Relations morphologie-cycle de l’azote au sein de l’épisolum humifère en futaie régulière pure de hêtre

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THESE
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Par
Jean TRAP

Relations morphologie-cycle de l'azote au sein de l'épisolum humifère en futaie régulière pure de hêtre

Equipe d'accueil : Laboratoire d'Ecologie, ECODIV, UPRES EA 1293, Université de Rouen
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Avant-propos

Cette thèse a été menée au Laboratoire d'Ecologie de l'Université de Rouen (UPRES EA 1293 ECODIV), dirigé par le Professeur Thibaud DECAËNS. Parallèlement, je fus régulièrement accueilli par l'équipe BIOSOL de l'ESITPA de Mont Saint Aignan, dirigée par Karine LAVAL. Enfin, une partie des analyses chimiques a été effectuée au sein de la plate-forme d'analyse chimique du Centre d'Ecologie Fonctionnelle et Evolutive de Montpellier. Mon allocation de recherche a été financée par la Région Haute-Normandie. Le fonctionnement de ce travail a été financé par le GIP ECOFOR, via le projet de recherche intitulé « Mise au point d'outils robustes d'estimation de la richesse minérale et de la production d'azote minéral du sol utilisant la valeur indicatrice de la flore, des formes d'humus et de la pédofaune » (VILFLORHUM, programme de recherche : « Typologie des stations », coordinateur : Dr. Michaël AUBERT), et par la Région Haute-Normandie au travers de son soutien au Grand Réseau de Recherche (Santé-Risque-Environnement).

Le présent manuscrit comprend une introduction générale, une synthèse bibliographique, six chapitres de résultats et une conclusion générale. Les chapitres de résultats ont été rédigés en anglais, sous la forme d'articles scientifiques. Tous les articles sont encadrés par une brève introduction et une synthèse rédigées en français, afin de faciliter la transition entre les différents chapitres.

Résumé

Relations morphologie-cycle de l'azote au sein de l'épisolum humifère en futaie régulière pure de hêtre

L'objectif de cette thèse est de contribuer à la compréhension (i) des relations morphologie/cycle de l'azote au sein de l'épisolum humifère, (ii) du cycle de l'azote au sein des différents horizons de l'épisolum humifère et (iii) des facteurs écologiques responsables du développement des formes d'humus et régulant le cycle de l'azote dans l'épisolum le long d'une chronoséquence de 130 ans en hêtraies pures.

Le cycle de l'azote. L'ammonification potentielle nette augmente avec l'âge des peuplements au sein des horizons organiques alors que la nitrification potentielle et *in situ* nette diminue au sein des horizons OL et A. La nitrification potentielle nette est essentiellement localisée au sein des horizons OF et OH et l'ammonification est toujours plus élevée au sein des horizons organiques. Les transformations fongiques dominent nettement au sein de l'horizon OL alors que les processus bactériens sont principalement localisés dans l'horizon A. Les résultats montrent que les processus en amont du cycle (apport d'azote, ammonification) sont favorisés au cours de la maturation des peuplements alors que les processus en aval du cycle (nitrification, dénitrification) diminuent le long de la chronoséquence. Le lessivage des nitrates varie peu le long de la chronoséquence alors que le prélèvement de l'azote minéral (surtout l'ammonium) et le lessivage de l'ammonium augmentent significativement. Nous avons également observé des corrélations significatives entre les variables morphologiques et la nitrification nette ou la teneur en nitrate au sein des horizons organiques. Certaines variables morphologiques (*i.e.* l'épaisseur de l'OF, le nombre de turricules de vers de terre, la structure de l'horizon A ou le pourcentage de feuilles blanchies au sein de l'horizon OLv) présentent un potentiel indicateur de production *in situ* d'azote minéral. Les variables morphologiques spécifiques à l'horizon OLv pourraient constituer des indicateurs robustes de production potentielle d'azote minéral.

Les facteurs de contrôle. Nous n'avons pas observé de variabilité significative des retombées de litière le long de la chronoséquence alors que la vitesse de décomposition de la litière diminue durant la phase de croissance des peuplements. De plus, la vitesse de décomposition de la litière est fortement corrélée à l'épaisseur des horizons OF et OH. La chute de la vitesse de décomposition de la litière serait donc responsable du changement mull-moder observé le long de la chronoséquence, alors que la production de litière jouerait un rôle secondaire mais contribuerait à un changement hemimoder-dysmoder. La chute de la vitesse de décomposition de la litière est en partie expliquée par des changements du profil structurel et fonctionnel des communautés microbiennes du sol le long de la chronoséquence. La biomasse fongique dans l'OL diminue le long de la chronoséquence. Le ratio biomasse fongique/biomasse bactérienne au sein des horizons OF et OH augmente le long de la chronoséquence. La diversité fonctionnelle des communautés bactériennes dans les horizons organiques est plus élevée dans les peuplements âgés. Ces changements fonctionnels au sein de l'épisolum humifère pourraient être sous le contrôle de la qualité de la litière de hêtre qui varie considérablement le long de la chronoséquence. Les résultats mettent en avant deux changements majeurs de la qualité de la litière. Le premier après 15 ans de vieillissement correspond à (1) une diminution des teneurs en Mg, en hémicellulose, en cellulose, en azote dans la lignine et (2) une augmentation des teneurs en Mn, en lignine, du C/N et du lignine/N. Le second après 95 ans de vieillissement correspond à (1) une baisse de la teneur en lignine, des cations et de l'azote dans la lignine et (2) une augmentation de la cellulose et de l'azote dans l'hémicellulose. Une approche expérimentale nous a permis de tester les effets de la litière de hêtre (apport et qualité) mais également des racines de hêtre (mycorhizées ou non) sur le cycle de l'azote et les communautés microbiennes du sol. La litière de hêtre, indépendamment de sa qualité initiale, inhibe la nitrification autotrophe et favorise la communauté fongique. Les racines, mycorhizées ou non, favorisent l'ammonification potentielle et les racines mycorhizées inhibent la nitrification autotrophe.

Mots clés : écologie fonctionnelle, sciences du sol, formes d'humus, épisolum humifère, minéralisation de l'azote, nitrification, lessivage, dénitrification, communautés microbiennes, production et décomposition de la litière, qualité de la litière, chronoséquence forestière, *Fagus sylvatica*, sol limoneux.

Abstract

Relationships between humus forms and soil N cycle in pure beech forest stands

The aims of the present thesis was to improve our knowledge on (i) the relationships between humus morphology and mineral nitrogen (N) production, (ii) N cycle and its regulation within the different soil layers and (iii) the environmental factors responsible for the development of humus forms and controlling soil N pathways along a chronosequence of 130 years of pure beech.

The nitrogen cycle. Potential net ammonification increases with stand age in the organic horizons, whereas both potential and net *in situ* nitrification decrease in OL and A horizons. Potential net nitrification takes mainly place in the OF and OH horizons with ammonification always higher in the organic horizons. The fungal N transformations clearly dominate in the OL horizon while bacterial processes appear to be mainly localized in the A horizon. In general, it appears that the intensity of the first steps of the cycle (*i.e.* N input and ammonification) are favored during the maturation of pure stands of beech while the latter process of the cycle (*i.e.* nitrification and denitrification) decrease along the chronosequence. Leaching of nitrate did not differ along the chronosequence, while the uptake of mineral N by roots (especially ammonium), and the leaching of ammonium significantly increased. We also observed several significant correlations between morphological variables and net nitrification or nitrate content within the organic horizons. Therefore, several morphological variables, such as the thickness of OF, density of earthworm casts, the structure of the A horizon or the percentage of bleached leaves in OLv, were found to be good predictors of *in situ* mineral N production. Furthermore, the morphological variables specific to the horizon OLv were also depicted as robust indicators of *ex situ* mineral N production. This work demonstrates that the shift *mull-moder* occurring along the chronosequence means an increase of ammonium production but a decrease in nitrification.

The driving factors. We did not find significant differences in litter production along the chronosequence, in opposite, the rate of litter decomposition decreased during the aggradation phase. Furthermore, litter decay rate was strongly correlated with the thickness of OF and OH layers. Thus, the decrease in litter decay rate appears to be responsible for the *mull-moder* shift observed during the chronosequence, while litter production would rather play a secondary role but may contribute to the second shift observed from *hemimoder* to *dysmoder* humus forms. The decrease in litter decomposition rate is partly explained by changes in both the structural and functional profiles of soil microbial communities. At the structural level, the fungal biomass in OL decreased from young to old stands. However, in OF and OH layers, the fungal to bacterial biomass ratio increased. Functional diversity of microbial community in organic horizons is higher in the oldest stands. Parallel to these changes, similar modifications were observed in litter quality. The results highlighted two major shifts. The first after 15 years corresponds to a decrease in Mg, hemicellulose, cellulose, lignin and N, and an increase in Mn content, lignin, C/N and lignin/N. The second after 95 years corresponds to a decrease in lignin, cations and N contents, lignin N, and an increase in cellulose N and hemicellulose N. An experimental approach allowed us to test the effects of beech litter (supply and quality), and roots (mycorhizal or not) on N cycling and soil microbial communities. Litter, regardless of initial quality, inhibits autotrophic nitrification and promotes fungal community. The roots promote ammonification potential while mycorhizal roots inhibit autotrophic nitrification. Therefore we hypothesized that litter quality may drive the soil microbial assemblages both at the functional and the structural level.

Keywords : functional ecology, soil sciences, humus forms, humic epipedon, N mineralization, nitrification, N leaching, denitrification, microbial community, litter production and decomposition, litter quality, forest chronosequence, *Fagus sylvatica*, loamy soil.

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Introduction Générale

FORÊT D'EAWY – PARCELLE 293 - JUIN 2008

1. Cadre de la thèse

Les écosystèmes forestiers représentaient en 2007 un peu moins de 4 milliards d'hectares, soit environ 30% de la superficie terrestre totale (FAO 2007). Entre 1990 et 2005, 3% du couvert forestier total ont été abattus, ce qui représente une perte moyenne de 0.2% par an (FAO 2007). En Europe, la superficie forestière déclarée sans la Fédération de Russie était de 193 millions d'hectares en 2005, soit une augmentation d'environ 7% depuis 1980. L'Europe est le seul continent à avoir connu une augmentation nette de son couvert forestier entre 1990 et 2005. Parallèlement à cette augmentation, les pays européens ont largement contribué à définir les critères de durabilité des politiques de gestion forestière. Une coopération (la « *Ministerial Conference on the Protection of Forest in Europe* ») matérialisée par une succession de conférences (Strasbourg 1990, Helsinki 1993, Lisbonne 1998, Vienne 2003, Varsovie 2007) et regroupant 46 pays européens, a permis l'adoption de déclarations et de résolutions concernant la protection et la gestion durable des forêts européennes.

> *« La gestion durable signifie la gérance et l'utilisation des forêts et des terrains boisés, d'une manière et à une intensité telles qu'elles maintiennent leur diversité biologique, leur productivité, leur capacité de régénération, leur vitalité et leur capacité à satisfaire, actuellement et pour le futur, les fonctions écologiques, économiques et sociales pertinentes aux niveaux local, national et mondial; et qu'elles ne causent pas de préjudices à d'autres écosystèmes. »*

Résolution H1, conférence interministérielle sur la protection des forêts en Europe, Helsinki, 1993

Gérer durablement les forêts implique donc une prise en compte de l'ensemble des fonctions de l'écosystème qui déterminent la productivité, la régénération et la santé des forêts. Parmi ces dernières, la pérennité de la fertilité minérale du sol et du recyclage biogéochimique des éléments nutritifs s'élève au premier rang.

L'azote est, avec le phosphore, l'un des deux éléments clef des écosystèmes forestiers, limitant la productivité et la croissance des peuplements (Schulze 2000; Dannenmann et al. 2009). L'absence d'apport d'azote par altération de la roche mère limite la disponibilité de cet élément alors que la forte solubilité des ions nitrates facilite les pertes d'azote par lessivage (Schulze 2000). Indispensable pour la croissance des organismes, l'azote est aussi l'objet d'intenses compétitions entre les bactéries autotrophes et hétérotrophes, les champignons saprophytes et mycorhiziens et les plantes (Kaye and Hart 1997; Hodge et al. 2000). La pérennité du recyclage de l'azote dans le sol et la production d'azote disponible pour les arbres sont par conséquent des facteurs majeurs qui déterminent la production de biomasse ligneuse. Gérer durablement les écosystèmes forestiers exige donc une compréhension exhaustive de la dynamique des transformations de l'azote dans les sols forestiers et des facteurs écologiques contrôlant son recyclage à long terme. C'est la raison pour laquelle de nombreux travaux sur la dynamique de l'azote ont été initiés durant ces dernières années (Fig. 1).

