim of trait-based approaches is to use the physiological, morphological or genomic characteristics at the individual level (e.g. physiological characteristics of strains) or aggregated at the community level (e.g. functional gene pools) to predict their functional potential under different environmental conditions (Violle et al., 2007, reviewed by Krause et al., 2014).

However, in practice this proves difficult as firstly, the gene phylogenies which are commonly applied in microbial ecology are often unable to resolve functional traits and second, experimental designs aimed at unravelling the link between biodiversity and ecosystem functioning are complicated to execute due to the interchangeable effects of biodiversity and

ecosystem processes. Shifts in biodiversity impact ecosystem processes but conversely it could also be shifts in ecosystem processes which induce the changes in biodiversity.

(2) Link biophysical approaches and biogeochemical cycles

The link between the biological (i.e. microbial activities) and physical (i.e. diffusion, dissolution) aspects associated with transient drying and rewetting has been the received a lot of attention (reviewed by Or et al., 2007 and Hinsinger et al., 2009). Several studies have also evaluated the impact of these biophysical aspects on soil trace gases fluxes (reviewed by Kin et al., 2011) and some advances have been made with including this in ecosystem process models (reviewed by Tecon and Or, 2017). The transient biophysical aspects of drying and rewetting, combined with the heterogeneous distribution of SOM within the soil matrix add to the immense temporal and spatial variability in biogeochemical processes within this complex environment. However, despite significant advances within the individual disciplines, a more coherent interdisciplinary framework is necessary to predict the impact of biophysical aspects associated with changing precipitation patterns on biogeochemical cycling.

(3) Modelling approaches

Most models predicting the effects of climate change on ecosystem processes do not include microbial ecology parameters. Relatively recently, microbial physiological properties such as drought tolerance, growth efficiency, dormancy and turn-over rates have been included to enhance ecosystem models (Manzoni and Katul., 2014; Wang et al., 2015), however, shifts in community structure are rarely considered (reviewed by Graham et al., 2016) . The work shown here, in concurrence with others, suggests that shifts in the composition of microbial communities may indeed be linked to variations in ecosystem processes and may thus enhance the predictive accuracy of the models.

8. References

1. Aanderud ZT, Lennon JT (2011). Validation of heavy-water stable isotope probing for the characterization of rapidly responding soil bacteria, Applied and Environmental Microbiology 77: 4589–4596.
2. Abid M, Tian Z, Ata-Ul-Karim ST, Cui Y, Liu Y, Jiang D, Dai T (2016). Nitrogen Nutrition Improves the Potential of Wheat (Triticum aestivum L.) to Alleviate the Effects of Drought Stress during Vegetative Growth Periods, Frontiers in Plant Science 7: 1–14.
3. Ashton IW, Miller AE, Bowman WD, Suding KN (2008). Nitrogen preferences and plant-soil feedbacks as influenced by neighbors in the alpine tundra, Oecologia 156: 625–636.
4. Augé RM, Toler HD, Saxton AM (2014). Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis, Mycorrhiza 25: 13–24.
5. Averill C, Waring BG, Hawkes CV (2016). Historical precipitation predictably alters the shape and magnitude of microbial functional response to soil moisture, Global Change Biology 22: 957–1964.
6. Bapiri A, Bååth, E, Rousk J (2010). Drying-Rewetting Cycles Affect Fungal and Bacterial Growth Differently in an Arable Soil, Microbial Ecology 60: 419-428.
7. Barnard RL, Osborne CA, Firestone MK (2013). Responses of soil bacterial and fungal communities to extreme desiccation and rewetting, ISME Journal 11: 2229-2241.
8. Barnard RL, Osborne CA, Firestone MK (2015). Changing precipitation pattern alters soil microbial community response to wet-up under a Mediterranean-type climate, ISME Journal 9: 946-957.
9. Bartlett MK, Klein T, Jansen S, Choat B, Sack L (2016). The correlations and sequence of plant stomatal, hydraulic, and wilting responses to drought, Proceedings of the National Academy of Sciences 113: 13098–13103.
10. Bates BC, Kundzewicz ZW, Wu S, Palutikof JP (2008). Climate Change and Water. Technical Paper of the Intergovernmental Panel on Climate Change, IPCC Secretariat, Geneva 1-210.
11. Birch HF (1958). The effect of soil drying on hummus decomposition and nitrogen availability, Plant and Soil 1: 9-31.
12. Blagodatskaya E, Kuzyakov Y (2013). Active microorganisms in soil: Critical review of estimation criteria and approaches, Soil Biology and Biochemistry 67: 192–211.
13. Blazewicz SJ, Barnard RL, Daly RA, Firestone MK (2013). Evaluating rRNA as an indicator of microbial activity in environmental communities: Limitations and uses, ISME Journal 7: 2061–2068.
14. Blazewicz SJ, Schwartz E, Firestone MK (2014). Growth and death of bacteria and fungi underlie rainfall-induced carbon dioxide pulses from seasonally dried soil, Ecology 95: 1162–1172.
15. Bloom AJ, Chapin FS, Mooney HA (1985). Plants-an Economic Analogy, Annual Review of Ecology and Systematics 16: 363-392.
16. Bonaterra A, Camps J, Montesinos E (2005). Osmotically induced trehalose and glycine betaine accumulation improves tolerance to desiccation, survival and efficacy of the

postharvest biocontrol agent Pantoea agglomerans EPS125, FEMS Microbiology Letters 250: 1-8.

17. Bonfante P, Genre A (2010). Mechanisms underlying beneficial plant - Fungus interactions in mycorrhizal symbiosis, Nature Communications 1: 1–11.

18. Boot CM, Schaeffer SM, Schimel JP (2013). Static osmolyte concentrations in microbial biomass during seasonal drought in a California grassland, Soil Biology and Biochemistry 57: 356-361.

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

20. Britto DT, Kronzucker HJ (2002). NH_4^+ toxicity in higher plants: a critical review, Journal of Plant Physiology 159: 567–584.

21. Brunner I, Herzog C, Dawes MA, Arend M, Sperisen C (2015). How tree roots respond to drought, Frontiers in Plant Science 6: 1–16.

22. Canarini A, Dijkstra FA (2015). Dry-rewetting cycles regulate wheat carbon rhizodeposition, stabilization and nitrogen cycling, Soil Biology and Biochemistry 81: 195-203.

23. Carini P, Marsden PJ, Leff JW, Morgan EE, Strickland MS, Fierer N (2016). Relic DNA is abundant in soil and obscures estimates of soil microbial diversity, Nature Microbiology 2: 1-6.

24. Cantarel AAM, Ommier T, Desclos-Theveniau M, Diquélou S, Dumont M, Grassein F, Kastl EM, Grigulis K, Lainé P, Lavorel S, Lemauviel-Lavenant S, Personeni E, Schloter M (2014). Using plant traits to explain plant-microbe relationships involved in nitrogen acquisition, Ecology 96: 788–799.

25. Chenu C, Roberson EB (1996). Diffusion of glucose in microbial extracellular polysaccharide as affected by water potential, Soil Biology and Biochemistry 28: 877–884.

26. Clein JS, Schimel JP (1994). Reduction in microbial activity in birch litter due to drying and rewetting events. Soil Biology and Biochemistry 26: 403–406.

27. Cole JA (1988) Assimilatory and dissimilatory reduction of nitrate to ammonia, in: The Nitrogen and Sulphur Cycles, edited by: Cole,J. A. and Ferguson, S. J., Cambridge University Press, Cambridge, 281–329

28. Costa E., Pérez J, Kreft JU (2006). Why is metabolic labour divided in nitrification? Trends in Microbiology 14: 213–219.

29. Cox PM, Betts RA, Jones CD, Spall SA, Totterdell IJ (2000). Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model, Nature 408: 184-187.

30. Csonka LN (1989). Physiological and genetic responses of bacteria to osmotic stress. Microbiological Reviews 53: 121-147.

31. Daims H, Lebedeva EV, Pjevac P, Han P, Herbold C, Albertsen M, Jehmlich N, Palatinszky M, Vierheilig J, Bulaev A, Kirkegaard RH, Von Bergen M, Rattei T, Bendinger B, Nielsen PH, Wagner M (2015). Complete nitrification by Nitrospira bacteria, Nature 528: 504–509.

32. de Boer W, Folman LB, Summerbell RC and Boddy L (2005). Living in a fungal world: Impact of fungi on soil bacterial niche development, FEMS Microbiology Reviews 29: 795-811.

33. de Boer W, Kowalchuck GA, van Veen JA (2006). Root-food and the rhizosphere microbial community composition, New Phytologist, 170: 3–6.

34. de Vries FT, Liiri ME, Bjørnlund L, Setälä HM, Christensen S and Bardgett RD (2012). Legacy effects of drought on plant growth and the soil food web, Oecologia 170: 821-833.

35. de Vries FT and Shade A (2013). Controls on soil microbial community stability under climate change, Frontiers in Microbiology 4: 1-16.

36. Dechesne A, Or D, Smets BF (2008). Limited diffusive fluxes of substrate facilitate coexistence of two competing bacterial strains, FEMS Microbiology Ecology 64: 1–8.

37. Dungait JAJ, Hopkins DW, Gregory AS, Whitmore AP (2012). Soil organic matter turnover is governed by accessibility not recalcitrance, Global Change Biology 18: 1781–1796.

38. Egerton-Warburton LM, Graham RC, Hubbert KR (2003). Spatial variability in mycorrhizal fungi and nutrient and water availability in a soil-weathered bedrock profile, Plant and Soil 249: 331–342.

39. Eilers KG, Debenport S, Anderson S, Fierer N (2012). Digging deeper to find unique microbial communities: The strong effect of depth on the structure of bacterial and archaeal communities in soil, Soil Biology and Biochemistry 50: 58–65.

40. Evans JR (1989). Photosynthesis and nitrogen relationships in leaves of C3 plants, Oecologia 78: 9–19.

41. Evans SE, Wallenstein MD (2012). Soil microbial community response to drying and rewetting stress: Does historical precipitation regime matter? Biogeochemistry 109: 101-116.

42. Eziz A, Yan Z, Tian D, Han W, Tang Z, Fang J (2017). Drought effect on plant biomass allocation: A meta-analysis, Ecology and Evolution 7: 11002–11010.

43. Feng S, Fu Q (2013). Expansion of global drylands under a warming climate, Atmospheric Chemistry and Physics 13: 10081–10094.

44. Fetzer I, Johst K, Schäwe R, Banitz T, Harms H, Chatzinotas A (2015). The extent of functional redundancy changes as species' roles shift in different environments, Proceedings of the National Academy of Sciences 112: 14888–14893.

45. Fierer N, Schimel JP (2002). Effects of drying - rewetting frequency on soil carbon and nitrogen transformations, Soil Biology Biochemistry 34: 777–787.

46. Fierer N, Schimel JP, Holden PA (2003). Influence of drying-rewetting frequency on soil bacterial community structure, Microbial Ecology 45: 63–71.

47. Fontaine S, Henault C, Aamor A, Bdioui N, Bloor JMG, Maire V, Mary B, Revaillot S, Maron PA (2011). Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect, Soil Biology and Biochemistry 43: 86–96.

48. Fontaine S, Mariotti A, Abbadie L (2003). The priming effect of organic matter: A question of microbial competition? Soil Biology and Biochemistry 35: 837–843.

49. Fontaine S, Bardoux G, Abbadie L, Mariotti A (2004). Carbon input to soil may decrease soil carbon content, Ecology Letters 7: 314–320.

50. Fontaine S, Barot S, Barré P, Bdioui N, Mary B, Rumpel C (2007). Stability of organic carbon in deep soil layers controlled by fresh carbon supply, Nature 450: 277–280.

51. Freedman ZB, Romanowicz KJ, Upchurch RA, Zak DR (2015). Differential responses of total and active soil microbial communities to long-term experimental N deposition, Soil Biology and Biochemistry 90: 275–282.