Nos connaissances sur les effets des conditions pédoclimatiques (Reich et al. 1997; Saetre et al. 1999; Zeller et al. 2007), des espèces d'arbres (Brierley et al. 2001a; Zeller et al. 2007) de la faune du sol (Schröter et al. 2003; Caner et al. 2004), des formes d'humus (Bottner et al. 1998; Hirobe et al. 2003), des propriétés physico chimiques du sol (Breland and Hansen 1996; Knoepp and Swank 2002; Grenon et al. 2004) ou encore des pratiques sylvicoles (Griffiths and Swanson 2001; Brais et al. 2002; Fotelli et al. 2002) sur les différentes transformations de l'azote dans le sol sont de plus en plus nombreuses et précises. Néanmoins, il s'agit d'un cycle biogéochimique très complexe dû, en partie, à une grande diversité des formes minérales (NH_3, NH_4^+, NO_3^-, NO_2, N_2O, N_2) et des organismes qui y sont impliqués (plantes, bactéries autotrophes et hétérotrophes, champignons, archées et pédofaune). Aussi, les facteurs écologiques impliqués dans la régulation du cycle de l'azote et leurs interactions sont nombreux et restent à être identifiés.

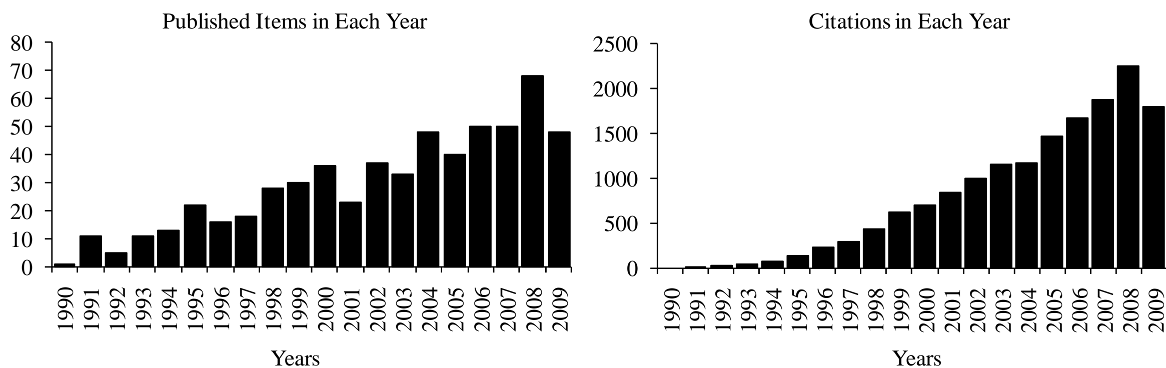

Figure 1. *Nombre de publications et de citations par an relatives au cycle de l'azote dans les sols forestiers (Web of Science, September 2009, Topic:* ***nitrogen cycle forest soil****; Time span=All Years. Databases=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ICi).*

2. Problématique de thèse

La problématique générale de cette thèse repose sur le constat suivant : Au cours de la maturation des peuplements forestiers semi-naturels, la morphologie de l'épisolum humifère, désignée par le terme « forme d'humus », évolue depuis des **mulls** sous les jeunes peuplements jusqu'à des formes d'humus de type **moder** sous les peuplements matures (Bernier and Ponge 1994a; Ponge and Delhaye 1995b; Arpin et al. 1998). En contexte géré, Aubert *et al.*. (2004a) ont observé l'apparition des formes d'humus de type moder le long d'une chronoséquence de 195 ans de hêtraie pure située en Forêt d'Eawy (Haute Normandie, France). Plus précisément, ce changement morphologique se traduit par :

-(i) l'apparition d'un horizon d'humification ou OH constitué de matière organique fine. La matière organique fine est principalement constituée d'amas de boulettes fécales et de micro débris végétaux et mycéliens non reconnaissables à l'œil nu;

-(ii) un épaississement des horizons holorganiques constitués de matériel organique peu modifié (horizon OL) ou fragmenté en mélange avec de la matière organique fine (horizon OF);

-(iii) un passage d'un horizon organo-minéral (A) biomacrostructuré (complexes argilo-humiques abondants) à un horizon **A** peu structuré, dit de juxtaposition (complexes argilo-humiques peu abondants).

Or, la traduction macro-morphologique de l'épisolum humifère présenterait un caractère indicateur en termes de recyclage de la matière organique. Les formes d'humus de type **mull** (horizon OL peu épais, absence d'horizon OH, horizon A épais à structure biologique grumeleuse ou microgrumeleuse) résulteraient d'un recyclage rapide de la matière organique**,** contrairement aux **moders** (horizon OL et OF épais, horizon OH présent, horizon A de juxtaposition peu épais)**,** qui reflèteraient un fonctionnement moins actif (Toutain 1987a; Ponge et al. 2002; Ponge 2003; Ponge and Chevalier 2006).

> *"**Mull** can be characterized by the rapid disappearance of leaf litter [...] and by a rapid cycling of nutrients. Mull is associated with the most fertile soils. In **moder** humus forms, organic matter accumulates in the form of three holorganic horizons OL (entire leaves or needles), OF (fragmented litter) and OH (humified litter). The cementation of organic matter by mineral particles is nil or poor, due to the scarcity of adhesive substances. Nutrients are sequestered in decaying plant debris, faeces of epigeic fauna and fungi, which form the bulk of the OF horizon where most organisms are living."*
>
> ***Ponge 2003:935***

Ce constat amène à s'interroger sur la nature et l'intensité des conséquences fonctionnelles de ces modifications morphologiques au sein de l'épisolum humifère au cours de la croissance des arbres.

3. Objectifs et hypothèses

L'objectif de ce travail de thèse est de contribuer à la compréhension (i) des relations morphologie/fonctionnement de l'épisolum humifère le long de la maturation des peuplements forestiers, (ii) du cycle de l'azote au sein des différents horizons de l'épisolum humifère, (iii) de la variabilité du cycle de l'azote le long de la maturation des peuplements forestiers et (iv) des facteurs écologiques responsables du développement des formes d'humus et régulant le cycle interne de l'azote dans le sol. Cette étude comprend trois objectifs distincts dont découlent plusieurs hypothèses déclinées en questions.

3.1. Premier objectif

L'objectif premier est de caractériser la dynamique du cycle interne de l'azote au sein de l'épisolum humifère le long d'une chronoséquence de hêtraie (*Fagus sylvatica* L.) pure de 130 ans et d'identifier les variables macro-morphologiques susceptibles d'être indicatrices des patrons de variations des transformations de l'azote. Ce travail se base sur une approche synchronique comparative visant à étudier les patrons de variations de la morphologie de l'épisolum humifère et du cycle de l'azote au cours du vieillissement des peuplements purs de hêtres.

<u>Hypothèse H1.</u> Le cycle interne de l'azote (*i.e.* ammonification et nitrification) au sein de l'épisolum humifère varie le long de la maturation des peuplements forestiers et cette variation est en adéquation avec les patrons de variations des formes d'humus.

> *Question 1.1.* Existe-t-il une variabilité temporelle de la production potentielle *ex situ* d'azote minéral au sein de l'épisolum humifère le long de la maturation des peuplements?

Question 1.2. Existe-t-il une variabilité temporelle de la production *in situ* d'azote minéral au sein de l'épisolum humifère le long de la maturation des peuplements?

Question 1.3. La variabilité temporelle des transformations de l'azote est-elle similaire dans les différents horizons de l'épisolum humifère?

Question 1.4. La variabilité morphologique de l'épisolum humifère est-elle corrélée à la variabilité fonctionnelle en termes de production d'azote minéral le long de la maturation des peuplements forestiers?

3.2. Second objectif

Le **second objectif** de ce travail de thèse est d'identifier les facteurs écologiques impliqués dans les changements morphologiques et fonctionnels (cycle de l'azote) de l'épisolum humifère le long de la chronoséquence de hêtraie pure. Ce travail se base sur une approche synchronique comparative visant à étudier les patrons de variations de la morphologie de l'épisolum humifère et des facteurs de contrôle.

Hypothèse H2. L'accumulation de matériels organiques transformés le long de la maturation des peuplements (apparition d'un horizon d'humification) résulte d'une augmentation de la production de litière de hêtres.

Question 2.1. Existe-t-il une variabilité temporelle des retombées de litière le long de la maturation des peuplements purs de hêtre?

Question 2.2. Les retombées de litière sont-elles le principal facteur de la variabilité morphologique?

Hypothèse H3. L'accumulation de matériels organiques transformés le long de la maturation des peuplements (apparition d'un horizon OH) résulte d'une diminution de la vitesse de décomposition de la litière.

Question 3.1. Existe-t-il une variabilité temporelle de la vitesse de décomposition de la litière le long de la maturation des peuplements purs de hêtre?

Question 3.2. Les vitesses de décomposition sont-elles le principal facteur de la variabilité morphologique?

D'après Ponge (2003), la diminution de la disponibilité des éléments dans le sol durant la phase de croissance intense des peuplements, est le principal facteur responsable de la diminution de la vitesse de décomposition de la litière et de l'apparition de l'horizon d'humification.

Hypothèse H4. La disponibilité des nutriments au sein de l'horizon organo-minéral diminue au cours de la maturation des peuplements.

Question 4.1. Est-ce que la disponibilité des éléments nutritifs au sein de l'horizon organo-minéral varie le long de la maturation des peuplements?

Question 4.2. Est-ce que la variation de la disponibilité des éléments nutritifs au sein de l'horizon organo-minéral est corrélée aux changements morphologiques et fonctionnels de l'épisolum humifère?

La vitesse de décomposition de la litière est sous le contrôle de nombreux facteurs, tels que le climat, le sol, la qualité des ressources et surtout l'activité biologique du sol. Néanmoins, dans des conditions climatiques et édaphiques similaires, les facteurs susceptibles de contrôler les processus de décomposition de la matière organique sont les organismes du sol et la végétation (Lavelle, 1993). Or, Hedde *et al..* (2007) ont montré peu de changement de la composition spécifique, de la densité et de la biomasse des communautés de macro-détritivores au sein de l'épisolum humifère le long d'une chronoséquence de hêtraie pure de 200 ans sur limon de plateau de Haute Normandie. Ainsi, ces résultats suggèrent que seules les communautés microbiennes du sol et/ou la qualité de la litière peuvent être responsables de l'apparition des moders.

Hypothèse H5. L'apparition d'un horizon OH le long de la maturation des peuplements suppose des modifications importantes des communautés microbiennes de l'épisolum humifère.

Question 5.1. Existe-t-il une variabilité temporelle du profil structurel et fonctionnel des communautés microbiennes au sein de l'épisolum humifère le long de la maturation des peuplements purs de hêtre?

Question 5.2. Existe-t-il une variabilité spatiale verticale du profil structurel et fonctionnel des communautés microbiennes au sein de l'épisolum humifère et temporelle le long de la maturation des peuplements purs de hêtre?

Question 5.3. La variabilité des communautés microbiennes de l'épisolum humifère est-elle corrélée à la variabilité morphologique le long de la maturation des peuplements purs de hêtre?

Question 5.4. Existe-t-il des variables macro-morphologiques indicatrices du profil structurel et fonctionnel des communautés microbiennes au sein de l'épisolum humifère le long de la maturation des peuplements purs de hêtre?

Hypothèse H6. L'apparition d'un horizon OH le long de la maturation des peuplements suppose des changements importants dans la qualité de la litière restituée au sol.

Question 6.1. La qualité de la litière de hêtre présente-t-elle une variabilité le long de la maturation des peuplements?

Question 6.2. La variation de la qualité de la litière de hêtre est-elle corrélée à la variation morphologique de l'épisolum humifère le long de la maturation des peuplements?

3.3. Troisième objectif

Le **troisième objectif** est de tester expérimentalement les facteurs écologiques impliqués dans le contrôle du recyclage de l'azote au sein de l'épisolum humifère. Nous

nous sommes intéressés aux effets (i) de l'apport et de la qualité de la litière et (ii) des racines ectomycorhizées sur le cycle de l'azote et les communautés microbiennes du sol.

Hypothèse H7. L'apport et la qualité de la litière influencent le cycle de l'azote dans le sol.

Question 7.1. L'apport de litière de hêtre affecte-t-il le cycle de l'azote via les communautés microbiennes du sol?

Question 7.2. La qualité de litière de hêtre affecte-t-elle le cycle de l'azote via les communautés microbiennes du sol?

Hypothèse H8. Les racines et la présence de champignons ectomycorhiziens influencent le cycle de l'azote.

Question 8.1. La présence de racines vivantes modifie-t-elle la production d'azote minéral?

Question 8.2. Les effets des racines vivantes ectomycorhizées sur la production d'azote minéral sont-elles différentes de celles des racines vivantes non ectomycorhizées?

Question 8.3. L'effet des racines ectomycorhizées ou non ectomycorhizées sur la production d'azote minéral intervient-il via les communautés microbiennes du sol?

Question 8.4. Existe-t-il des effets antagonistes ou synergiques litière/racine sur la production d'azote minéral et les communautés microbiennes du sol?

4. Organisation du manuscrit

Le manuscrit comprend une introduction générale, un chapitre de synthèse bibliographique, six chapitres de résultats répartis en trois parties et une conclusion générale. Les chapitres de résultats ont été rédigés en anglais sous la forme d'articles scientifiques, ce qui induit inévitablement une certaine redondance entre les chapitres. Chaque article est encadré par une courte présentation et une synthèse rédigées en français.

Les sources bibliographiques concernant les formes d'humus, le cycle de l'azote et sa régulation dans les sols forestiers, sont nombreuses. Celles-ci sont mentionnées au sein du **chapitre 1** qui dresse un bilan des connaissances actuelles sur les formes d'humus et le cycle de l'azote dans les sols forestiers ainsi que sur les facteurs régulant les transformations de l'azote dans le sol. Un accent est porté sur les patrons de variations du cycle de l'azote et des facteurs de contrôle abiotiques et biotiques le long de la maturation des forêts.

L'objectif 1, abordé au sein de la **partie I**, est de caractériser les patrons de variations de la minéralisation de l'azote et de la nitrification au sein de l'épisolum humifère le long d'une chronoséquence de 130 ans de hêtraie pure. La partie I comprend deux chapitres (chapitres 2 et 3).

L'hypothèse H1 a été testée en conditions contrôlées et sur le terrain. Une caractérisation *ex situ* a été réalisée par la méthode d'incubation aérobie à 28°C pendant 28 jours développée par Hart *et al.*. (1994). Les résultats sont présentés au sein du

chapitre 2. L'utilisation d'inhibiteurs sélectifs, l'acétylène et le captan, nous a permis (i) d'identifier les organismes responsables des processus de production d'azote minéral et (ii) de suivre l'évolution de leur contribution dans la production d'azote minéral le long de la chronoséquence de hêtraie pure. Une caractérisation complémentaire *in situ* mensuelle pendant un an des patrons de variations du cycle de l'azote a également été réalisée par incubation de carottes intactes d'épisolum. Les résultats sont présentés au sein du **chapitre 3**.

L'objectif 2, abordé dans la **partie II,** est d'identifier les facteurs écologiques responsables des patrons de variations des formes d'humus et des transformations de l'azote dans le sol, le long de la chronoséquence. La deuxième partie se compose de trois chapitres (chapitres 4, 5 et 6).

Les hypothèses 2, 3 et 4 sont testées au sein du **chapitre 4** dont l'objectif est d'identifier les facteurs responsables de l'apparition des moders le long de la chronoséquence. L'apparition de l'horizon OH résulte soit d'une augmentation de la production de la litière de hêtres et/ou d'une diminution de la vitesse de décomposition de la litière de hêtres le long de la maturation du peuplement. L'hypothèse 5 est testée au sein du **chapitre 5** qui s'attache à la caractérisation des communautés microbiennes (bactéries et champignons) au sein de l'épisolum humifère le long de la chronoséquence. La composition et la biomasse des communautés microbiennes constituent les principaux facteurs responsables de la vitesse de décomposition des litières Ce chapitre expose les patrons de variations des profils structurels (empreintes génétiques et indices biochimiques de biomasse) et fonctionnels (profil physiologique de type *Biolog*) des communautés microbiennes du sol. L'hypothèse 6 est testée au sein du **chapitre 6.** Ce chapitre expose les patrons de variations de la qualité (teneurs en fibres et éléments minéraux) de la litière de hêtre le long de la chronoséquence.

L'objectif 3, abordé au sein de **la partie III,** est d'identifier les facteurs écologiques qui contrôlent le cycle de l'azote dans le sol. La partie III comprend un chapitre (chapitre 7).

Les hypothèses 7 et 8 sont testées au sein du **chapitre 7** qui s'intéresse aux effets de la litière (apport et qualité) et des racines de hêtres (ectomycorhizées ou non) sur la production d'azote minéral et sur les communautés microbiennes du sol. Il s'agit d'une expérimentation sous serre de 6 mois basée sur la présence/absence (i) de litière de hêtre issue d'un peuplement de 20 ans ou de 90 ans, et (ii) de racines ectomycorhizées ou non ectomycorhizées.

L'ensemble des résultats est synthétisé et discuté au sein de la **Conclusion Générale et Perspectives**.

CHAPITRE 1
Synthèse bibliographique

I. Présentation du chapitre 1

Le cadre conceptuel de cette étude s'appuie sur des notions relatives aux sciences du sol, *e.g.* la morphologie de l'épisolum humifère, la dynamique de la matière organique, le cycle de l'azote, l'activité biologique. Dans cette partie, je présente, dans un premier temps, les caractéristiques macro-morphologiques des deux types de formes d'humus rencontrées lors de l'étude, *i.e.* le mull et le moder. Dans un second temps, je présente les différentes transformations du cycle de l'azote au sein des écosystèmes forestiers, *i.e.* apport d'azote, formes de l'azote, minéralisation, ammonification, nitrification, dénitrification, lessivage. Dans un troisième temps, j'expose les facteurs écologiques susceptibles d'être impliqués dans le contrôle du cycle interne de l'azote dans le sol. Enfin, je replace ces notions dans le contexte dynamique des systèmes forestiers. Dans un souci d'harmoniser le vocabulaire entre les différents chapitres du manuscrit, la synthèse bibliographique a été rédigée en anglais.

II. Synthèse bibliographique

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1. Humus forms

The soil is an essential component of terrestrial ecosystems, encompassing mineral materials, plant roots, microbial and animal biomass, organic matter in various states of decay, as well as water and a gaseous atmosphere (Brêthes *et al.* 1995; Gobat *et al.* 2004). Soil organic matter from litter, roots and dead animals is either mineralized and re-used by organisms or transformed and stabilized into humus (*i.e.* humification processes). According to the biological activity efficiency, organic matter accumulated or not on the top of soil, leading to different soil macro-morphological profiling; *i.e.* the humus forms. I will here present the main forest humus forms.

1.1. Definition and nomenclature of humus forms

The "**humic epipedon**" is defined as the group of the organic and organic-enriched mineral horizons at the soil surface (SSAJ 1997). The macro-morphological description of the humic epipedon (*i.e.* the vertical sequence of its constitutive horizons) is called the "humus form" (Brêthes *et al.* 1995; Jabiol *et al.* 2009). Soil horizons containing organic matter (OM) can be divided in: (i) holorganic horizons (O horizons) almost without mineral material and (ii) organo-mineral horizon (A horizon) below. Organic horizons can be divided into three types according the degree of litter transformation (Jabiol *et al.* 2007):

(1) The "**OL layer**" consisting of almost unmodified leaf and woody fragments. Most of the original biomass structures are easily discernible. This horizon can be divided into: OLn consisting of litter less than one year old without obvious decomposition; OLv consisting of litter more than one year old with coloration changes, cohesion and hardness mainly due to fungal activity; OLt consisting of litter more or less fragmented, recognizable to the naked eye, with earthworms casts but without humified OM.

(2) The "**OF layer**" consisting of a mixture of coarse plant fragments with fine organic matter (FOM). This layer is characterized by an accumulation of partly decomposed OM derived mainly from leaves and woody materials. Depending on the percentage of FOM, this horizon can be divided into OFr (less than 30% FOM) and OFm (30-70% FOM). As earthworm's activity is reduced, leaf transformations are attributed to the activity of soil epigeic fauna and fungi.

(3) The "**OH layer**" is an organic horizon characterized by an accumulation of faecal pellets and fine plants fragments (Jabiol *et al.* 2009). The original structures and materials are not discernible. FOM amounts to more than 70 % by volume. The term "humus layer" can also be found in the literature.

The **A horizon** corresponds to the organo mineral horizon below the organic layers. The chemical nature of OM and its assemblage with mineral matter (clay-humus complexes) in the A horizon depend on the biological activity. We distinguish three main pathways used to distinguish the different humus forms (Fig. 1.1.) (Brêthes *et al.* 1995) :

(a) **Biomacrostructured** A horizon characterizing **MULL** humus forms (including "Eumull", "Mesomull", "Oligomull" and "Dysmull"). Clay-mineral complexes may be cementing due to the mixing activity of soil-dwelling earthworms (Bernier and Ponge 1994). The division of the MULL humus type is based on the presence/absence of the different O layers (Fig. 1.1).

(b) **Insolubilisation** A horizon characterizing the “Oligomull mycogène”. Soluble metabolic products of white-rot fungi may precipitate on clay-iron particles. The insolubilisation A is weak. Earthworm activity is weak while this of fungi is strong.

(c) **Juxtaposition** A horizon characterizing **MODER** humus forms (including “Hemimoder”, Eumoder and Dysmoder). Inherited OM made of plant cell walls and fungal mycelium recognizable in transmission or even light microscopy may be present in faecal pellets of many small animals (litter-dwelling earthworms, arthropods, enchytraeids), side by side with mineral grains. The division of the MODER humus type is based on the presence/absence of the different O layers (Fig. 1.1).

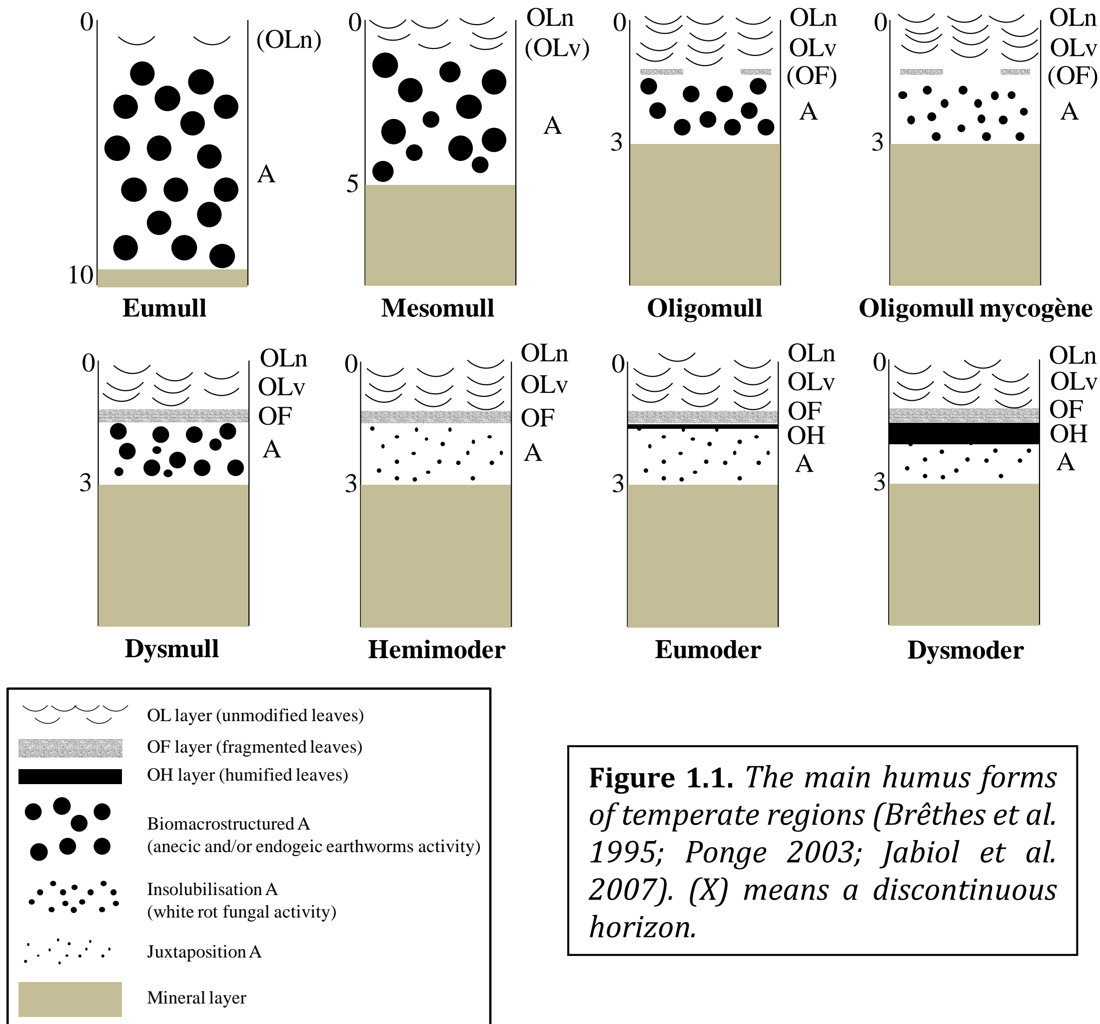


Figure 1.1. *The main humus forms of temperate regions (Brêthes et al. 1995; Ponge 2003; Jabiol et al. 2007). (X) means a discontinuous horizon.*

1.2. Potential indicators of ecosystem functioning

The vertical organization of these constitutive horizons is considered to be a great integrator of forest soil biological activity which conditions the rate of decomposition processes and nutrient cycling (Gobat *et al.* 2004; Jabiol *et al.* 2007). Humus forms are the result of litter input and decomposition processes. Nutrient cycling is governed by these processes and the resulting humus forms can be considered as a reflection of plant–soil interactions.