52. Fuchslueger L, Bahn M, Hasibeder R, Kienzl S, Fritz K, Schmitt M, Watzka M, Richter A (2016). Drought history affects grassland plant and microbial carbon turnover during and after a subsequent drought event, Journal of Ecology 104: 1453–1465.

53. Galloway JN, Cowling EB, Seitzinger SJ, Socolow R (2002). Reactive nitrogen: too much of a good thing? AMBIO: A Journal of the Human Environment 31: 60–63.

54. Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, Karl DM, Michaels AF, Porter JH, Townsend AR, Vo CJ (2004). Nitrogen cycles: past, present and future, Biogeochemistry 70: 153-226.

55. Giorgi F, Lionello P (2008). Climate change projections for the Mediterranean region, Global and Planetary Change 63: 90–104.

56. Global Carbon Budget 2017, by Le Quéré C et al, Earth System Science Data Discussions [www.globalcarbonproject.org/carbonbudget].

57. Gordon H, Haygarth PM, Bardgett RD (2008). Drying and rewetting effects on soil microbial community composition and nutrient leaching, Soil Biology and Biochemistry, 40: 302–311.

58. Gougoulias C, Clark JM, Shaw LJ (2014). The role of soil microbes in the global carbon cycle: Tracking the below-ground microbial processing of plant-derived carbon for manipulating carbon dynamics in agricultural systems, Journal of the Science of Food and Agriculture 94: 2362–2371.

59. Grant K, Kreyling J, Dienstbach LFH, Beierkuhnlein C, Jentsch A (2014). Water stress due to increased intra-annual precipitation variability reduced forage yield but raised forage quality of a temperate grassland, Agriculture, Ecosystems and Environment 186: 11–22.

60. Griffiths BS, Philippot L (2013). Insights into the resistance and resilience of the soil microbial community, FEMS Microbiology Reviews 37: 112–129.

61. Harrison KA, Bol R, Bardgett RD (2007). Preferences for Different Nitrogen Forms by Coexisting Plant Species and Soil Microbes, Ecological Society of America 88: 989–999.

62. Hegde RS, Fletcher JS (1996) Influence of plant growth stage and season on the release of root phenolics by mulberry as related to development of phytoremediation technology. Chemosphere 32: 2471–2479.

63. Hettich LR, Pan C, Chourey K, Giannone RJ (2013). Metaproteomics: Harnessing the power of high performance mass spectrometry to identify the suite of proteins that control metabolic activities in microbial communities, Analytical Chemistry 85: 4203–4214.

64. Hodge A, Robinson D, Fitter A (2000). Are microorganisms more effective than plants at competing for nitrogen? Trends in Plant Science 5: 304–308.

65. Hofer D, Suter M, Buchmann N, Lüscher A (2017). Nitrogen status of functionally different forage species explains resistance to severe drought and post-drought overcompensation, Agriculture, Ecosystems and Environment 236: 312–322

66. Högberg P, Read D (2006). Towards a more plant physiological perspective on soil ecology Trends in Ecology and Evolution 21: 548–554

67. Huang L, He B, Chen A, Wang H, Liu J, Lu A, Chen Z (2016). Drought dominates the interannual variability in global terrestrial net primary production by controlling semi-arid ecosystems, Scientific Reports 6: 1–7.

68. Iovieno P, Bååth E (2008). Effect of drying and rewetting on bacterial growth rates in soil, FEMS Microbiology Ecology 65: 400–407.

69. IPCC (2007). Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.

70. IPCC (2013). Climate Change 2013: Chapter 6. Carbon and Other Biogeochemical Cycles Chapter 6 of the Fifth Assessment Report of the Intergovernmental Panel on Climate Change 465-570.

71. Jackson LE, Schimel JP, Firestone MK (1989). Short-term partitioning of ammonium and nitrate between plants and microbes in an annual grassland. Soil Biology and Biochemistry 21: 409–415.

72. Jones CM, Spor A, Brennan FP, Breuil M, Bru D, Lemanceau P, Griffiths B, Hallin S, Philippot L (2014). Recently identified microbial guild mediates soil N_2O sink capacity, Nature Climate Change 4: 801–805.

73. Jones SE, Lennon JT (2010). Dormancy contributes to the maintenance of microbial diversity, Proceedings of the National Academy of Sciences 107: 5881–5886.

74. Kramer C, Gleixner G (2008). Soil organic matter in soil depth profiles: Distinct carbon preferences of microbial groups during carbon transformation, Soil Biology and Biochemistry 40: 425–433.

75. Kuzyakov Y (2010). Priming effects: Interactions between living and dead organic matter, Soil Biology and Biochemistry 42: 1363–1371.

76. Kuzyakov Y, Xu X (2013). Competition between roots and microorganisms for nitrogen: Mechanisms and ecological relevance, New Phytologist 198: 656–669.

77. Lado-Monserrat L, Lull C, Bautista I, Lidón A, Herrera R (2014). Soil moisture increment as a controlling variable of the "Birch effect". Interactions with the pre-wetting soil moisture and litter addition, Plant and Soil 379: 21–34.

78. Landesman WJ, Dighton J (2011). Shifts in Microbial Biomass and the Bacteria: Fungi Ratio Occur Under Field Conditions Within 3 h After Rainfall, Microbial Ecology 62: 228–236.

79. Lehmann J, Solomon D, Kinyangi J, Dathe L, Wirick S, Jacobsen C (2008). Spatial Complexity of Soil Organic Matter Forms at Nanometre Scales, Nature Geoscience 1: 238-242.

80. Lennon JT, Jones SE (2011). Microbial seed banks: The ecological and evolutionary implications of dormancy, Nature Reviews Microbiology 9: 119–130.

81. Lennon JT, Muscarella ME, Placella S (2017). How, when, and where relic DNA biases estimates of microbial diversity, Pre-print article.

82. Levy-Booth DJ, Campbell RG, Gulden RH, Hart MM, Powell JR, Klironomos JN, Pauls KP, Swanton CJ, Trevors JT, Dunfield KE (2007). Cycling of extracellular DNA in the soil environment, Soil Biology and Biochemistry 39: 2977–2991.

83. Maathuis FJ (2009). Physiological functions of mineral macronutrients, Current Opinion in Plant Biology 12: 250–258.

84. MacLean RC (2005). Adaptive radiation in microbial microcosms, Journal of Evolutionary Biology 18: 1376–1386. .

85. Makino A (2011). Photosynthesis, Grain Yield, and Nitrogen Utilization in Rice and Wheat, Plant Physiology 155: 125–129.

86. Malik AA, Chowdhury S, Schlager V, Oliver A, Puissant J, Vazquez PGM, Jehmlich N, von Bergen M, Griffiths RI, Gleixner G (2016). Soil fungal:bacterial ratios are linked to altered carbon cycling, Frontiers in Microbiology 7: 1–11.

87. Månsson K, Bengtson P, Falkengren-Grerup U, Bengtsson G (2009). Plant-microbial competition for nitrogen uncoupled from soil C:N ratios, Oikos 118: 1908–1916.

88. Manzoni S, Schimel JP, Porporato A (2012). Responses of soil microbial communities to water stress: Results from a meta-analysis, Ecology 93: 930–938.

89. Manzoni S, Katul G (2014). Invariant soil water potential at zero microbial respiration explained by hydrological discontinuity in dry soils, Geophysical Research Letters 41: 7151–7158.

90. Miranda JD, Armas C, Padilla FM, Pugnaire FI (2011). Climatic change and rainfall patterns: Effects on semi-arid plant communities of the Iberian Southeast, Journal of Arid Environments 75: 1302–1309.

91. Moreau D, Pivato B, Bru D, Busset H, Deau F, Faivre C, Matejicek A, Strbik F, Philippot L, Mougel C (2015). Plant traits related to nitrogen uptake influence plant- microbe competition, Ecology 96: 2300–2310.

92. Morillas L, Portillo-Estrada M, Gallardo A (2013). Wetting and drying events determine soil N pools in two Mediterranean ecosystems, Applied Soil Ecology 72: 160–170.

93. Mortimore M, Anderson S, Cotula L, Davies J, Faccer K, Hesse C, Morton J, Nyangena W, Skinner J, Wolfangel C (2009). Dryland opportunities: a new paradigm for people, ecosystems and development, IUCN, Gland, Switzerland; IIED, London, UK and UNDP/DDC, Nairobi, Kenya.

94. Moyano FE, Vasilyeva N, Bouckaert L, Cook F, Craine J, Curiel Yuste J, Don A, Epron D, Formanek P, Franzluebbers A, Ilstedt U, Kätterer T, Orchard V, Reichstein M, Rey A, Ruamps L, Subke JA, Thomsen IK, Chenu C (2012). The moisture response of soil heterotrophic respiration: Interaction with soil properties, Biogeosciences 9: 1173–1182.

95. Näsholm T, Kielland K, Ganeteg U (2009). Uptake of organic nitrogen by plants, New Phytologist 182: 31–48.

96. Nielsen KM, Johnsen PJ, Bensasson D, Daffonchio D (2007). Release and persistence of extracellular DNA in the environment, Environmental Biosafety Research 6: 37–53.

97. Or D, Smets BF, Wraith JM, Dechesne A, Friedman SP (2007). Physical constraints affecting bacterial habitats and activity in unsaturated porous media - a review, Advances in Water Resources 30: 1505–1527.

98. Owens H, LaFantasie J, Adler PB (2012). Mycorrhization rates of two grasses following alterations in moisture inputs in a southern mixed grass prairie, Applied Soil Ecology 60: 56–60.

99. Padilla FM, Aarts BHJ, Roijendijk YOA, de Caluwe H, Mommer L, Visser EJW, de Kroon H (2013). Root plasticity maintains growth of temperate grassland species under pulsed water supply, Plant and Soil 369: 377–386.

100. Philippot L, Hallin S, Börjesson G, Baggs EM (2009). Biochemical cycling in the rhizosphere having an impact on global change, Plant and Soil 321: 61–81.

101. Philippot L, Andert J, Jones CM, Bru D, Hallin S (2011). Importance of denitrifiers lacking the genes encoding the nitrous oxide reductase for N_2O emissions from soil, Global Change Biology, 17: 1497–1504.

102. Pietramellara G, Ascher J, Borgogni F, Ceccherini MT, Guerri G, Nannipieri P (2009). Extracellular DNA in soil and sediment: Fate and ecological relevance, Biology and Fertility of Soils 45: 219–235.

103. Placella SA, Brodie EL, Firestone MK (2012). Rainfall-induced carbon dioxide pulses result from sequential resuscitation of phylogenetically clustered microbial groups, Proceedings of the National Academy of Sciences 109: 10931–10936.

104. Pohlon E, Fandino AO, Marxsen J (2013). Bacterial community composition and extracellular enzyme activity in temperate streambed sediment during drying and rewetting, PLoS ONE 8: e83365.

105. Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L (2012). Tansley review Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control, New Phytologist 193: 30–50.

106. Prosser JI (2012). Ecosystem processes and interactions in a morass of diversity, FEMS Microbiology Ecology 81: 507–519.

107. Radajewski S, Ineson P, Parekh NR, Murrell JC (2000). Stable-isotope probing as a tool in microbial ecology, Nature 403: 646–649.

108. Ravishankara AR, Daniel JS, Portmann RW (2009). Nitrous Oxide (N_2O): The Dominant Ozone-Depleting Substance Emitted in the 21st Century, Science 123: 2007–2010.

109. Rayner PJ, Scholze M, Knorr W, Kaminski T, Giering R, Widmann H (2005). Two decades of terrestrial carbon fluxes from a carbon cycle data assimilation system (CCDAS), Global Biogeochemical Cycles 19: 1-20.

110. Ruamps LS, Nunan N, Chenu C (2011). Microbial biogeography at the soil pore scale, Soil Biology and Biochemistry 43: 280–286.