As such, humus forms are indicators of OM and soil quality, soil biological activity, and nutrient supply. Thus, the succession of the three main humus forms mull-moder-mor is usually considered as a decreasing gradient of OM recycling.

*"**MULL** can be characterized by the rapid disappearance of leaf litter under the influence of burrowing animals and/or white rots, and by the homogenization of humified organic matter with mineral particles within macro-aggregates. The hemorganic A horizon is the place where most soil organisms are living, plant roots included. Fungi are present, both as saprophytic and mycorhizal species, but bacteria abound due to the numerous mineral particles at the surface of which they adhere and primer effects from animal mucus and root exudates. Mull fauna exhibit a high biomass and a high species richness including megafauna (moles, small rodents), macrofauna (earthworms, large arthropods, molluscs), mesofauna (mites, springtails, enchytraeids) and microfauna (nematodes, protozoa).*

*In **MODER** humus forms macrofauna are smaller and reduced in abundance and diversity compared to mull, thus organic matter accumulates in the form of three holorganic horizons OL (entire leaves or needles), OF (fragmented litter) and OH (humified litter). The cementation of organic matter by mineral particles is nil or poor, due to the scarcity of adhesive substances such as mucoproteins or bacterial and root polysaccharides. Most microbial biomass is fungal, due to more acid conditions than in mull."*

Ponge (2003:935-937)

Humus formation is influenced by various factors such as climatic conditions (Aerts 1997b; Ponge *et al.* 1998), topography (Gallardo *et al.* 1995; Ettema and Wardle 2002), parent material (Kindel and Garay 2002), forest stands age and density (Bernier and Ponge 1994; Gallardo *et al.* 1995; Chauvat *et al.* 2007), stands composition (Muys 1995; Aubert *et al.* 2004), chemical composition of the litter (Aerts 1997b; Loranger *et al.* 2002; Berg and McClaugherty 2003), soil chemical and physical properties (Kindel and Garay 2002), and soil biota (Loranger *et al.* 2002; Ponge 2003; Chauvat *et al.* 2007). Therefore, the humus forms and their macro morphological properties, which are assumed to be indicative of chemical and functional properties (Brêthes *et al.* 1995; Jabiol *et al.* 2007), can reveal important ecosystem functions. Among these functions, the recycling of nitrogen (N) in forest upper soil layers appears central since N is usually limited and few available in soil for trees.

2. Nitrogen (N) cycle in forest soils

2.1. Soil N forms

Forest soil includes both organic (protein, nucleic acid, amino acid, wall materials such as chitin, and mineral (NH_4^+, NO_3^-, NO_2, N_2O, N_2, etc.) N forms. However, great part of N is in organic form (Zeller 1998; Knops *et al.* 2002), *i.e.* more than 90%. Indeed, the total organic N pool in the soil is the biggest N storage pool amounting to 9–15 tons of N ha^{-1}. For instance, Berg and McClaugherty (2003) showed that Scots pine humus possessed about 11 g-N kg^{-1} ash-free OM and Silver fir humus contained about 38 g-N kg^{-1}. In a pure beech stands of 130-160 years old in Germany, Meiwes *et al.* (2009) measured decreasing N contents with soil depth, *i.e.* 13–24 g-N kg^{-1} in the OL and OF layers, 15 g-N kg^{-1} in the OH layers, 2.5–5.4 g-N kg^{-1} in the 0–10 cm depth and further to 0.5–1.7 g-N kg^{-1} in the 40–80 cm soil depth. N storage in trees and understory can reach 0.6–1.4 tons, contained in needles/leaves, wood, twigs, branches and bark, coarse and fine roots (Zechmeister-Boltenstern and Zechmeister-Boltenstern 2007). The soil microbial N pool contains 30–300 kg N ha^{-1}; it is easily mineralized and can be partly

released after extreme weather events. Finally, the extractable soil inorganic N pool is transient and may contain 10–40 kg N ha^{-1}, some of which is absorbed to clay minerals and humus surfaces.

2.2. Soil N fluxes

The cycling of N in forest ecosystem can be divided into an external N cycle and an internal N one (Hart *et al.* 1994).

"The external cycle includes processes that add or remove N from ecosystems, such as: dinitrogen fixation, dry and wet deposition, N fertilization, N leaching, denitrification and ammonia volatilization. The internal N cycle consists of those processes that convert N from one chemical form to another or transfer N between ecosystem pools. Processes of the internal cycle: plant assimilation of N, return of N to soil in plant litterfall and root turnover, N mineralization, microbial N immobilization and nitrification."

Hart *et al.* (1994:999)

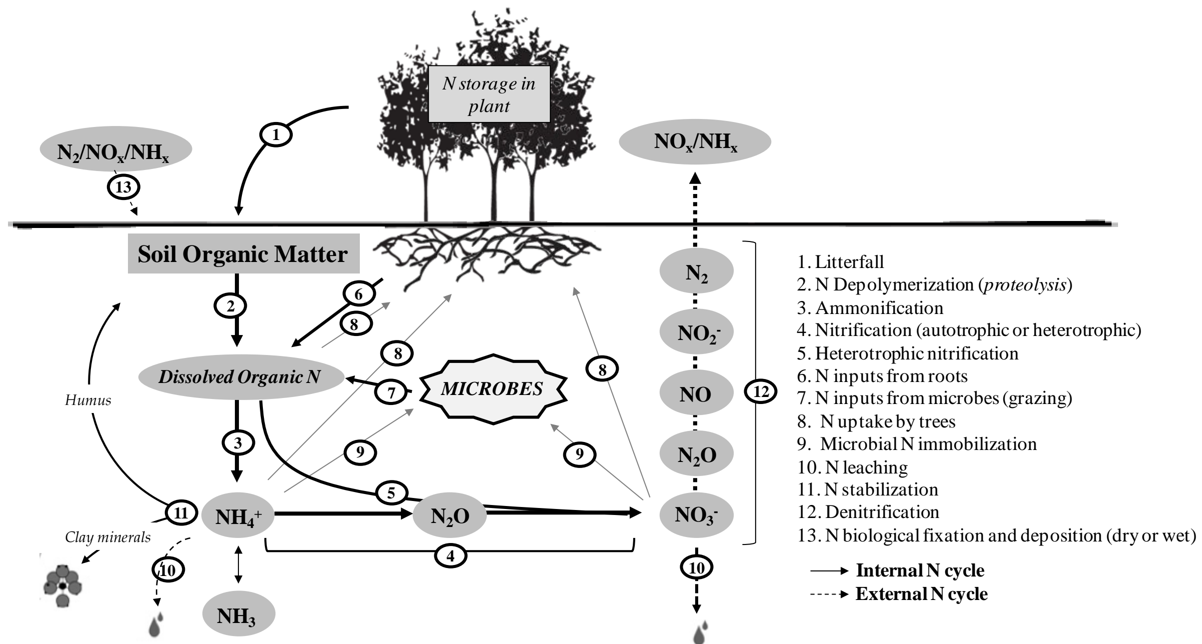


Figure 1.2. *N cycle in forest soil (modified from Hart et al. 1994; Jussy 1998; Schulze 2000; Schimel and Bennett 2004). Full arrows correspond to the internal N fluxes while dotted ones correspond to external N cycle.*

Both external (N deposition, N leaching, denitrification) and internal N fluxes (N mineralization, nitrification) occurred in forest soils (Fig. 1.2). Soil N cycle undergoes a variety of redox reactions performed in different ways by different organisms, mainly bacteria and fungi.

All of **the reduction N reactions** are performed by bacteria, archaea and some specialized fungi. There is only one remarkable exception, the assimilatory NO_3^- reduction, which also occurs in plants. In assimilatory NO_3^- reduction, NO_3^- is reduced via nitrite (NO_2^-) to ammonium. In general, In general, NH_4^+ is then used for the synthesis of glutamine as the first organic N-containing molecule formed. Glutamine is the N-donor for the synthesis of other amino acids and heterocyclic N-compounds. NO_3^- can also serve as an electron acceptor through denitrification. This pathway uses NO_3^- rather than oxygen (O_2) as the respiratory electron acceptor under anaerobic conditions.

In the first step, NO_3^- is reduced to NO_2^- in a reaction catalyzed by several different types of reductases. Nitrite is reduce to nitric oxide (NO), nitrous oxide (N_2O) and finally to dinitrogen (N_2). Nitrite can be also reducing to NH_4^+. This process is called NO_3^-/NO_2^- ammonification.

The oxidation N reactions are also performed by bacteria, archaea and some specialized fungi. Specialized organisms can oxidize either NH_4^+ or NO_2^- to meet their demands for energy and reducing equivalents by using a pathway called nitrification (developed further). A poorly studied subject is the depolymerization of organic N compounds (proteolysis*)*, through which organisms degrade proteins and other N-containing compounds to meet both their N-demand and presumably also their energy requirement. The final connection in the N-cycle is N_2 fixation, which allows some bacteria and archaea to reduce N_2 to NH_4^+ to provide their N-requirement. This reductive reaction is catalyzed by the enzyme complex called nitrogenase. N_2 fixation proceeds not only in free-living prokaryotes but also in bacteria in symbiosis with plants. The main reactions mentioned above as well as the factors implicated in the control of these reactions are extensively presented and discussed in this chapter.

2.2.1. N inputs

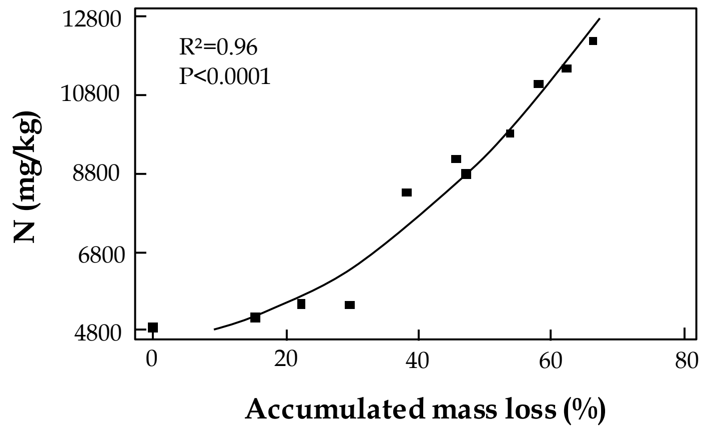
In forest ecosystems, litterfall is the main source of organic N inputs into the soil. Root litter, N_2 assimilation, atmospheric N deposition (dry and wet) and throughfall inputs contribute lesser in soil N inputs. For instance, Bonito *et al.* (2003) reported N inputs in a high elevation northern hardwood forest at Coweeta with 3.62 g-N m^2 y^{-1} from litterfall, 1.94 g-N m^2 y^{-1} from root litter, 0.89 g-N m^2 y^{-1} from wet deposition and 0.706 g-N m^2 y^{-1} from throughfall. Nevertheless, dry and wet atmospheric N deposition in N saturated forests can altered significantly the soil N cycle, leading to soil acidification, alteration of fauna and plant communities or higher nitrate and aluminum leaching. Total N input into the forest soil through litterfall varies according to litter production and initial N concentrations in litter. Lebret *et al.* (2001) measured, in an Atlantic beech forest, a total leaf litter production between 2.1 and 4.7 t ha^{-1} yr^{-1} according to the age of trees. Pederson and Bille-Hansen (1999) measured 3.1, 3.5 and 3.4 t ha^{-1} y^{-1} in beech, Sitka spruce and Norway spruce forest, respectively. N concentrations in litter vary greatly with species (Berg and McClaugherty 2003; Sariyildiz *et al.* 2005a). For instance, Hirobe *et al.* (2006) found 5.4 mg N g dry litter of *Pinus nigra* while 12.7 mg N were found per g dry litter of *Quercus robur*. Finally, total N inputs through litterfall depend on total litter inputs and N concentration in litter.

2.2.2. Litter N dynamics during litter decomposition

The release of N from leaves or needles into the soil occurred during litter decomposition. The rate of N release is function of litter decomposition rates. In fact, during litter decomposition, the concentration of N increases in litter (Fig. 1.3.). This increase in N concentration is a general phenomenon, also described as a decrease in the C/N ratio. The increase is normally linearly related to accumulated litter mass loss, usually with a high R^2 value, irrespective of the initial N concentration and of how the absolute amount of N changes during decomposition (Berg and Laskowski 2006c). However, the increase in N concentration in litter during litter decomposition differs between tree species, resulting in different final N concentrations in litter. For instance, Berg *et al.* (2006a) reported that in Scots pine needle litter, the N concentration may increase at least 3 times during decomposition (from about 4 mg N g^{-1} litter to about 12

mg N g^{-1} litter. In contrast, in grey alder leaves, N concentration changed from about 30 to 51 mg N g^{-1} litter.

Figure 1.3. *Changes in N concentration during decomposition of Scots pine needle litter. (Berg and Laskowski. 2006a). During litter decomposition, litter N concentration increases with accumulated mass loss (%).*



Litter decomposition affected N concentration and in return, N contents affect litter decomposition (Fig. 1.4.). Namely, the decomposition of water-soluble substances and unshielded cellulose or hemicellulose is stimulated by high levels of N (early stage, phase 1). When all unshielded holocellulose is decomposed, only lignin-encrusted holocellulose and lignin remain. In this late stage (phase 2), the degradation of lignin rules the litter decomposition rate. The degradation of lignin is hampered by N, and higher N levels suppress its decomposition whereas Mn has a stimulating effect on the degradation of lignin. Finally, in the humus-near stage (phase 3), the lignin level is about constant, the litter decomposition rate approaches zero, and the accumulated mass loss reaches its limit value. During litter decomposition, the dynamics of the amounts of N may be divided into three different steps or phases (Fig. 1.5.). In the first case, there is a short leaching of N followed by a net uptake and a net release (Fig. 1.5.A). In another case, there may be a net uptake followed by a net release (Fig. 1.5.B), and in a third case, only a net release is observed (Fig. 1.5.C). Thus, all three phases are not always present and not always clearly distinguished.

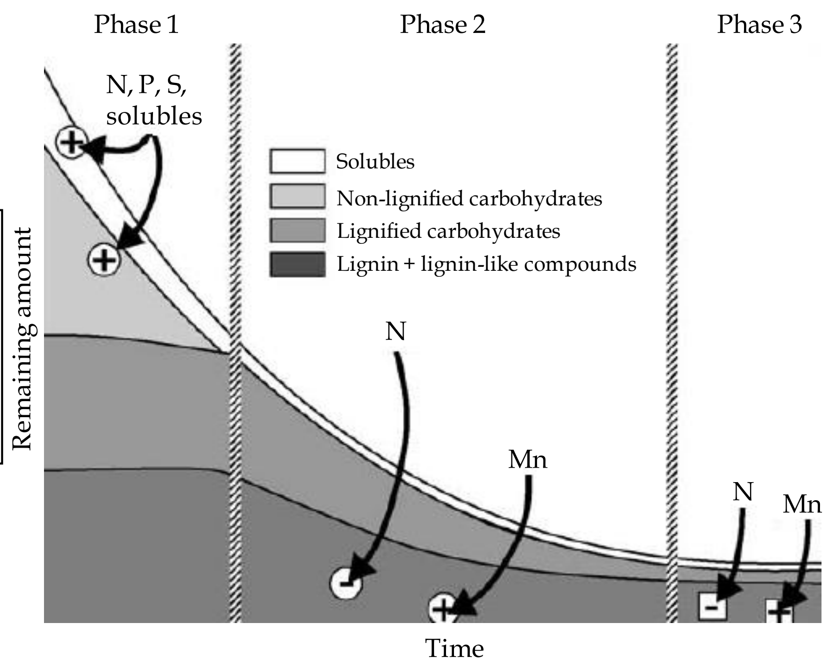


Figure 1.4. *Model for chemical changes and rate-regulating factors during litter decomposition (Berg and Laskowski 2006c).*

This first phase of N dynamics corresponds to a rapid release of initially leachable N in litter. A possible factor which determines the amount leached in the field would be rainfall and the movement of water, *i.e.* more intensive water movements promoting high leaching. Another factor may be freeze–thaw cycles, in which the freezing followed by thawing breaks tissue and cell structures and causes a release of N and other nutrients. In field experiments, the leaching phase relates to a net loss of N. At the same time as N is released, the fungal biomass transports N into the litter. This means that we have two counteracting processes, *i.e.* N is leached from decomposing litter during a short initial period after the incubation with a simultaneous transport of N into the litter structure. Such an accumulation phase has been established for a number of litter species (Berg and Laskowski 2006c).

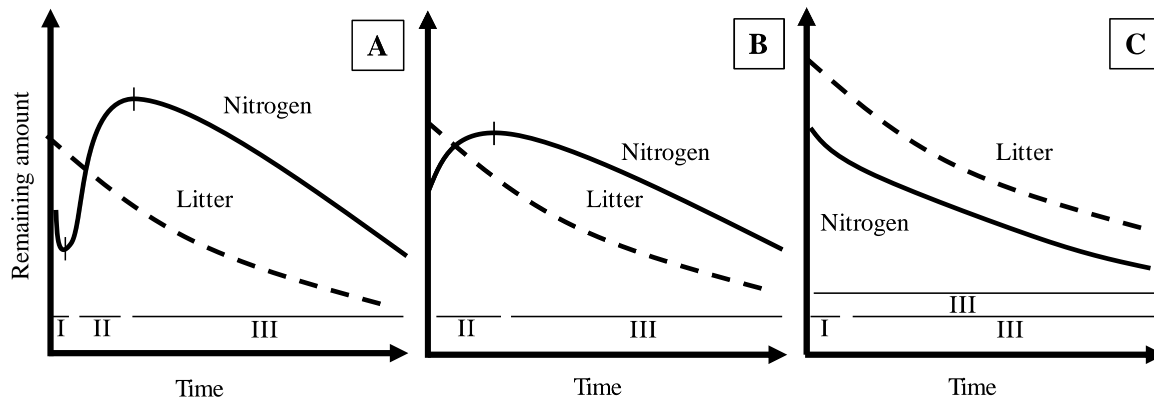


Figure 1.5. *Three separate phases may be distinguished for the change in amount of litter N over time. The accumulation phase could be missing, especially in litter with high N concentrations. (A) A leaching phase (I) is followed by an accumulation (II) and a release phase (III). (B) An accumulation (phase II) is followed by a release (phase III). (C) Only a release is seen (phase III or phase I + phase III) (Berg and Laskowski 2006c).*

The accumulation phase starts early in the decomposition process, sometimes directly after an initial leaching, and sometimes without a preceding leaching phase. A net N accumulation in litter means an uptake of N from its immediate environment. The uptake could be, in part, due to N_2 fixation by microorganisms present in the litter, but in investigated cases in temperate and boreal forests, this process appeared too slow to account for the observed net increases in amounts of N in needles and leaf litter. Such a net increase is mainly due to uptake by fungal hyphae from the surroundings of the litter. The initial concentration of N in litter has definitely an influence on whether there will be a net accumulation of N or not. When foliar litter species with different initial N concentrations were incubated in the same forest floor, the more nutrient-poor ones clearly accumulated N. For example, in leaf litter of European ash and durmast oak, a high initial N concentration of 15 mg g^{-1} N resulted in a net release, while in durmast oak litter with 7.5 mg g^{-1} N, a clear uptake took place. The point at which N release from litter begins has often been related to a particular or ‘‘critical’’ C/N ratio of the litter. But, the concentration of a given nutrient is not the sole determinant of its uptake or release in decomposing litter.

2.2.3. N mineralization

The large reservoir of organic N compounds in the soils is decomposed (N depolymerization) and can be converted into ammonium (ammonification). Ammonium can be converted via nitrite to nitrate (nitrification). N mineralization corresponded to the production of mineral N, including both ammonification and nitrification. In Norway spruce forest soil, annual net N mineralization was estimated to be between 0.5 and 5.0% of the total amount of N, *i.e.* 35-105 kg N ha^{-1} y^{-1} (Persson and Wirén 1995). Jussy *et al.* (2000) measured in situ N mineralization which reached 200.7, 130.7, 221.8 kg N ha^{-1} y^{-1} in a 20, 40 and 60 years old stand of Douglas-fir in France, respectively.

Schimel and Bennett reported in 2004 the evolution of the thinking about the soil N cycle and proposed a new paradigm of N cycling in soil (Fig. 1.6.). Firstly, microbial mineralization of organic N in soil was considered as the bottleneck in the internal cycling of N because it is an essential intermediate step between the transfer of organic N to the soil and the availability of inorganic N for subsequent plant growth (Neff *et al.*, 2003). This approach was based on the accepted view that (1) plant use only inorganic materials for their nutrition (mineral-nutrition theory of Lieibig, 1842) (2) plants are poor competitors for available soil N relative to microbes. Consequently, these assumptions established *net* mineralization as the key step in soil N cycling. This thinking led to the development of new methods, such as “buried bag” and “resin core” incubations, widely used and considered as an adequate “**measure”** of plant-available N. In the 1990s, numerous studies highlight the limitations of net rates as measures of N cycling dynamics. As a result, net N mineralization is now considered as an “**index”** rather than as a “**measure”** of plant-available N.

“The net accumulation of inorganic N in the absence of plant roots is thought to provide a good index of N availability to plants”

Hart *et al.* (1994:999)

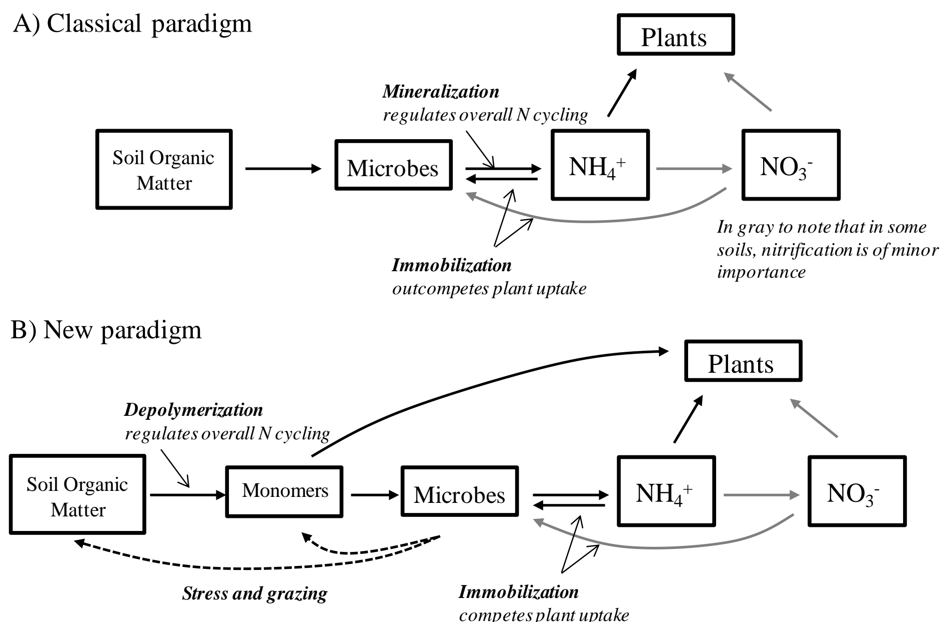


Figure 1.6. *The changing paradigm of the N cycle. (A) The dominant paradigm of N cycling up through the middle 1990s. (B) The paradigm as it developed in the late 1990s (Schimel and Bennett 2004).*

At the same time, several studies suggested that in low-N ecosystems, plants appear (i) to use organic N as a significant N source and (ii) to compete effectively against soil microbes for available N thanks to mycorhizae, which act as "microbial mercenaries" and microsite dynamics in soil. These results allow the development of a new paradigm which recognizes that the critical point in the N cycle is the depolymerization of N-containing compounds.

The main enzymes involved in the mineralization of N are hydrolases such as laccases and chitinases (Courty *et al.* 2006) breaking down complex phenolic compounds by oxidation making polyphenol-bound soil proteins more accessible, protease and peptidase, amidase such as arylamidase (or α-aminoacyl-peptidase hydrolase) catalyzing N terminal amino acid release from peptide, amide or arylamide (Acosta-Martinez and Tabatabai 2002; Ekenler and Tabatabai 2004), urease, a ubiquitous extracellular enzyme mineralizing urea in NH_3 and CO_2 (Marzadori *et al.* 2000), and oxidoreductase such as membrane-bound ammonia monooxigenase (De Boer and Kowalchuk 2001) (Table 1.1). The release of amino acids through N depolymerization was placed as the key N process regulating overall N cycling (Schimel and Bennett 2004). Indeed, extra cellular enzymes cleaved polymers not available and release monomers (amino acids, amino sugars, nucleic acids, etc.).