111. Rütting T, Boeckx P, Müller C, Klemedtsson L (2011). Assessment of the importance of dissimilatory nitrate reduction to ammonium for the terrestrial nitrogen cycle, Biogeosciences 8: 1779–1791.

112. Saneoka H, Moghaieb REA, Premachandra GS, Fujita K (2004). Nitrogen nutrition and water stress effects on cell membrane stability and leaf water relations in Agrostis palustris Huds, Environmental and Experimental Botany 52: 131–138.

113. Schimel JP, Braswell BH, McKeown R, Ojima DS, Parton WJ, Pulliam W (1996). Climate and Nitrogen controls on the geography and timescales of terrestrial biogeochemical cycling, Global Biogeochemical Cycles 10: 677-692

114. Schimel JP, Bennet J (2004). Nitrogen mineralisation: Challenges of a changing paradigm, Ecology 85: 591–602.

115. Schimel J, Balser TC, Wallenstein M (2007). Microbial Stress-Response Physiology and Its Implications for Ecosystem Function, Ecology 88: 1386–1394.

116. Schimel JP, Schaeffer SM (2012). Microbial control over carbon cycling in soil, Frontiers in Microbiology 3: 1–11.

117. Schimel J (2013). Soil carbon: Microbes and global carbon, Nature Climate Change 3: 867–868.

118. Schmidt MWI, Torn MS, Abiven S, Dittmar T, Guggenberger G, Janssens IA, Kleber M, Kögel-Knabner I, Lehmann J, Manning DAC, Nannipieri P, Rasse DP, Weiner S, Trumbore SE (2011). Persistence of soil organic matter as an ecosystem property, Nature 478: 49–56.

119. Schwartz E (2007). Characterization of growing microorganisms in soil by stable isotope probing with $H_2^{18}O$, Applied and environmental microbiology 73: 2541–2546.

120. Schwartz E, Van Horn DJ, Buelow HN, Okie JG, Gooseff MN, Barrett JE, Takacs-Vesbach CD (2014). Characterization of growing bacterial populations in McMurdo Dry

Valley soils through stable isotope probing with ^{18}O-water, FEMS microbiology ecology 89: 415–425.

121. Schwartz E, Hayer M, Hungate BA, Koch BJ, McHugh TA, Mercurio W, Morrissey EM, Soldanova K (2016). Stable isotope probing with ^{18}O-water to investigate microbial growth and death in environmental samples, Current Opinion in Biotechnology 41: 14–18.

122. Schwinning S, Sala OE (2004). Hierarchy of responses to resource pulses in arid and semi-arid ecosystems, Oecologia 141: 211–220.

123. Shi A, Marschner P (2014). Drying and rewetting frequency influences cumulative respiration and its distribution over time in two soils with contrasting management, Soil Biology and Biochemistry, 72: 172–179.

124. Sistla SA, Schimel JP (2012). Stoichiometric flexibility as a regulator of carbon and nutrient cycling in terrestrial ecosystems under change, New Phytologist 196: 68–78.

125. Six J, Bossuyt H, Degryze S, Denef K (2004). A history of research on the link between (micro) aggregates, soil biota, and soil organic matter dynamics, Soil and Tillage Research 79: 7–31.

126. Six J, Frey SD, Thiet RK, Batten KM (2006). Bacterial and Fungal Contributions to Carbon Sequestration in Agroecosystems, Soil Science Society of America Journal 70: 555-569.

127. Skinner RH, Comas LH (2010). Root distribution of temperate foliage species subjected to water and nitrogen stress, Crop Science 50: 2178-2185.

128. Smith KA, Ball T, Conen F, Dobbie KE, Massheder J, Rey A (2003). Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes, European Journal of Soil Science 54: 779–791.

129. Stark JM, Firestone MK (1995). Mechanisms for Soil Moisture Effects on Activity of Nitrifying Bacteria, Applied and Environmental Microbiology 61: 218–221.

130. Steenwerth KL, Jackson LE, Calderón FJ, Scow KM, Rolston DE (2005). Response of microbial community composition and activity in agricultural and grassland soils after a simulated rainfall, Soil Biology and Biochemistry 37: 2249–2262.

131. Tecon R, Or D (2017). Biophysical processes supporting the diversity of microbial life in soil, FEMS microbiology reviews 41: 599–623.

132. Thinh NC, Shimono H, Kumagai E, Kawasaki M (2017). Effects of elevated CO_2 concentration on growth and photosynthesis of Chinese yam under different temperature regimes, Plant Production Science 20: 227–236.

133. Thomas F, Hehemann JH, Rebuffet E, Czjzek M, Michel G (2011). Environmental and gut Bacteroidetes: The food connection, Frontiers in Microbiology 2: 1–16.

134. Tückmantel T, Leuschner C, Preusser S, Kandeler E, Angst G, Mueller CW, Meier IC (2017). Root exudation patterns in a beech forest: Dependence on soil depth, root morphology, and environment, Soil Biology and Biochemistry 107: 188–197.

135. Urich T, Lanzén A, Qi J, Huson DH, Schleper C, Schuster SC (2008). Simultaneous assessment of soil microbial community structure and function through analysis of the meta-transcriptome, PLoS ONE 3: e2527.

136. Van Diepen LTA, Frey SD, Landis EA, Morrison EW, Pringle A (2017). Fungi exposed to chronic nitrogen enrichment are less able to decay leaf litter, Ecology 98: 5–11.

137. Van Kessel MAHJ, Speth DR, Albertsen M, Nielsen PH, Op Den Camp HJM, Kartal B, Jetten MSM, Lücker S (2015). Complete nitrification by a single microorganism, Nature 528: 555–559.

138. Violle C, Navas ML, Vile D, Kazakou E, Fortunel C, Hummel I, Garnier E (2007). Let the concept of trait be functional! Oikos 116: 882–892.

139. Vos M, Wolf AB, Jennings SJ, Kowalchuk GA (2013). Micro-scale determinants of bacterial diversity in soil, FEMS microbiology reviews 37: 936–954.

140. Vu JCV, Allen LH, Boote KJ, Bowes G (1997). Effects of elevated CO_2 and temperature on photosynthesis and Rubisco in rice and soybean, Plant, Cell and Environment 20: 68–76.

141. Wallenstein MD, Hall EK (2012). A trait-based framework for predicting when and where microbial adaptation to climate change will affect ecosystem functioning, Biogeochemistry 109: 35–47.

142. Wang Y, Yu S, Wang J (2007). Biomass-dependent susceptibility to drought in experimental grassland communities, Ecology Letters 10: 401–410.

143. Wang Y, Jiang Q, Yang Z, Sun W, Wang D (2015). Effects of water and nitrogen addition on ecosystem carbon exchange in a meadow steppe, PLoS ONE 10: 1–16.

144. Wardle DA, Van der Putten WH (2002). Biodiversity, ecosystem functioning and above-ground-below-ground linkages. Loreau M, Naeem S,Inchausti P (eds), Biodiversity and Ecosystem Functioning, Synthesis and Perspectives 155-168.

145. Wedderburn ME, Crush JR, Pengelly WJ, Walcroft JL (2010). Root growth patterns of perennial ryegrasses under well-watered and drought conditions, New Zealand Journal of Agricultural Research 53: 377–388.

146. Wood NJ, Alizadeh T, Bennett S, Pearce J, Ferguson SJ, Richardson DJ, Moir JWB (2001). Maximal expression of membrane-bound nitrate reductase in Paracoccus is induced by nitrate via a third fnr-like regulator named NarR, Journal of Bacteriology 183: 3606–3613.

147. Xiang SR, Doyle A, Holden PA, Schimel JP (2008). Drying and rewetting effects on C and N mineralization and microbial activity in surface and subsurface California grassland soils, Soil Biology and Biochemistry 40: 2281–2289.

148. Yachi S, Loreau M (1999). Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis, Proceedings of the National Academy of Sciences 96: 1463–1468.

149. Yang Y, Guo J, Wang G, Yang L, Yang Y (2012). Effects of drought and nitrogen addition on photosynthetic characteristics and resource allocation of Abies fabri seedlings in eastern Tibetan Plateau, New Forests 43: 505–518.

150. Young IM, Crawford JW, Nunan N, Otten W, Spiers A (2008) Microbial distribution in soils: physics and scaling. Advances in Agronomy 100: 81–121.

151. Young JPW (1996). Phylogeny and taxonomy of rhizobia, Plant and Soil 186: 45–52.

152. Zhan J, Sun Q (2012). Diversity of free-living nitrogen-fixing microorganisms in the rhizosphere and non-rhizosphere of pioneer plants growing on wastelands of copper mine tailings, Microbiological Research 167: 157–165.

153. Zhou J, Xia B, Treves DS, Wu L, Marsh TL, Neill RVO, Palumbo AV, Tiedje JM (2002). Spatial and Resource Factors Influencing High Microbial Diversity in Soil Spatial and Resource Factors Influencing High Microbial Diversity in Soil, Applied and Environmental Microbiology 68: 326–334.

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10. Supplementary material

Fig. S1. Soil CO_2 efflux rate (a) and cumulative emissions (b) over 120 hours after rewetting, of soil with a history of dry (blue) and wet (red) watering regimes. Bars indicate $\pm$1s.e. (n=3).

Table S1. PERMANOVA results for the UniFrac pairwise dissimilarity of the relative abundance of bacterial sequences, based on 16S gene, and the Bray-Curtis distance of the relative abundance of fungal sequences, based on ITS gene Illumina MiSeq sequencing, using a non-parametric permutational approach. The explanatory variables are activity, precipitation pattern, depth and their interaction. The analysis is nested by mesocosm.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F value	R2	P value
Bacteria						
Mesocosm level						
Activity	1	0.754	0.754	92.33	0.611	<0.001
Precip. pattern	1	0.014	0.014	1.667	0.011	0.176
Activity × Precip. pattern	1	0.003	0.003	0.39	0.003	0.745
Residuals	22	0.180	0.008		0.146	
Depth level						
Depth	2	0.049	0.024	3.185	0.040	0.021
Depth × Activity	2	0.021	0.011	1.389	0.017	0.225
Depth × Precip. pattern	2	0.023	0.012	1.531	0.019	0.186
Depth × Activity × Precip. pattern	2	0.014	0.007	0.915	0.011	0.413
Residuals	36	0.275	0.008		0.223	
Fungi						
Mesocosm level						
Activity	1	0.813	0.813	11.956	0.093	<0.001
Precip. pattern	1	0.323	0.323	4.748	0.037	<0.001
Activity × Precip. pattern	1	0.071	0.071	1.047	0.008	0.347
Residuals	22	1.495	0.068		0.171	
Depth level						
Depth	2	1.246	0.623	5.622	0.143	<0.001
Depth × Activity	1	0.071	0.071	0.643	0.008	0.929
Depth × Precip. pattern	2	0.517	0.258	2.333	0.059	0.001
Depth × Activity × Precip. pattern	2	0.148	0.074	0.668	0.017	0.974
Residuals	36	3.988	0.111		0.457	

Table S2. PERMANOVA results for the UniFrac pairwise dissimilarity and the Bray-Curtis distance of the relative abundance of bacterial and fungal sequences, based on 16S and ITS gene Illumina MiSeq sequencing, respectively, both in the active and the inactive communities, using a non-parametric permutational approach. The explanatory variables are precipitation pattern, depth and their interaction. The analysis is nested by mesocosm.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F value	R2	P value
Active bacterial community						
Precip. pattern	1	0.010	0.010	0.998	0.032	0.096
Depth	2	0.038	0.019	1.837	0.119	0.038
Mesocosm	8	0.103	0.013	1.243	0.322	0.163
Precip. Pattern × Depth	2	0.024	0.012	1.133	0.073	0.324
Residuals	14	0.146	0.010		0.453	
Inactive bacterial community						
Precip. pattern	1	0.006	0.006	1.880	0.041	0.106
Depth	2	0.032	0.016	4.632	0.200	<0.001
Mesocosm	8	0.062	0.008	2.272	0.393	<0.001
Precip. Pattern × Depth	2	0.010	0.005	1.447	0.063	0.089
Residuals	14	0.048	0.003		0.303	
Active fungal community						
Precip. pattern	1	0.250	0.250	1.515	0.050	0.002
Depth	2	0.853	0.427	2.585	0.170	0.001
Mesocosm	8	1.222	0.153	0.926	0.243	0.002
Precip. Pattern × Depth	2	0.395	0.197	1.196	0.078	0.176
Residuals	14	2.311	0.165		0.459	
Inactive fungal community						
Precip. pattern	1	0.144	0.144	1.704	0.050	0.017
Depth	2	0.588	0.294	3.485	0.204	0.001
Mesocosm	8	0.708	0.088	1.048	0.245	0.393
Precip. Pattern × Depth	2	0.268	0.134	1.589	0.093	0.009
Residuals	14	1.182	0.084		0.409	