Table 1.1. *Main enzymes involved in the soil N cycle.*

Forms	Enzymes	Examples	References
Complex compounds	Hydrolase	*Laccase*	Courty et al., 2005
			Courty et al., 2006
			Snajdr, 2008
		Chitinase	Courty et al., 2005
		N-Acetyl-β-D-glucosaminidase	Ekenler and Tabatai, 2002
		Chitobiosidase	Tabatai, 2003
		Endochitinase	
Peptides	Protease	*Endoprotease*	Caldwell, 2005
	Peptidase	*Aminopeptidase*	Saiya-Cork et al. 2002
		Carboxypeptidase	Caldwell, 2005
Non-peptides	Oxidoreducatse	*L-amino acid dehydrogenase*	Caldwell, 2005
	Amidase or amidohydrolase	*Urease*	Sinsabaugh et al., 2000
		Glutaminase	Marzadori et al., 2000
		Asparaginase	Acosta-Martinez & Tabatabai, 2000
		Arylamidase	
	Nucleic acid deaminase	*(Adénosine)désaminase*	
Inorganic N	Oxidoreductase	*Hydroxylamine oxidoreducatse*	De Boer and Kowalchuk, 2001
		Ammonia monooxigenase	De Boer and Kowalchuk, 2001
		Nitrogenase	Rosh et al., 2002
		Nitrate reductase	Hayatsu et al., 2008

Organic N depolymerization and ammonification are achieved by numerous soil enzymes produced by different groups of microorganisms (Schimel *et al.* 2005), *i.e.* fungi and bacteria, plants (particularly via roots exudation) and animals (Gobat *et al.* 2004). Nevertheless, according to their biomass and high metabolic activity, microorganisms are considering as the main source of soil enzymes. The available DON (Dissolved Organic N, *e.g.* N-containing monomers) pool can be recycled and reused through the microbial system or taken up by trees. DON cycle is an increasingly important focus of studies that attempt to understand the controls over N cycling within ecosystems.

Neff *et al.* (2003) reviewing studies focusing on DON have developed a new concept of the terrestrial N cycling including DON dynamics. They formulated two hypotheses. (1) DON can be a **"short circuit"** in the terrestrial N cycle: plants take up some organic forms of N directly from soil solution without the need of microbial mineralization. Plants directly absorb and generally prefer amino acids over inorganic N. Amino acid uptake by plants occurs in many species including non-mycorhizal and mycorhizal plants. (2) DON can **"leak"** from ecosystems, despite high demand of N by plants and microbes when some forms of DON are flushed from ecosystems due to their recalcitrance or during rapid rates of leaching. They suggest that DON can play multiple roles because it contains compounds that are both labile (easily degraded by plants or microorganisms) and recalcitrant (not easily utilized by plants or microorganisms) which behave in fundamentally different ways. DON availability in soils is controlled by both biological and physical processes (Fig. 1.7.).

2.2.4. Nitrification

Nitrification is a two-step process corresponding to the conversion of ammonium via nitrite to nitrate (De Boer and Kowalchuk 2001). It is a key soil process since it allows (i) the production of easily available mineral N for plant and microbes and (ii) N losses by denitrification and leaching (Persson *et al.* 2000). Indeed, contrary to nitrate, ammonium is hardly mobile due to its positive charge responsible for it clay mineral bounding. Nitrate production in forest soils showed substantial variations between studies. Persson *et al.* (2000) found potential net nitrification from about 50 to 172 kg N ha^{-1} y^{-1} in European beech stand. Jussy *et al.* (2000) measured *in situ* nitrification which reached 165.9, 112.1, 192.9 kg N ha^{-1} y^{-1} in a 20, 40 and 60 years old stand of Douglas-fir in France, respectively.

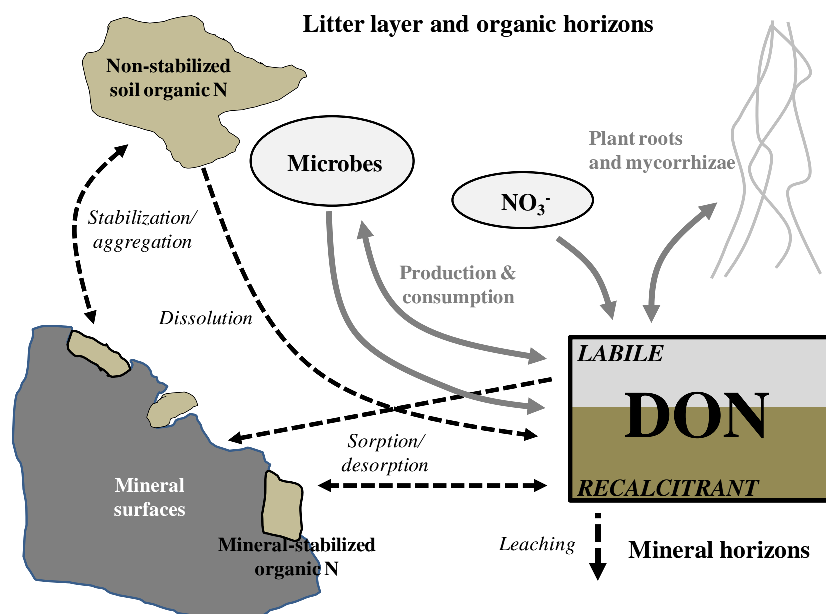


Figure 1.7. *Mechanistic controls over the soil DON cycle. Solid grey arrows and text show fluxes dominated by biological processes; black dashed lines and italics text indicate physically controlled processes. Note that only DON cycling processes are shown. The arrow from NO_3^- to DON shows the possibility of a biotic or abiotic incorporation of NO_3^- into dissolved organic matter (Neff et al. 2003).*

In forest soils, nitrification was long considered to be carried out mainly by autotrophic bacteria (Persson and Wirén 1995; De Boer and Kowalchuk 2001). Autotrophic nitrification is a two-step process, *i.e.* the oxidation of ammonia to nitrite, followed by the oxidation of nitrite to nitrate (Figure 1.8). The first step requires energy and involves the membrane-bound enzyme ammonia mono-oxygenase. This enzyme, for which the substrate is ammonia rather than ammonium, forms hydroxylamine. Hydroxylamine is oxidized to nitrite by the soluble enzyme hydroxylamine oxidoreductase, generating energy. Finally, nitrite oxidation is carried out by the soluble enzyme nitrite oxidoreductase.

The two steps are performed by different groups of aerobic bacteria, the ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). Gram-negative bacteria of the family *Nitrobacteraceae* are responsible for autotrophic nitrification. The AOB found in the soil belong to the genera *Nitrosospira, Nitrosomonas*, *Nitrosolobus*, *Nitrosovibrio* and *Nitrosococcus*, while the genus *Nitrobacter* is regarded as the dominant NOB. Besides autotrophic bacteria, nitrate can be also produced by fungi and heterotrophic bacteria, *i.e.* heterotrophic nitrification (De Boer and Kowalchuk 2001). Biochemical pathways of heterotrophic nitrification were reported by de Boer and Kowalchuk (2001).

"Two biochemical pathways have been proposed for the oxidation of ammonium by heterotrophs. The first pathway is known from heterotrophic nitrifying bacteria (Paracoccus denitrificans, Thiosphaera pantotropha, Pseudomonas putida and Alcaligenes faecalis). These bacteria possess ammonia- and hydroxylamine oxidizing enzymes that have strong similarities with those of autotrophic nitrifiers. Some of the heterotrophic nitrifying bacteria, such as Thiosphaera pantotropha, combine their nitrifying activity with aerobic denitrification […]. The other heterotrophic pathway for ammonium oxidation is also known as fungal nitrification. Wood (1987) suggested that nitrogen compounds react with hydroxyl radicals that are produced when hydrogen peroxide and superoxide are both present. These conditions for hydroxyl radical formation are likely to occur during cell lysis and lignin degradation when oxidases and peroxidases are released into the environment."

de Boer and Kowalchulk (2001:854)

The ecological significance of heterotrophic nitrification in forest ecosystem is uncertain. It has been generally considered that heterotrophic nitrifiers are unimportant in the formation of nitrate in soils. In contrast to AOB, oxidation of ammonium by heterotrophs is not linked to cellular growth. However, large numbers or high biomass of heterotrophic bacteria and fungi might compensate for their relative inefficiency. In fact, heterotrophic nitrifiers have been considered to be responsible for significant nitrification in acidic forest soils (Duggin *et al.* 1991; Papen and von Berg 1998; Pedersen *et al.* 1999; Trap *et al.* 2009). One reason why heterotrophic nitrification may be important in these soils is that autotrophic nitrification requires a higher pH. Nevertheless, recent studies have shown that autotrophic nitrification does occur in acidic forest soils (Rudebeck and Persson 1998; Trap *et al.* 2009).

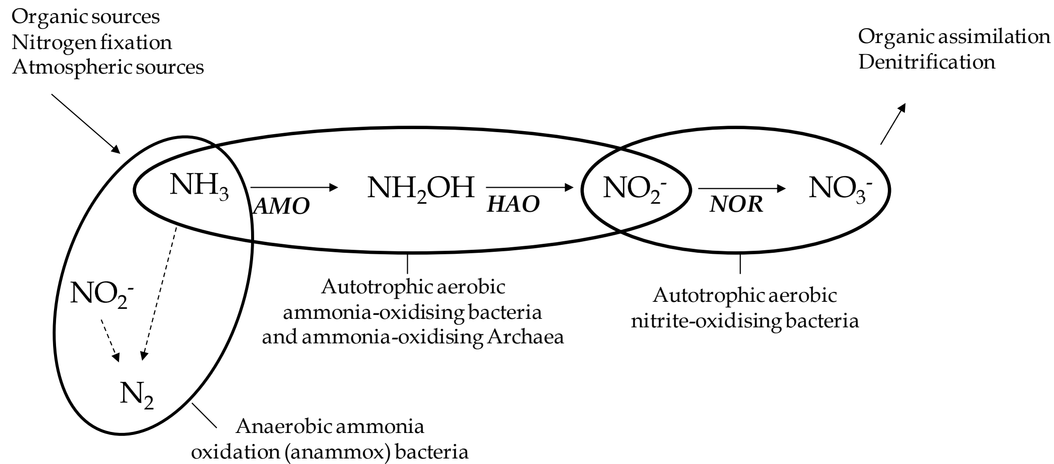


Figure 1.8. *Autotrophic nitrification, a two-step process. Autotrophic ammonia oxidation during nitrification. Ammonia-oxidising organisms convert ammonia to nitrite through hydroxylamine using the enzymes ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO). Autotrophic nitrite-oxidising bacteria subsequently use the enzyme nitrite oxidoreductase (NOR) to convert nitrite to nitrate, which can be assimilated or subjected to denitrification processes. In anaerobic environments, ammonia can be converted to molecular nitrogen by the anammox process by several enzymatic steps, represented by dashed arrows (Nugroho, 2006).*

2.2.5. N uptake by trees

Trees roots can access both organic and inorganic N forms. Some works showed that trees can take up organic N compounds directly via their roots (Kielland 1994) or in association with some types of mycorhizal fungi (Nasholm *et al.* 1998; Wallenda and Read 1999; Nasholm *et al.* 2009). Total N uptake in kg N per ha per year depends to climate conditions, species or soils. For instance, Jussy *et al.* (2000) measured Douglas-fir N uptake in France which reached 130.9, 118.3, 177.5 kg N ha^{-1} in a 20, 40 and 60 years old stand, respectively. In a low elevation oak pine and a high elevation northern hardwood forests at Coweeta, 4.39 and 7.11 g-N m^2 yr^{-1} were taken up by trees, respectively (Bonito *et al.* 2003). In consequence to the low availability of N in soils limiting trees growth and productivity, trees developed strong associations with fungi, *i.e.* the mycorhizal symbiosis. Mycorhizal associations are involved in the absorption of nutrients from soil, and especially N (Wallenda and Read 1999; Nasholm *et al.* 2009). Our understanding of the N uptake by trees from soils has been improved by the recognition that the forms in which the element may be available to mycorhizal fungi in forests soil are diverse (Nasholm *et al.* 1998; Gallet-Budynek *et al.* 2009; Nasholm *et al.* 2009). The view that mineral N was the only important source of the element predominated during much of the following century. Many studies emphasize the potential importance of organic N as a central N source for the nutrition of forest trees in symbiosis with mycorhizal fungi (Nasholm *et al.* 1998; Wallenda and Read 1999; Finzi and Berthrong 2005; Nasholm *et al.* 2009). The rate and the preference of both organic and mineral N forms uptake by trees depend to species and fungal species associations. Overall, acidophilus species such as coniferous take up ammonium preferentially (Kronzucker *et al.* 1996; Gessler *et al.* 1998) while calcareous species take up nitrate preferentially. But, since ammonium is the major source of mineral N in most forest soils, the majority of ECM fungi preferentially use the ammonium when grown in culture.

2.2.6. Denitrification and leaching

Denitrification and leaching are the main mineral N losses from forest soils (external N cycle). Under anaerobic conditions, some forest soils showed denitrification. NO_3^- can serve as an electron acceptor through dissimilatory NO_3^- reduction or denitrification. This pathway uses NO_3^- rather than oxygen O_2 as the respiratory electron acceptor under anaerobic conditions. In the first step, NO_3^- is reduced to NO_2^- in a reaction catalyzed by several different types of reductases. The subsequent reduction of NO_2^- to nitric oxide (NO), nitrous oxide (N_2O) and finally to dinitrogen (N_2) involves the action of a sequence of specific enzymes. Denitrification is carried out by facultative anaerobic species, predominantly heterotrophic bacteria, the most common being species of the genera *Pseudomonas* and *Alcaligenes*. Most of denitrifying bacteria require anaerobic conditions, but some species continue to denitrify at varying levels of dissolved oxygen. Some nitrifiers also can denitrify. Nitrobacter cells are able to grow by denitrification under anaerobic environments and *Nitrosomonas europaea* has been shown to reduce nitrite to gaseous N compounds (NO, N_2O, N_2) under conditions of oxygen stress by simultaneous oxidation of ammonium (Nugroho, 2006). In addition to denitrification and autotrophic nitrification, also heterotrophic nitrifiers and fungi have been suggested to participate in N_2O production in acidic forest soils. Leaching occurs especially if the soil is N saturated. In a low elevation oak pine and a high elevation northern hardwood forests at Coweeta, 0.31 and 0.14 g-N m^2 y^{-1} were losses by leaching to a depth of 10 cm, respectively (Bonito *et al.* 2003). In a 20, 40 and 60 years old stand of Douglas-fir in France, N leaching reached 76.7, 32.1, 60.2 kg N ha^{-1} at 15 cm depth, respectively (Jussy *et al.* 2000).

3. Ecological factors driving soil N cycle

Soil processes are generally controlled by: (i) climatic conditions (mainly temperature and moisture), (ii) chemical (resource quality) and biological (soil organisms) factors that operate at different spatial and temporal scales. Several factors, both biotic and abiotic, are known to influence soil N transformations. It includes (i) temperature and moisture (Knoepp *et al.* 2000), pH (De Boer and Kowalchuk 2001; Ste-Marie and Houle 2006), ionic environment (Lavelle and Spain 2001), soil resources (Satti *et al.* 2003), soil microbial communities (Chapman *et al.* 2006), herbaceous vegetation (Lata *et al.* 2004), allelophatic compounds (White 1988; Paavolainen *et al.* 1998) or soil compaction (Breland and Hansen 1996). Lavelle *et al.* (1993) proposed a hierarchical model of the abiotic and biotic factors determining organic matter decomposition in terrestrial ecosystems (Figure 1.9.). The model includes four hierarchical levels: (i) climatic factors (moisture and temperature regimes); (ii) edaphic properties such as the content of clay in soils; (iii) the physical and chemical characteristics of the decomposing resources; and (iv) biological regulation through interactions between macro- and micro-organisms. Soil processes are determined by interactions among these four groups of factors which are not equally important since they operate at different spatial and temporal scales and may have opposing influences on decomposition processes.

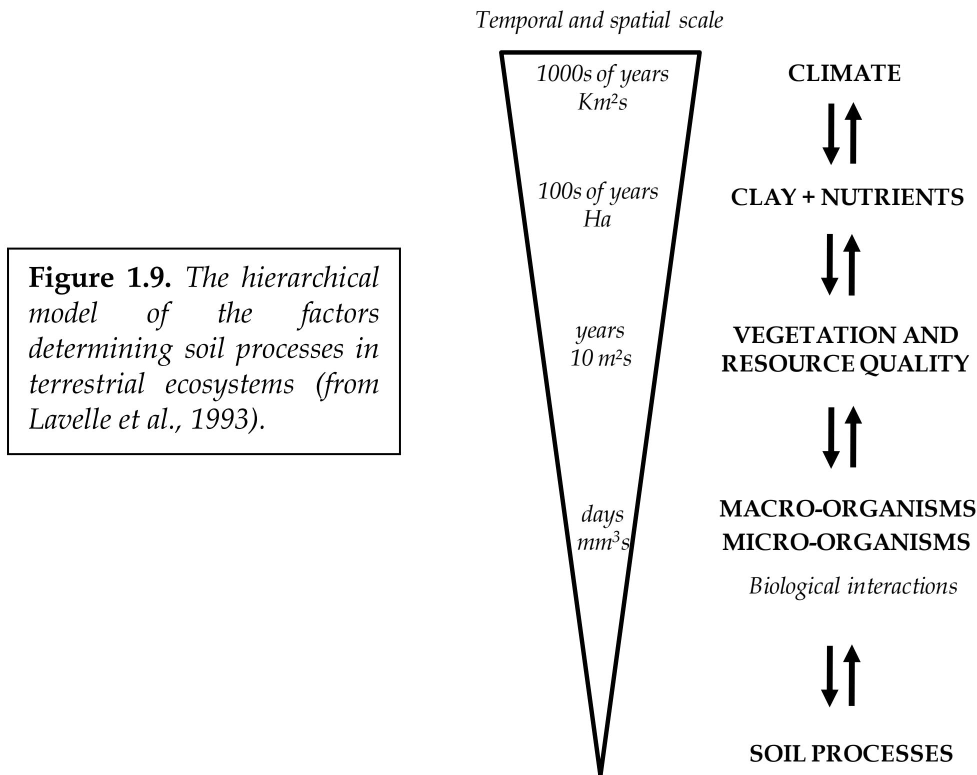


Figure 1.9. *The hierarchical model of the factors determining soil processes in terrestrial ecosystems (from Lavelle et al., 1993).*

In this model, all hierarchical levels interact and the position of a factor only indicates the probability that it may have a dominating effect over a factor operating at a lower level. This model was used as a frame to present the ecological factors recognized as influencing the soil N cycle.

3.1. Climate

At high temperatures, microbial activity is expected to increase, resulting in greater N mineralization and nitrification. Cookson *et al.* (2007) showed that 14 days-incubation temperature (5 or 25°C) greatly increased N dynamics. DON, ammonium and nitrate pools, gross N mineralization, nitrification and immobilization rates were higher at 25°C compared to 5°C. Besides temperature, microbial activity and N fluxes are known to be maximal for a certain percentage of humidity allowing a good aeration. Soil moisture content used in the literature to maximize microbial activity was often set at 60% of the soil holding capacity (Frostegard *et al.* 1996; Öhlinger 1996c; Martinez-Toledo 1997; Rudebeck and Persson 1998; Priha and Smolander 1999). In high moisture content, nitrate is used as an alternative electron acceptor resulting in the conversion of NO_3 to N_2 or N_2O via denitrification (Figure 1.10) (Franzluebbers 1999). Compton et al (2002) showed that low soil moisture inhibited mineral N immobilization without influencing N production processes. They explained these results by reduced diffusion of substrate, dehydration of microorganisms or shifts in microbial community composition, while extracellular enzymes released maintained the production. Also, until recently, ammonia oxidation was thought to be a strictly aerobic process, requiring molecular O_2. Under anoxic conditions, some AOB (*N. eutropha* and *N. europaea*) can oxidize ammonia in the presence of pyruvate, and with nitrite as electron acceptor or with NO_2 gas.

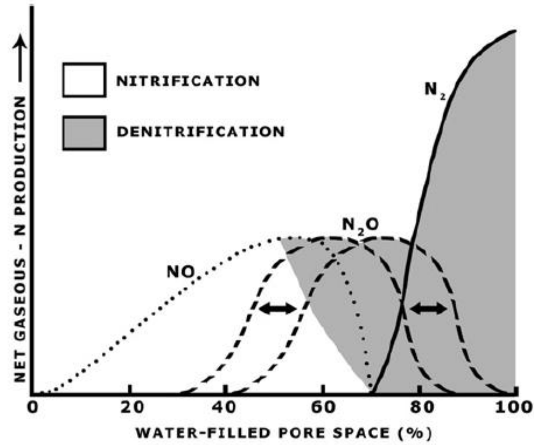


Figure 1.10. *A conceptual model describing losses of NO, N_2O and N_2 from nitrification and denitrification as a function of soil moisture (Zechmeister-Boltenstern and Zechmeister-Boltenstern 2007).*

3.2. Soil, nutrients and ionic environment

Soil physical and chemical properties influence greatly microbial activity and consequently N recycling (Coté *et al.* 2000; Hirobe *et al.* 2003). For instance, soil structural aggregates may prevent resource accessibility and clay mineralogy influence the adsorption of (i) enzymes limiting their activity and (ii) natural inhibitor preventing specific microbial function (Lavelle *et al.* 1993). Soil types have a great influence on N mineralization (Coté *et al.* 2000). The fine texture of clay soil reduces the amount of N mineralized. Protection of soil organic matter by fine particles is well known (Gobat *et al.* 2004). Bauhus *et al.* (1998) found a higher proportion of total soil N incorporated into microbial biomass in clay than in till soils.

Soil pH appears as one of the most important abiotic factors especially towards the nitrification process. For instance, Rudebeck and Persson (1998) showed that acidification with H_2SO_4 reduced net nitrification whereas an addition of $CaCO_3$ caused an increase of this process in the OF and OH layers of coniferous forests. Ste Marie and Paré (1999) found within organic layers of boreal forest a negative correlation between pH and net nitrification rates. An increase of pH in the organic layers had a positive effect on net nitrification while acidification depressed this process. De Boer and Kowalchuk (2001) distinguish four patterns of nitrification according to soil pH :(i) No nitrification at either pH, (ii) Acid-sensitive nitrification (nitrate production at pH 6 not at 4), (iii) Acid-tolerant pH-dependent nitrification (nitrate production both at pH 4 and 6, with a production 1.5 times faster at pH 6) and (iv) Acid-tolerant pH-independent. Besides nitrification, differences in gross N fluxes between land use (forest, grassland and arable soils) were found by Cookson *et al.* (2007) and explained by variations in pH. Soil N recycling is also greatly influenced by the ionic environment (Lavelle *et al.* 1993). For instance, manganese is a coenzyme essential for the activity of Mn-peroxidase, a lignin-degrading enzyme with Mn as part of the functioning enzyme. High Mn concentrations enhance its production (Berg and Laskowski 2006b). Coté *et al.* (2000) observed a significant linear correlation between exchangeable Mn and mineralized C and N mineralization in the forest floor. Higher base cations (Ca^{2+}, Na^+, K^+, Mg^{2+}) pools in forest soils favor high pH and consequently microbial activity and N recycling. Also, P availability has been reported as a factor controlling nitrification (Brais *et al.* 1995). Adams (1986) said that low P availability has been suggested as a possible explanation for the absence of nitrification in some forest soils.

4.3. Tree species

Soil N transformations have been shown to be affected by the dominant tree species (Reich *et al.* 1997; Bauhus *et al.* 1998; Priha and Smolander 1999; Coté *et al.* 2000; Graystone and Prescott 2005; Zeller *et al.* 2007). For instance, Nugroho *et al.* (2006) observed higher net nitrification rates in spruce, fir and larch soil compared to pine soils, while net N mineralization was higher in these last ones. Paré and Bergeron (1996) observed high net nitrification rates in deciduous stands of 29 and 44 years old forests originating from fire while no nitrate accumulation was found under spruce stands or in older forests of any composition. Ste-Marie and Paré (1999) observed high nitrate accumulations in the forest floor of deciduous boreal forest than coniferous. Aubert *et al.* (2005) showed lower net nitrification both in the field and in the laboratory for mixed beech-hornbeam stand than pure beech stands.

Usually, tree species affects concurrently soil nutrients availability, soil processes and organisms. For instance, Birch (*Betula* sp.) has a reputation in forestry history as a soil-improving species, especially compared to conifers such as spruce. Conifers have been found to change soil fertility gradually in an unfavorable direction by lowering the soil pH, decomposition rates and concentration of exchangeable nutrients, by increasing soil C/N ratio and by enhancing podzolisation (Kanerva, 2007). Tree species differ in total N inputs by litter and total N influence greatly soil N mineralization and nitrification. Some works observed significant correlations between soil N mineralization and total N (Booth *et al.* 2005) or total C (Barrett and Burke 2000).