Fig. S1. Photograph of one of the mesocosms used in the study.
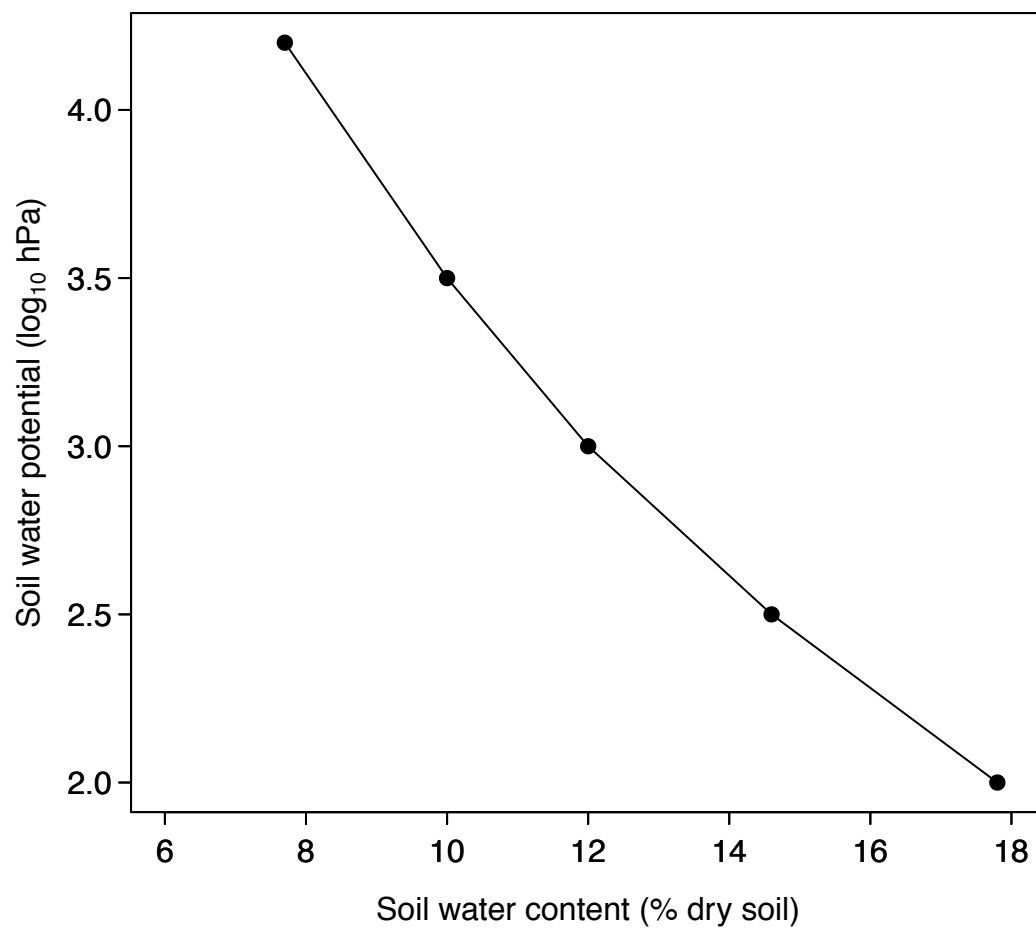
Fig. S2. Theoretical soil water retention curve for our soil, based on pedotransfer functions that used soil texture, bulk density as well as soil horizon (Al Majou *et al.*, 2008).
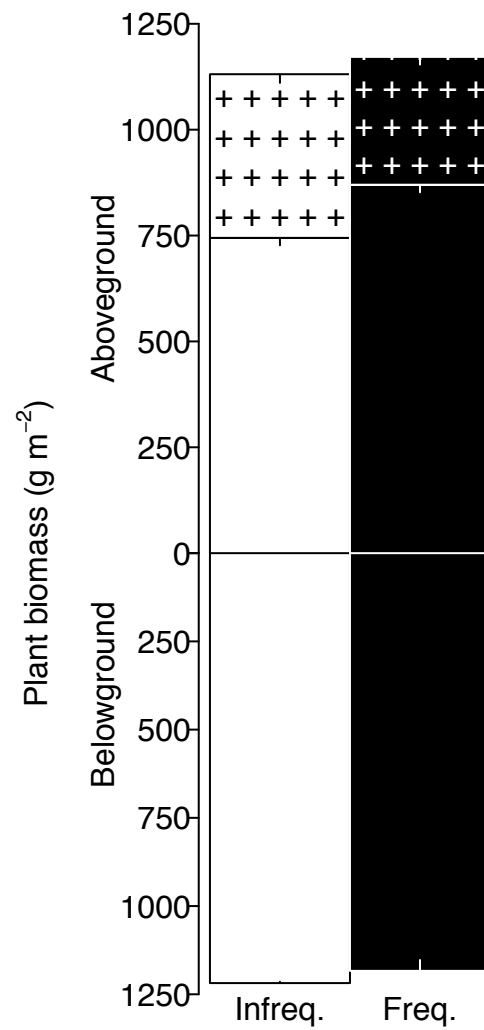
Fig. S3. Plant biomass in the infrequent watering (open bars) and frequent watering (closed bars) treatments. The polygons filled with crosses indicate dead aboveground biomass. Bars indicate mean, ticks inside the bars indicate standard error (n=5).
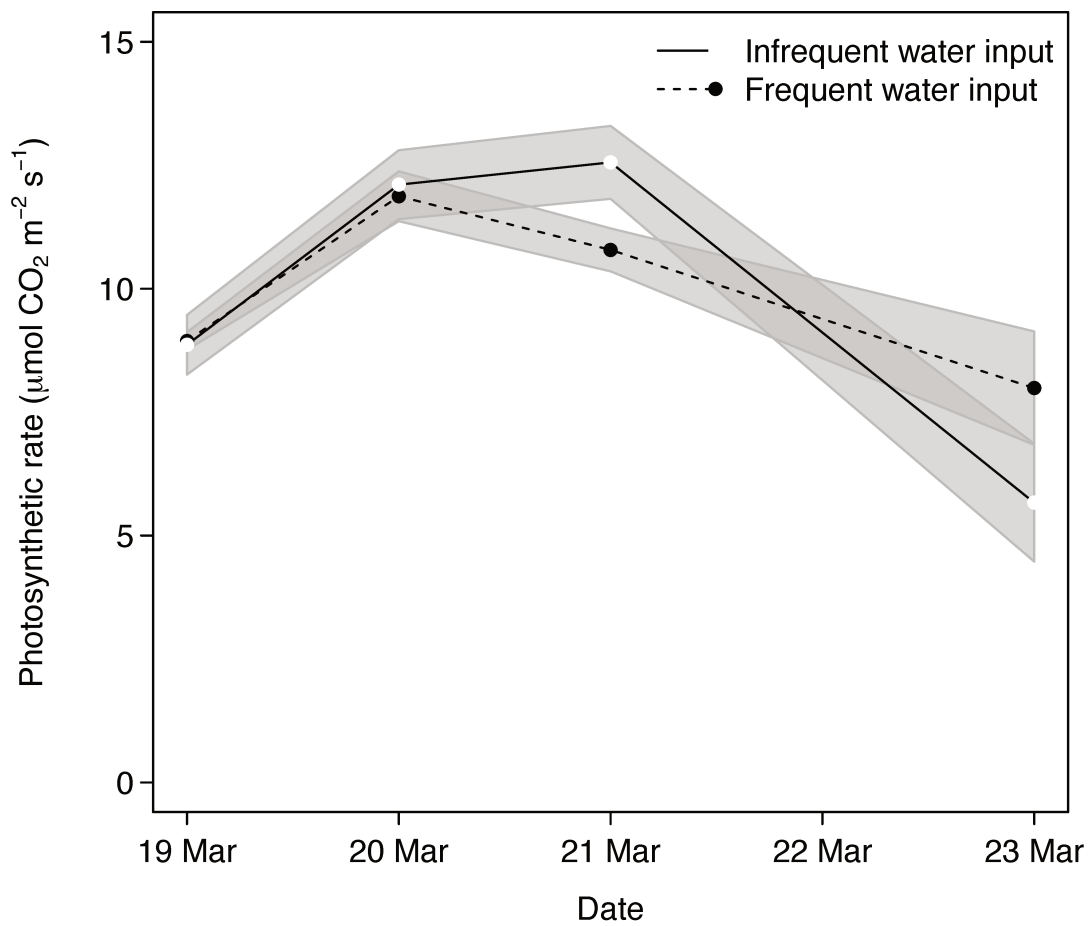
Fig. S4. Plant photosynthetic rate, scaled by plant leaf area for each experiment treatment (infrequent and frequent water input, full and dashed curves, respectively). Shaded polygons indicate ±standard error of the mean.
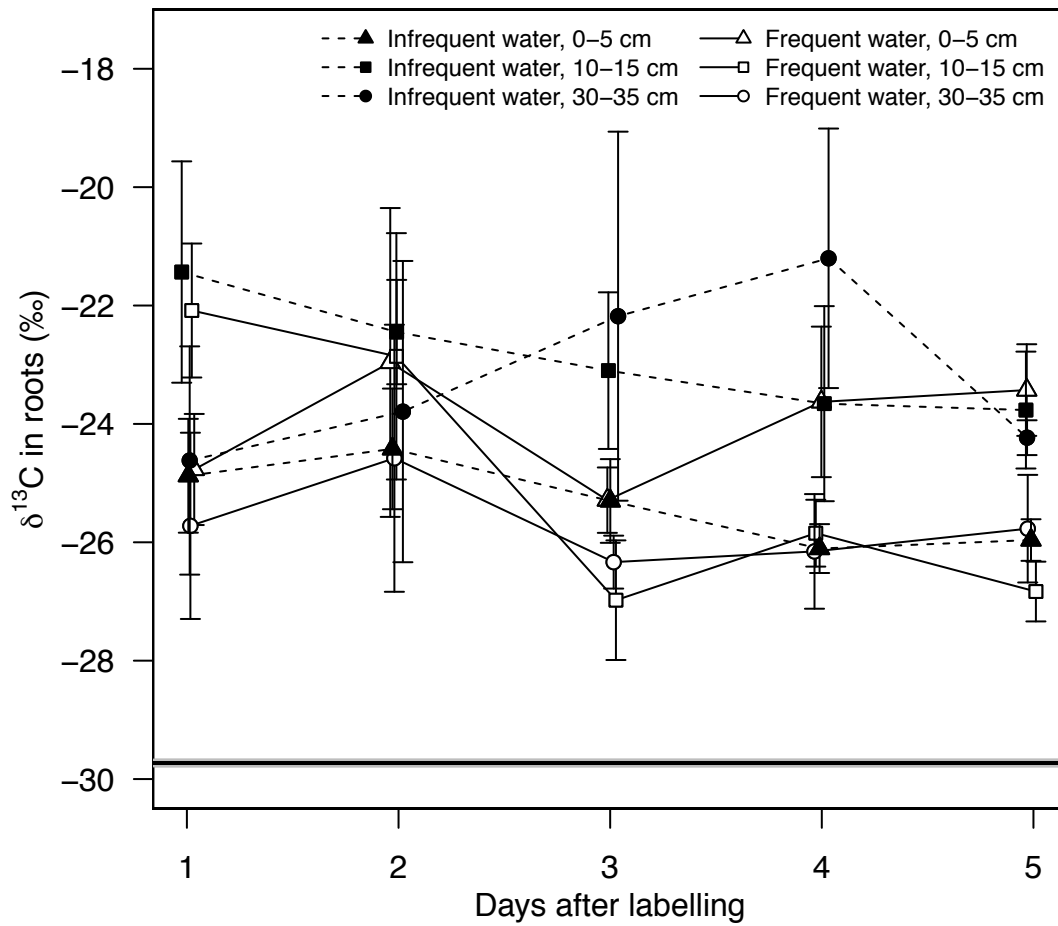
Fig. S5. ^{13}C signature in roots (mean±se), over 5 days after plant ^{13}C-CO$_2$ labeling, in the infrequent (open symbols) and frequent (closed symbols) water input treatments, at three depths (top: 0-5 cm, triangles; middle: 10-15 cm, squares, bottom: 30-35 cm, circles). Background root ^{13}C signature mean ± standard error is shown (horizontal line and grey polygon).
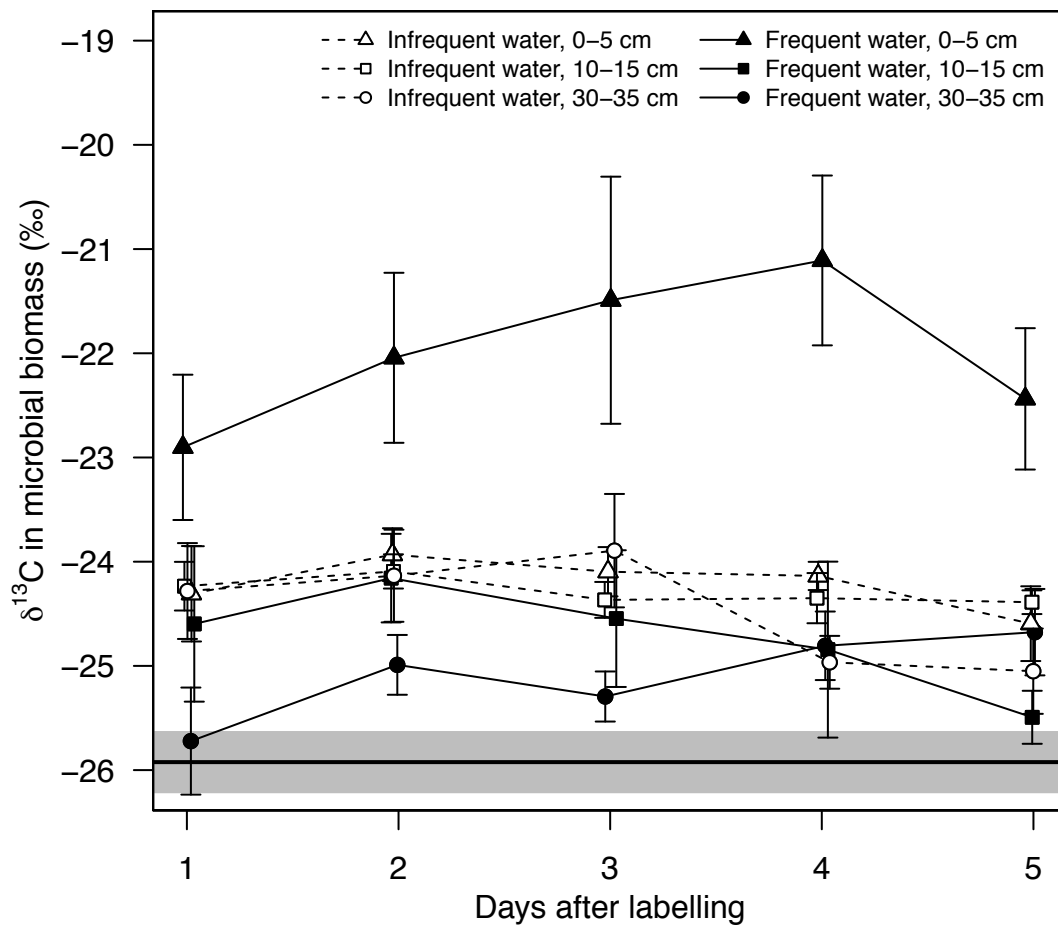
Fig. S6. ^{13}C signature in microbial biomass (mean±se), over 5 days after plant ^{13}C-CO_2 labeling, in the infrequent (open symbols) and frequent (closed symbols) water input treatments, at three depths (top: 0-5 cm, triangles; middle: 10-15 cm, squares, bottom: 30-35 cm, circles). Background microbial biomass ^{13}C signature mean ± standard error is shown (horizontal line and grey polygon).
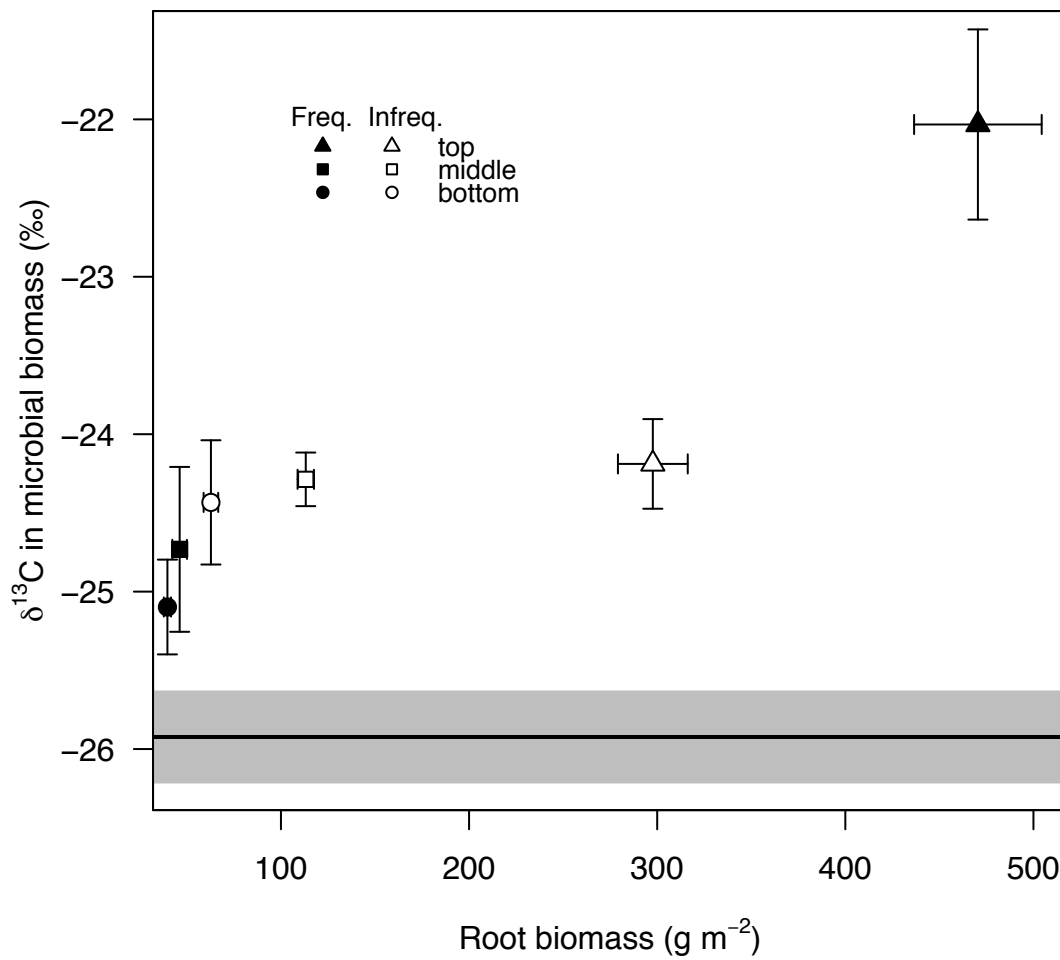
Fig. S7. [13]C signature in microbial biomass against root biomass (mean ± standard error) in the frequent (closed symbols) and infrequent (open symbols) water input treatments, at three depths (top: 0-5 cm, triangles; middle: 10-15 cm, squares, bottom: 30-35 cm, circles). Microbial biomass $\delta^{13}C$ was averaged over 5 days after plant $^{13}C\text{-}CO_2$ labeling. Background microbial biomass ^{13}C signature is shown (line and grey polygon indicate mean ± standard error).
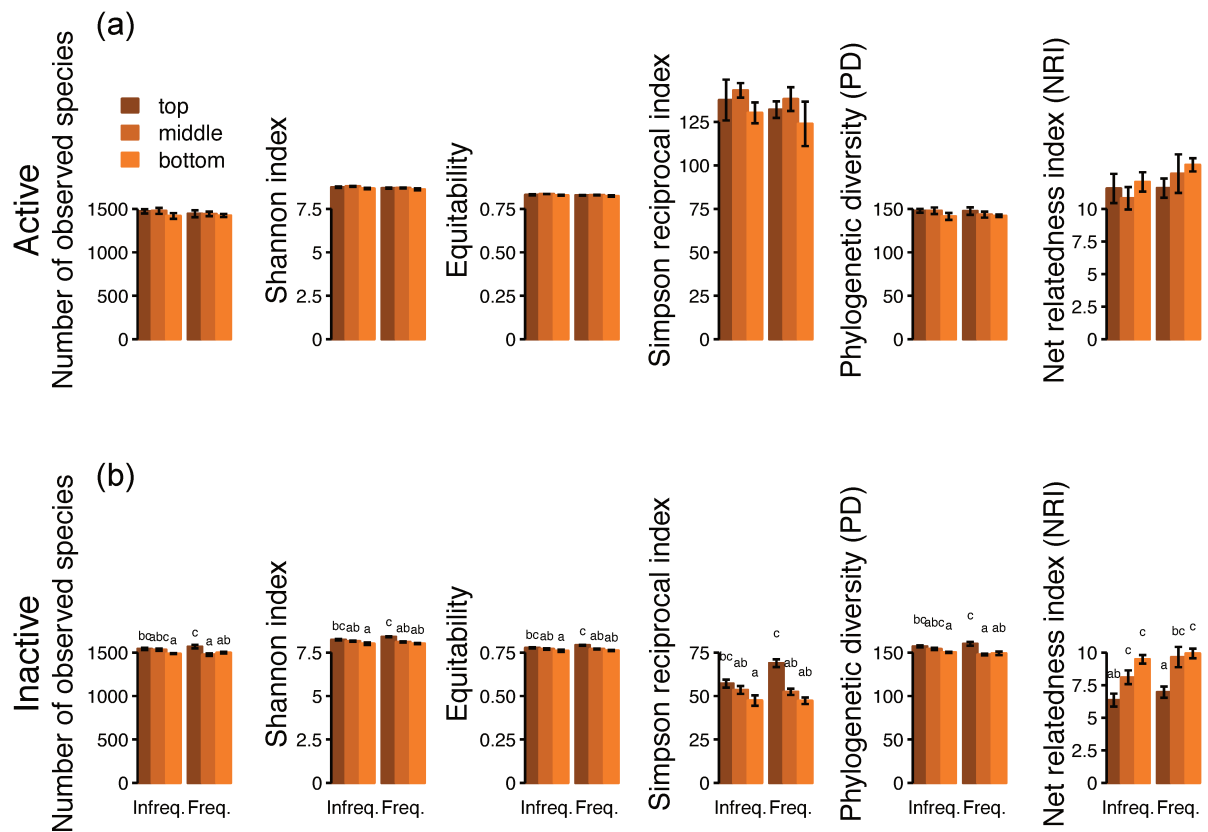
Fig. S8. Bacterial diversity indices in the active (a) and inactive (b) bacterial communities in the infrequent watering (left bar triplets) and frequent watering (right bar triplets) treatments, at three soil depths (top: 0-5 cm, brown; middle: 10-15 cm, light brown, bottom: 30-35 cm, orange). Within an index, bars that share a letter are not significantly different. Bars indicate mean ± standard error.
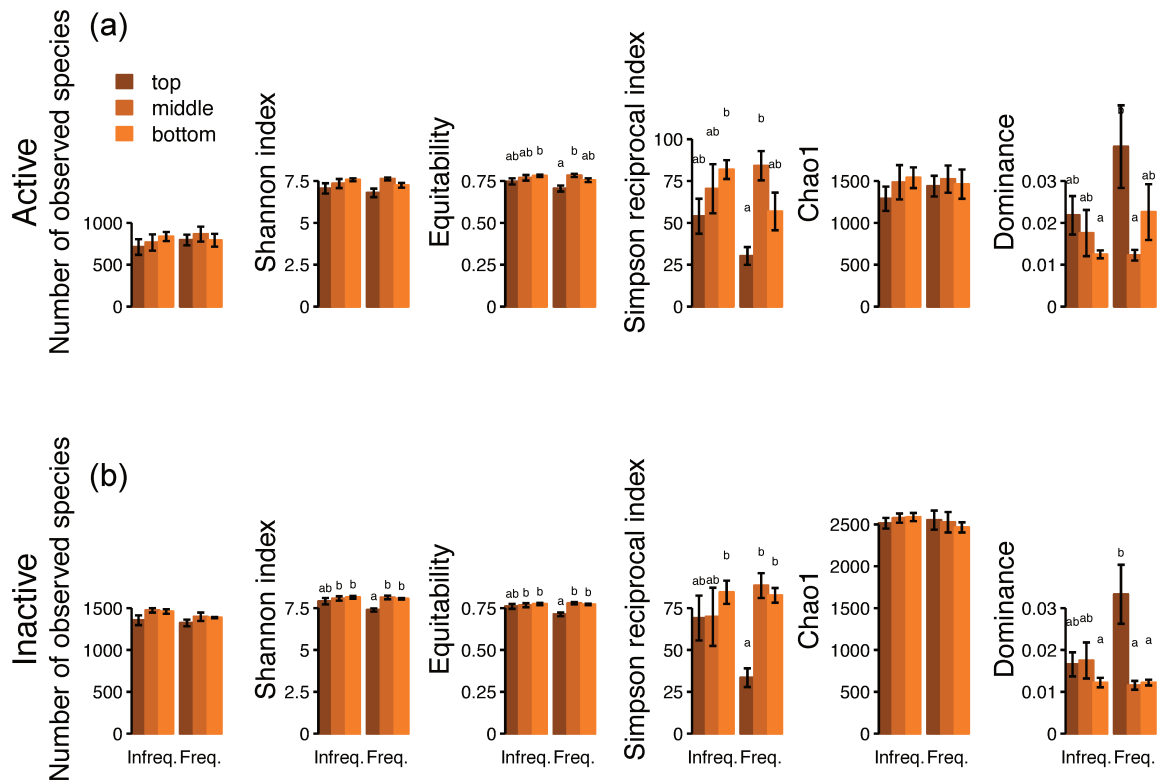
Fig. S9. Fungal diversity indices in the active (a) and inactive (b) fungal communities in the infrequent watering (left bar triplets) and frequent watering (right bar triplets) treatments, at three soil depths (top: 0-5 cm, brown; middle: 10-15 cm, light brown, bottom: 30-35 cm, orange). Within an index, letters indicate significant differences among the means: bars that share a letter are not significantly different. Bars indicate mean ± standard error.
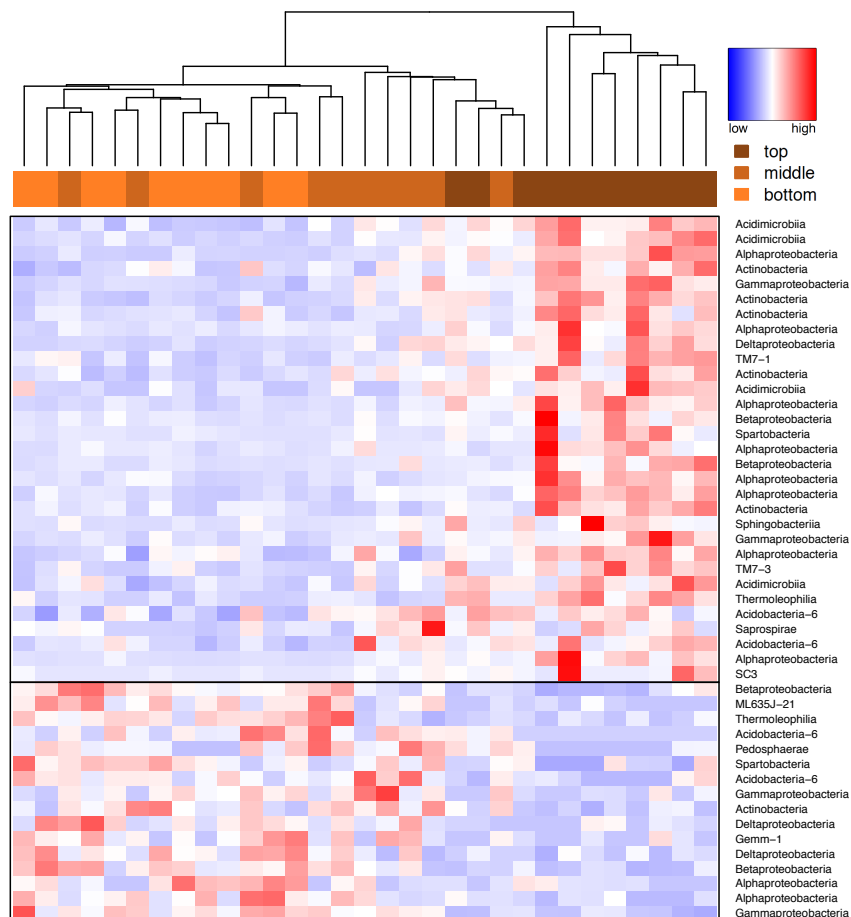
Fig. S10. Heatmap of the OTUs (labeled by class) that responded significantly to soil depth in the active bacterial community (lines, class assignment provided), across all samples (top: 0-5 cm, brown; middle: 10-15 cm, light brown, bottom: 30-35 cm, orange). The relative abundance of the OTUs across samples ranges from blue to white to red (low to medium to high relative abundance). The OTUs clustered into a "top" group and a "bottom" group, delineated by a black line.
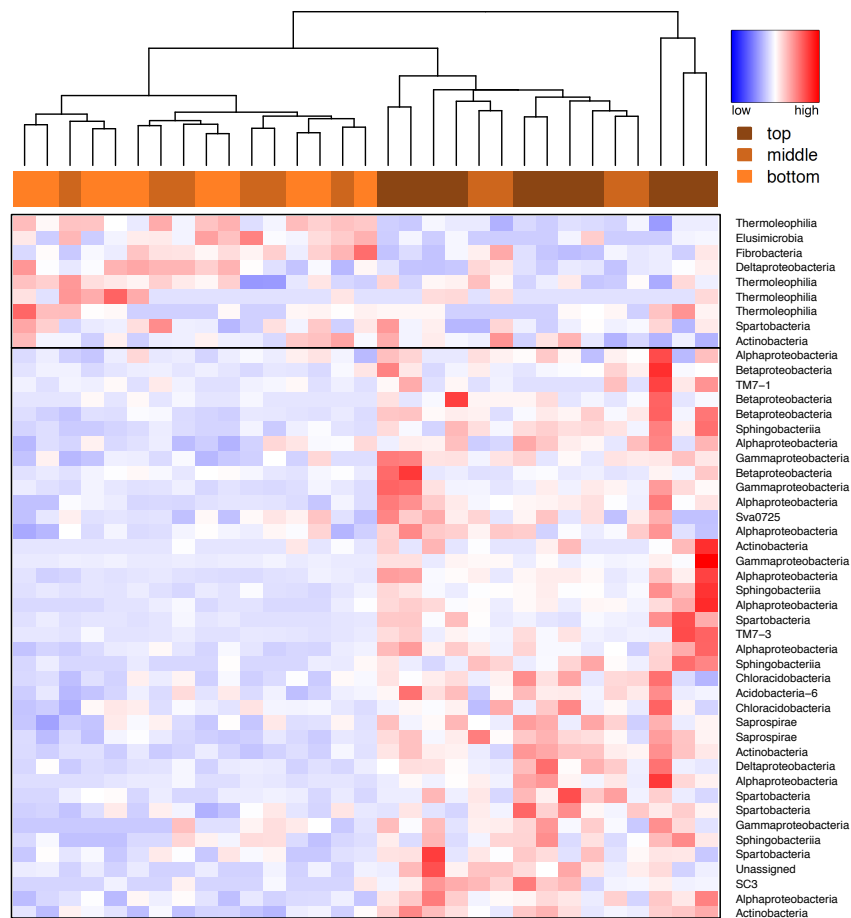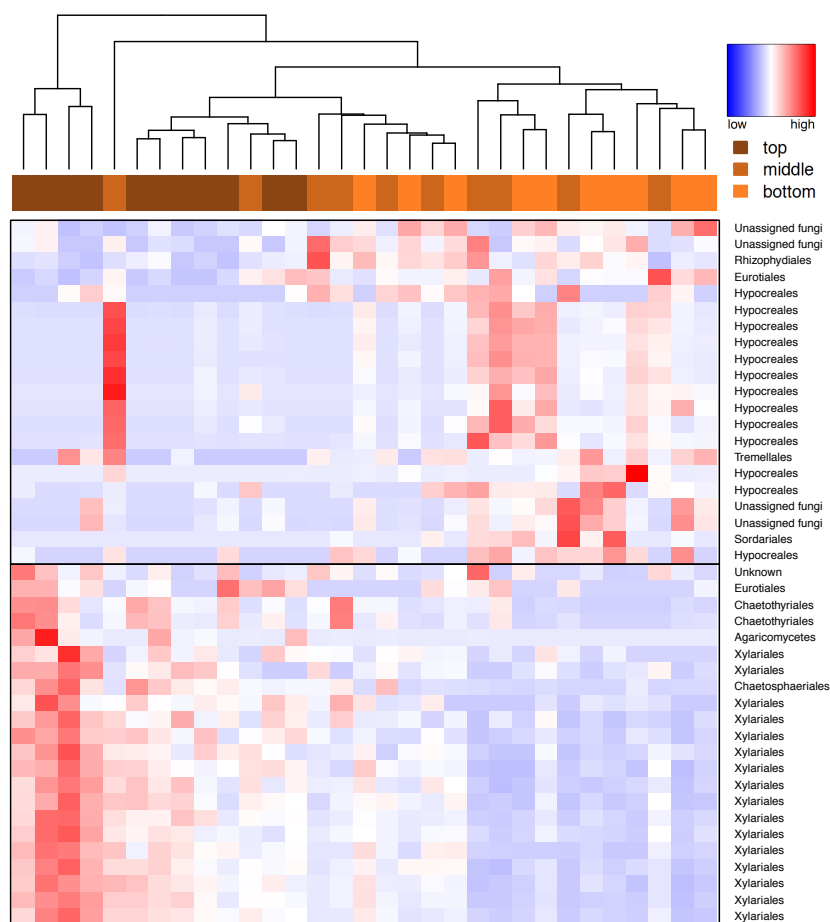
Fig. S11. Heatmap of the OTUs (labeled by class) that responded significantly to soil depth in the inactive bacterial community (lines, class assignment provided), across all samples (top: 0-5 cm, brown; middle: 10-15 cm, light brown, bottom: 30-35 cm, orange). The relative abundance of the OTUs across samples ranges from blue to white to red (low to medium to high relative abundance). The OTUs clustered into a "bottom" group and a "top" group, delineated by a black line.
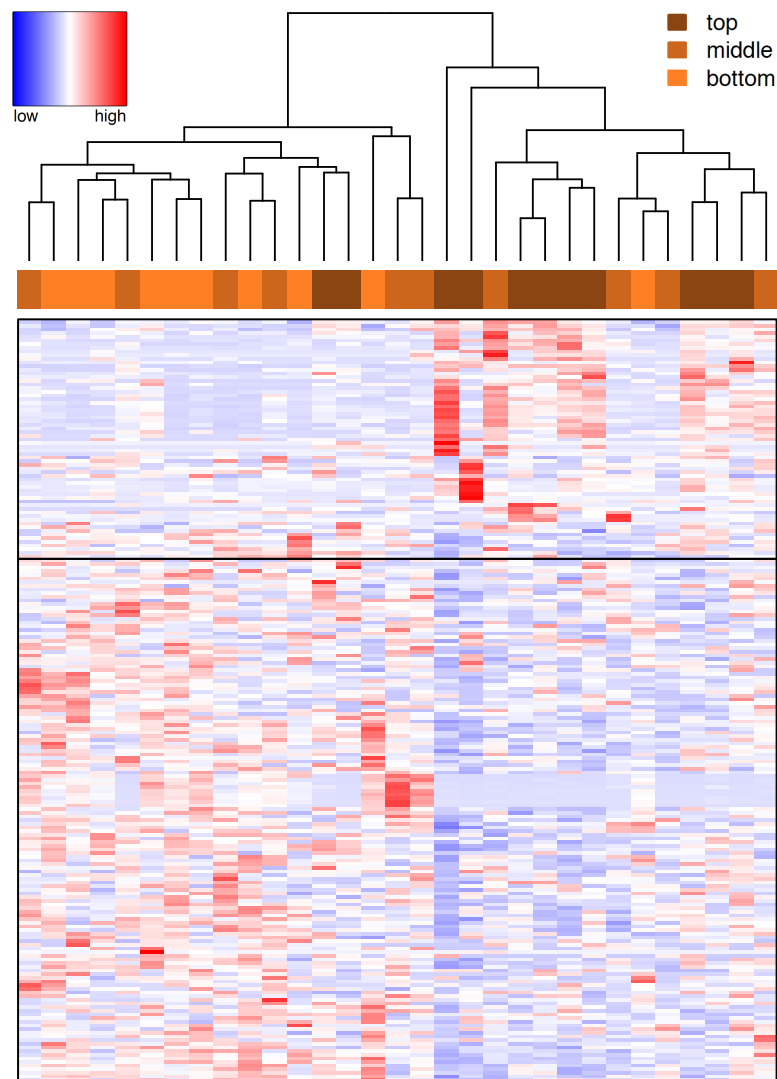
Fig. S12. Heatmap of the OTUs (labeled by order) that responded significantly to soil depth in the active fungal community (lines, class assignment provided), across all samples (top: 0-5 cm, brown; middle: 10-15 cm, light brown, bottom: 30-35 cm, orange). The relative abundance of the OTUs across samples ranges from blue to white to red (low to medium to high relative abundance). The OTUs clustered into a "top" group and a "bottom" group, delineated by a black line.

Fig. S13. Heatmap of the OTUs (no label for clarity) that responded significantly to soil depth in the inactive fungal community (lines, class assignment provided), across all samples (top: 0-5 cm, brown; middle: 10-15 cm, light brown, bottom: 30-35 cm, orange). The relative abundance of the OTUs across samples ranges from blue to white to red (low to medium to high relative abundance). The OTUs clustered into a "bottom" group and a "top" group, delineated by a black line.
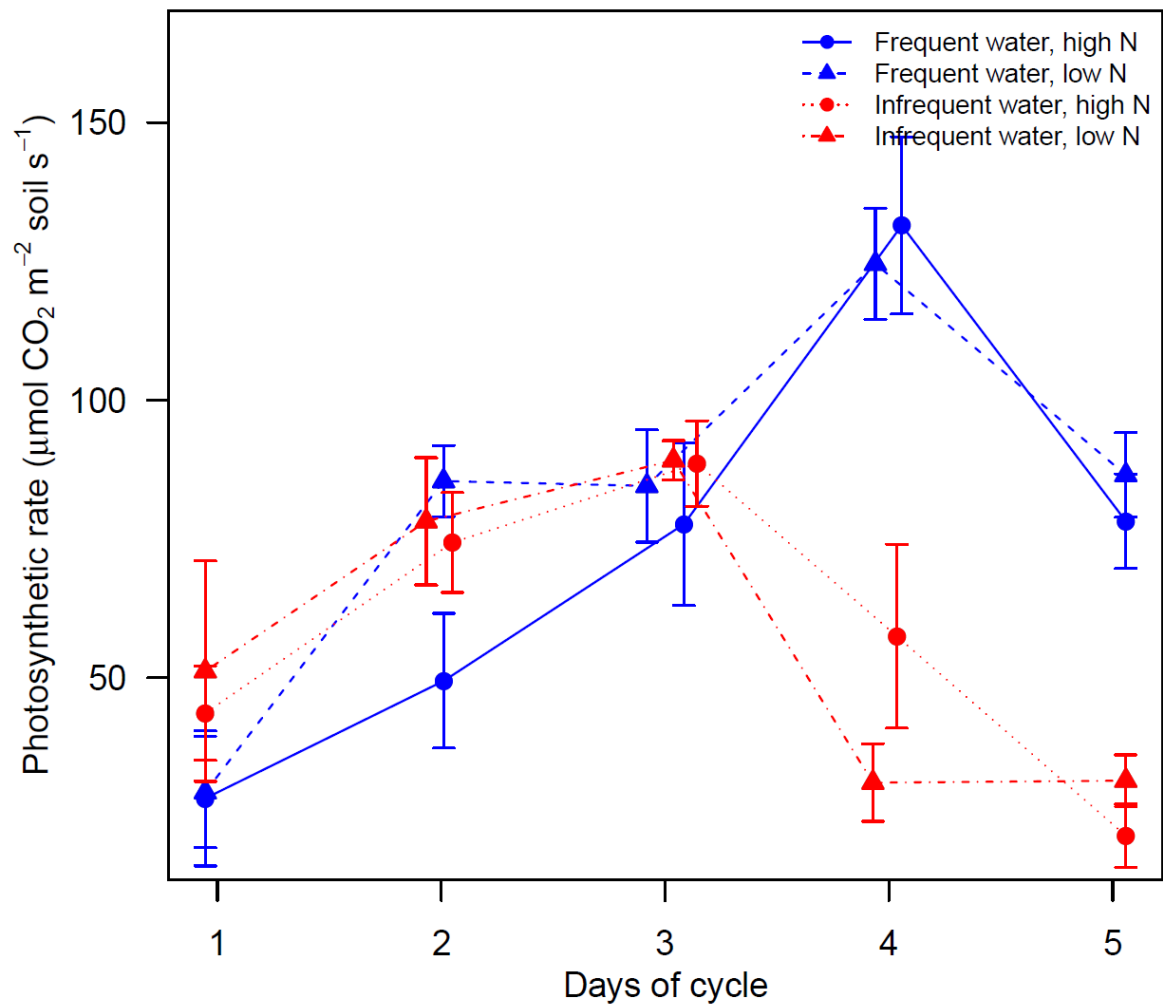
Fig. S1. Photosynthetic rate in μmol CO_2 per m_2 of soil surface, over a 5 day watering cycle. On day 1 all plants had received their respective water input right before photosynthesis rate was measured and all frequently watered plants (blue) had received their daily input prior to subsequent measurements, whilst the infrequently watered plants (red) did not receive another input within this time period. Bars indicate mean $\pm$ standard error (n=5).
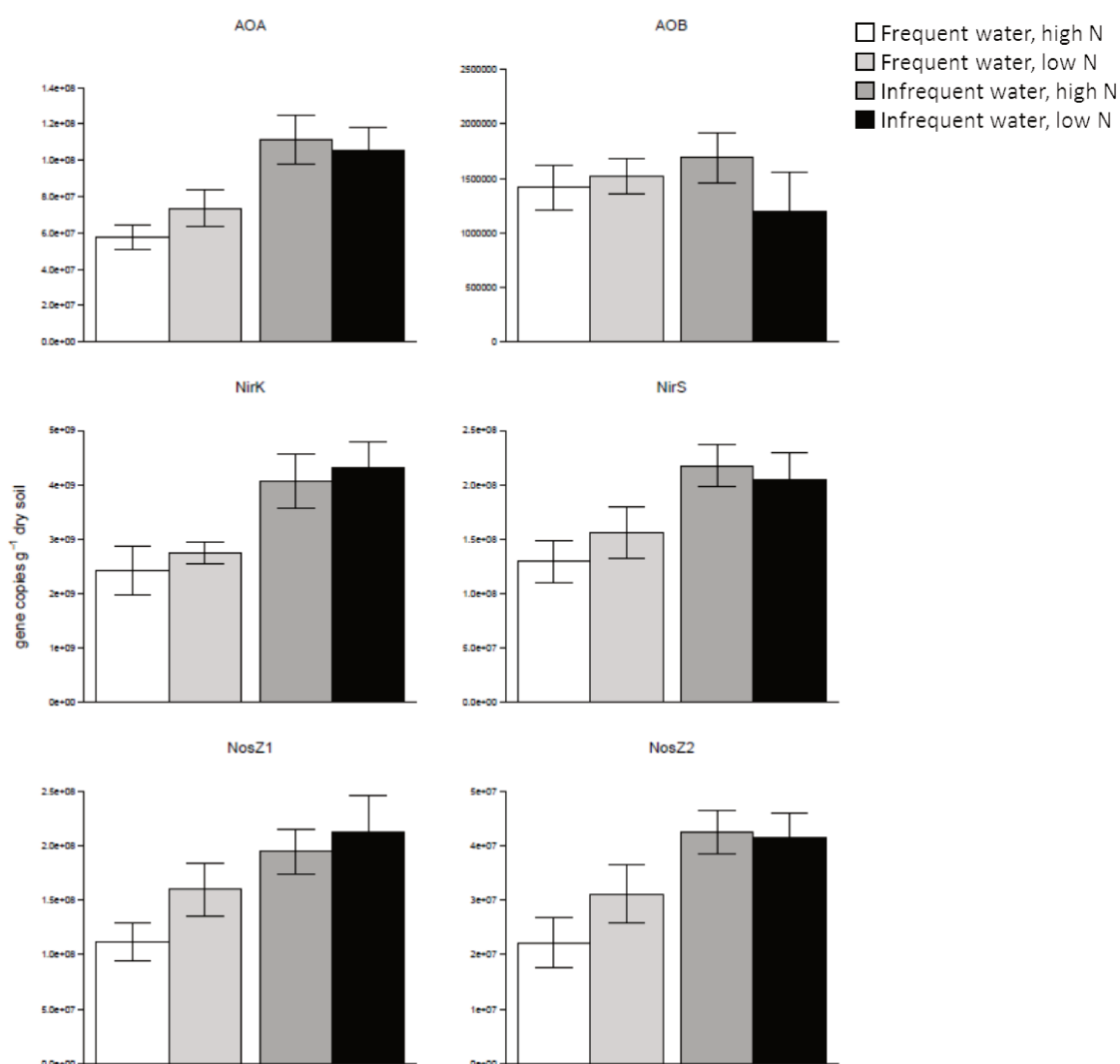
Fig. S2. N transformation marker gene abundances for each of the precipitation and N input combinations. The top row shows marker genes for the oxidation of NH4 to NO3, the middle row marker genes for the reduction of NO3 to N2O and the bottom row marker genes for the reduction of N2O to N2. All except AOB are significantly higher under infrequent water input (dark grey and black) than frequent water input (white and light grey), but were not significantly affected by N input. Note different scale of the y-axes due to highly variable abundances between the different marker genes. Bars indicate mean ± standard error (n=5).
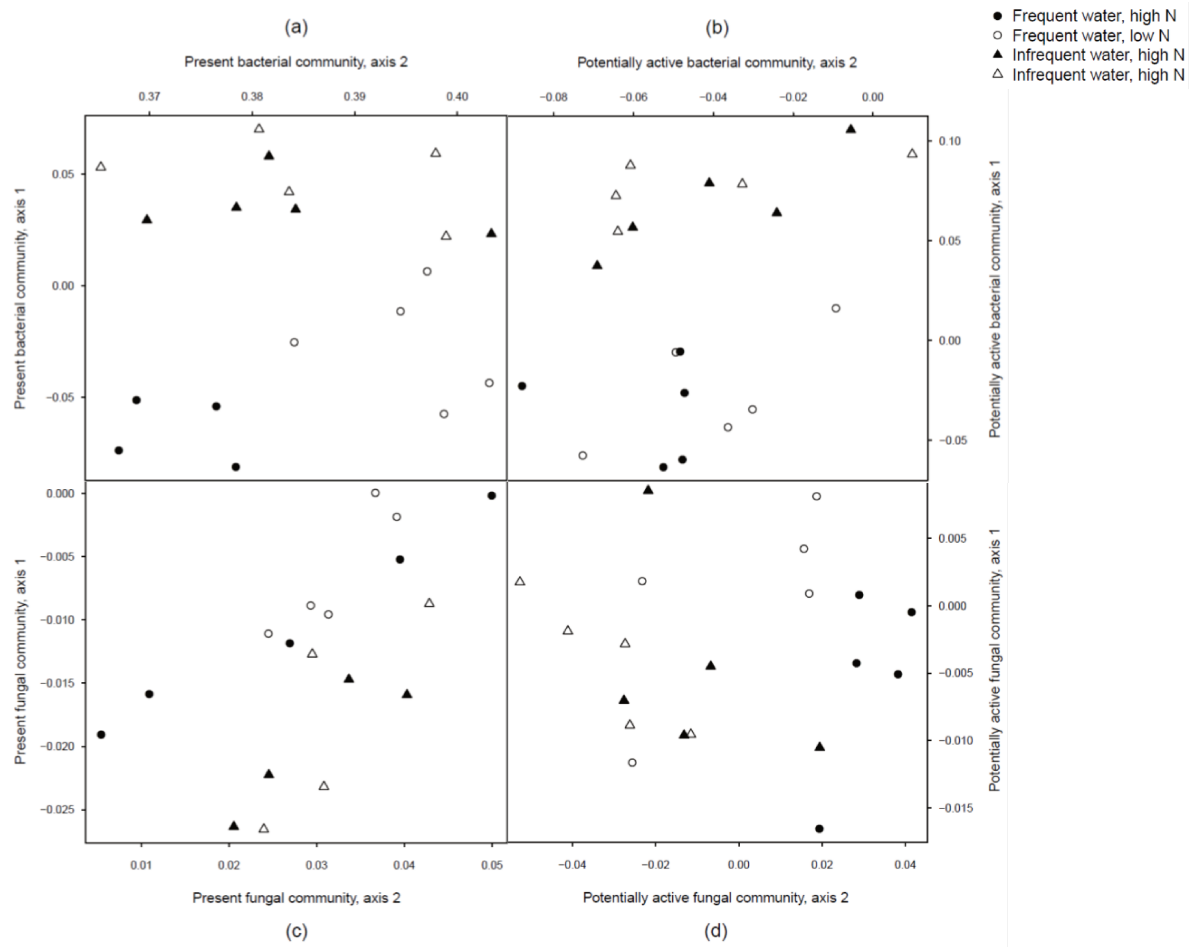
Fig. S3 (a-b). Principal coordinates analysis (PCoA) of the UniFrac pairwise dissimilarity of the relative abundance of bacterial 16S sequences in present (DNA based, a) and potentially active communities (RNA based, b) and fungal 18S sequences in present (DNA based, c) and potentially active communities (RNA based, d) before the final rewetting event.
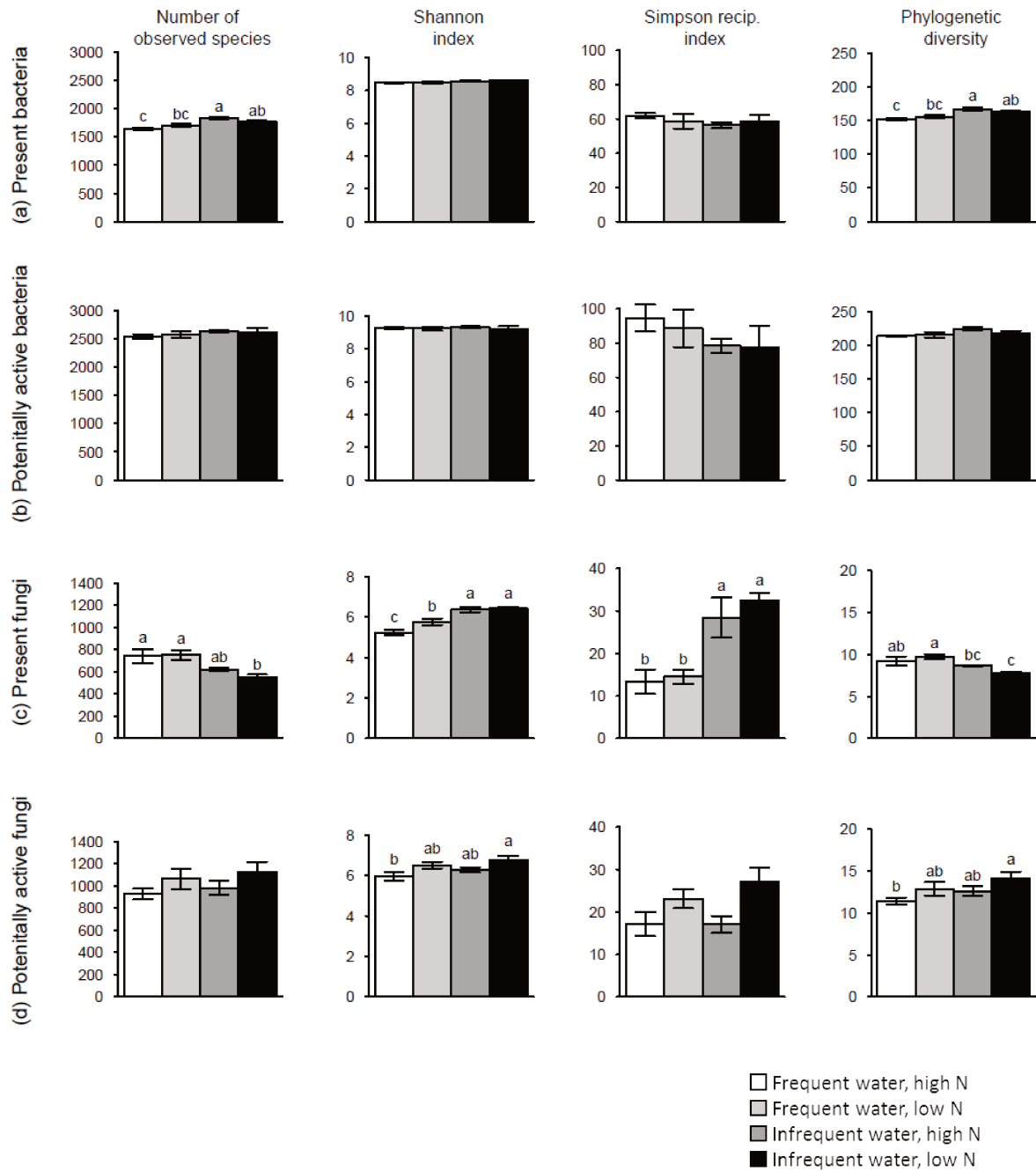
Fig. S4 (a-d). Present and potentially active bacterial (a and b respectively) and fungal (c and d respectively) richness (Observed species and Shannon), evenness (Shannon and Simpson reciprocal) and phylogenetic diversity indices for different precipitation and N input histories, prior to rewetting. Letters indicate significantly different groups and bars indicate mean ± standard error (n=5). Note the different scales of the y-axis between different indices and between present and potentially active communities due to high level of variation.
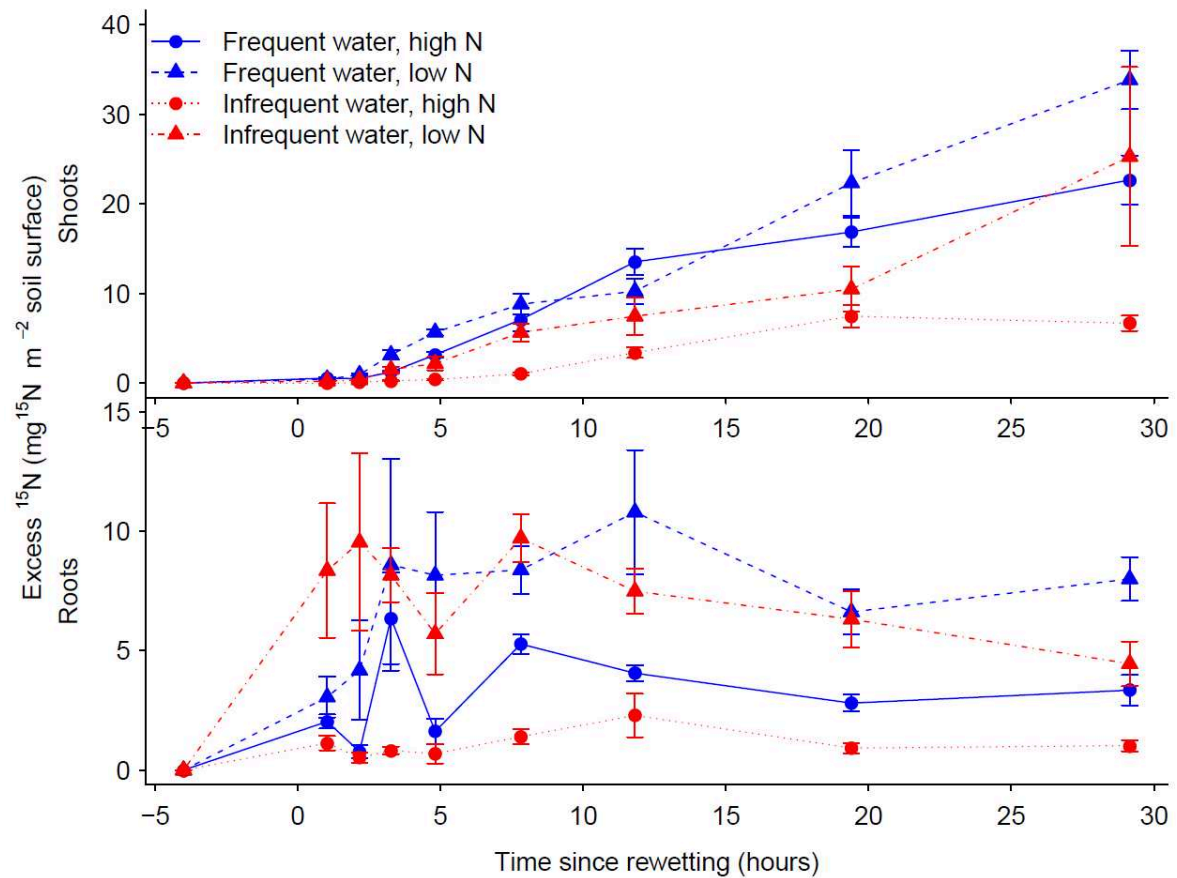
Fig. S5. Excess 15N signal in plant shoots (above) and roots (below) over time (hours) post rewetting, for each of the precipitation and N input histories. Bars indicate mean ± standard error (n=5).