Besides quantity, litter quality is considered as the key plant trait that influences soil N cycle (Wedin and Tilman 1990; Stump and Binkley 1993; Aerts 1997b; Satti *et al.* 2003). The availability of organic C and total N inferred by the C/N ratio influence directly N transformations rates (Hungate *et al.* 1999). Most of microorganisms are directly dependent on easy available C and due to the heterotrophic nature of the decomposers, the availability of labile C was often assumed to be the main factor constraining soil microbial activity and soil N recycling. Côté *et al.* (2000) found significant negative linear correlations between the C/N ratio and the amount of N mineralized and the rate of N mineralization in the forest floor of boreal mixed wood stands. Currie (1999) reported NITREX (European Nitrogen Experiment project) results that showed a negative relationship between C/N and net nitrification measured by field incubations, *i.e.* high forest floor C/N ratio was observed with low net nitrification. Some studies have shown greater immobilization in litter with low initial N concentrations (Pastor *et al.* 1987; Hobbie 2000). Other studies suggest that initial lignin concentration determines N-immobilization (Melillo *et al.* 1982).

Inhibitory compounds present in litter or released by roots into the soil could affect soil microorganisms and consequently soil N cycle (Leckie 2005). Strauss and Lamberti (2002) formulated two hypotheses on nitrification control by the quality of DOC. The first corresponds to a **competition hypothesis**. Under high C/N ratio, higher quality organic carbon should provide a better substrate for heterotrophic organisms increasing their demand and competition for ammonium, reducing nitrification rates. In return, poorer quality lead to lower heterotrophic activity, decreasing the competition. The second refers to an **allelopathy hypothesis**. Allelopathy refers to inhibitory and stimulatory interactions between organisms and was defined as “the direct or indirect harmful or beneficial effects of one on another through the production and release of chemical compounds”. All stages of the N cycling could be affected by allelopathy but most studies focused on nitrification because only two groups of bacteria are implicated.

Allelopathic chemicals are generally secondary plant products as terpenes or phenolic compounds. Dissolved organic carbon leached from leaves likely varies in quality in regard of species composing the forest stands (Strauss and Lamberti 2002) affecting differently soil N cycling. For instance, a species which possesses the ability to use efficiently ammonium *versus* nitrate may able to inhibit nitrification by allelopathy and increase the ammonium/nitrate ratio in the soil in order to prevent the loss of energy during the reduction of nitrate to ammonium and to reduce interspecific competition. For instance, Ward *et al.* (1997) showed that ammonia oxidation was inhibited by redwood monoterpenes (limonene, α-pinen, β-pinene, γ-pinene, sabinen, myrcene).

3.4. Soil organisms

Since most soil N transformations are carried out by bacteria and fungi, microorganisms play important roles in the nitrogen cycle of various ecosystems. In consequence, the structure and biomass of soil microbes are directly related with soil N availability and recycling (Smolander and Kitunen 2002; Booth *et al.* 2005; Finzi and Berthrong 2005; Myrold and Posavatz 2007). In general, soils with low N availability and turnover are mainly dominated by fungi (Leckie 2005). The species composition of the microbial community may vary with the general properties of the soil/litter properties, such as nutrient status and pH (Berg and Laskowski 2006b). In fact, all factors influencing microbial activity may impact soil N processes. Forest maturation may certainly change the microbial community structure and thus soil N transformations along the rotation. For more information, Hayatsu *et al.* (2008) reviewed the microbial N transformations: "*Various players in the nitrogen cycle: Diversity and functions of the microorganisms involved in nitrification and denitrification*". Even if the soil N transformations are performed by microorganisms, soil fauna plays an important role in soil N cycle (Lavelle and Spain 2001; Schröter *et al.* 2003; Berg and Laskowski 2006b). In fact, microorganisms are inactive for most of time due to their relative inability to move and the discontinuous distribution of resources. Thus, they constitute a largely dormant population, i.e the "Sleeping Beauty Paradox" named by Lavelle (1996). The presence and nature of structures produced by invertebrates that act as incubators for microbial activities provide the division of invertebrates into three major functional groups, micropredators, litter transformers and ecosystem engineers (Lavelle 1996).

(i) Among micropredators, protozoa and nematodes do not build any structure. Anyway, increased grazing by protozoa allow the release of N immobilized and further enhance bacterial turnover to finally, enhance N mineralization (Schröter *et al.* 2003; Postma-Blaauw *et al.* 2006). Also, it was showed that different nematode species can contribute either differently (De Mesel *et al.* 2003) or similarly (Djigal *et al.* 2004) to N mineralization. Nematode species can influence the population sizes of other ones, which may affect the N mineralization in the system (Postma-Blaauw *et al.* 2006). Furthermore, interactions between species may lead to resource partitioning, and consequently to a more intensive use of resources and increased N mineralization (Postma-Blaauw *et al.* 2006).

(ii) Litter transformers build holorganic structures that act as incubators for microbial activities. In these organic fecal pellets, N mineralization may be enhanced in short periods. Faecal pellet formation results in an increase in availability of nutrients such as N and P to microorganisms (Rawlins *et al.* 2006). A study on the role of the pill millipede (*Glomeris marginata*) showed that sterol, short chain fatty acids,

triacylglycerols, carbohydrates and amino acids decreased in the faecal pellets of these macro arthropods, but high concentrations of lignin, wax esters and triterpenoids were likely to be mediated by microorganisms (Rawlins *et al.* 2006). However, these compact structures limit aeration and water storage in the longer term, resulting in a significant decrease of mineralization (Lavelle 1996).

(iii) The size of ecosystem engineers allows the development of anisosymbiotic relationships with microflora in their proper gut. Effects of ecosystem engineers are multiple, *i.e.* bioturbation, dissemination of spores, regulation of structural porosity, water storage capacity, aeration, infiltration rates, nutrient cycling, surface structures, etc. These structures have an effect on selection on litter transformers, on associated microfauna and microflora and on root development. Earthworms are known to increase N mineralization (Zhang *et al.* 2000; Lavelle and Spain 2001). Nevertheless, earthworm species does not affect similarly N transformations. Indeed, Postma-Blaauw *et al.* (2006) showed that earthworm species had different effects on N mineral concentrations according to their ecological groups (epigeic, anecic or endogeic). They observed a positive effect on N mineralization by *L. rubellus* and *L. terrestris* but not by *A. caliginosa*. Moreover, the combination of *L. rubellus* and *A. caliginosa* facilitated N bacterial immobilization. Their results showed that N dynamics are affected as well as by the ecological traits of the earthworm species and by the earthworm community composition.

4. Nitrogen cycle along forest maturation

4.1. Forest cycling

Forest ecosystems may be considered as a mosaic of sylvigenetic phases (Lemée 1989, Oldeman, 1990. According to Oldeman's nomenclature, each gap opening (silvigenetic cycle) or clear-cutting (sylvicultural cycle) constitutes the "zero event" of a given eco-unit. It is the beginning of an internal dynamics of the forest ecosystem which lead gradually, in a variety of ways, to the canopy closure and later phases of growth (growth or aggradation phase), maturing (mature or biostatsis), then senescence (regeneration cutting). Ponge *et al.* (1998) described the natural silvigenetic cycle of semi-natural spruce forests as a biphasic cycle (Bernier and Ponge 1994). The first phase is a tree growth phase characterized by (i) organic matter accumulation on the organic layers, (ii) strong nutrients uptakes; leading to the development of moder humus forms (**autotrophic phase**). The second one is a mineralization phase characterized by an increasing activity of decomposers (**heterotrophic phase**).

4.2. Chronosequence as "space for time" substitution

From a technical point of view, the study of soil N transformations during the whole forest cycle is rather difficult if not impossible, *i.e.* the length of the forest cycle can reach 100 years for coniferous tree species and more than 150 years for deciduous species in managed systems. To explore the soil compartment changes involving with forest development, it is nevertheless necessary to investigate the complete forestry cycle, *i.e.* to focus on important stages of development like regeneration, aggradation (intense growth phase), mature and senescence stages. Chronosequence offers the opportunity to simultaneously investigate forest sites of different age, enabling the 'space for time' substitution as a surrogate for long-term studies (Pickett 1989).

This synchronic approach is still the most suitable way to study the dynamic aspect of forest ecosystem function (Trofymow and Porter 1998)

"Chronosequence research offers scientists the opportunity to examine, over a period of a few years, long-term changes in forest succession"

Trofymow and Porter (1998:8)

"Space-for-time substitution has been a very widely used approach to the study of long-term phenomena in ecology. It has been used most often, and most successfully, in systems acknowledged to have strong successional dynamics. It works in these cases"

Pickett (1989:128)

Chronosequences are false time series which integrate independent forest stands with their distinct history and variability into one unit. Hence, stands within a chronosequence should be as identical as possible in their characteristics in order to minimize the resulting variability. In an experience based on unique stand per silvicultural phase or age class, it is impossible to allocate the differences measured for a variable to stand age effect. Since every stand is the only representative of its silvicultural phase, the risk of confusion is high: a stand can have been polluted or not well managed. In other words, there is not real replication of treatments but pseudo-replications (Hurlbert 1984).

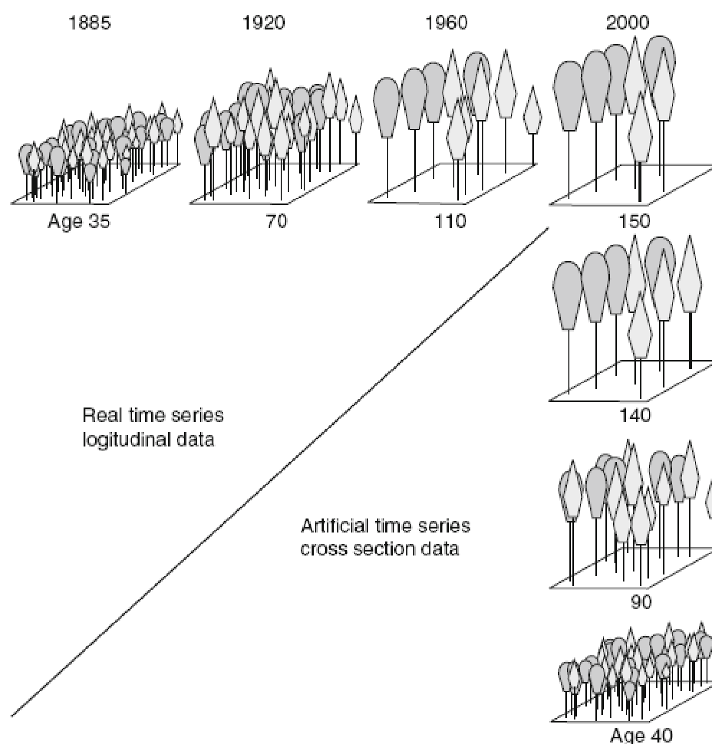


Figure 1.11. *Comparison of the principles of real time series and artificial time series (chronosequence as a space-for-time substitution).*
If stand development is recorded regularly from 1885 to 2000, then the sequence of data produces a real time series with surveys in 1885, 1920, 1960, and 2000 (row). In contrast, an artificial time series is constructed from spatially adjacent stands in different development phases (e.g. age 40, 90, 140, and 150) with comparable site conditions (column) (Pretzschi 2009).

The independent selection of several stands by silvicultural phases allows acquiring a measure of within silvicultural phase variance. This allows separating the intra-silvicultural phase variance from the inter-silvicultural phase variance. Finally, experimental design should take into account intra-stands variability by pseudo-replication in each stand (intra-stands variance) and inter-stands variability within each silvicultural phase by replication of stands (inter-stands variance or intra-silvicultural phase variability). Stand selection should minimize stand characteristic differences by

choosing neighboring stands in the same climate and altitude, on the same soil parent materials and with a similar topography, historic and management. Obviously, forest stands are open systems and are affected by past litter removal or by atmospheric nutrients input that can cause specific growth trends. But, unlike short-lived agricultural crops, forest stands cannot be investigated in climate chambers or phytotrons under controlled and managed environmental conditions. Instead, forest maturation research must accept the environmental influences at a given site and record local conditions and they can be taken into account in the analysis.

4.3. Humus forms succession: indices of long-term N changes

The initial studies suggesting forest soil functioning changes at the rotation scale focused on humic epipedon morphology; *i.e.* humus forms (Bernier and Ponge 1994; Ponge and Delhaye 1995; Aubert *et al.* 2004). Indeed the cyclic patterns of humus forms are strongly connected with soil fauna structure and activity and the life cycle of forest ecosystem (Bernier and Ponge 1994) (Figure 1.12).

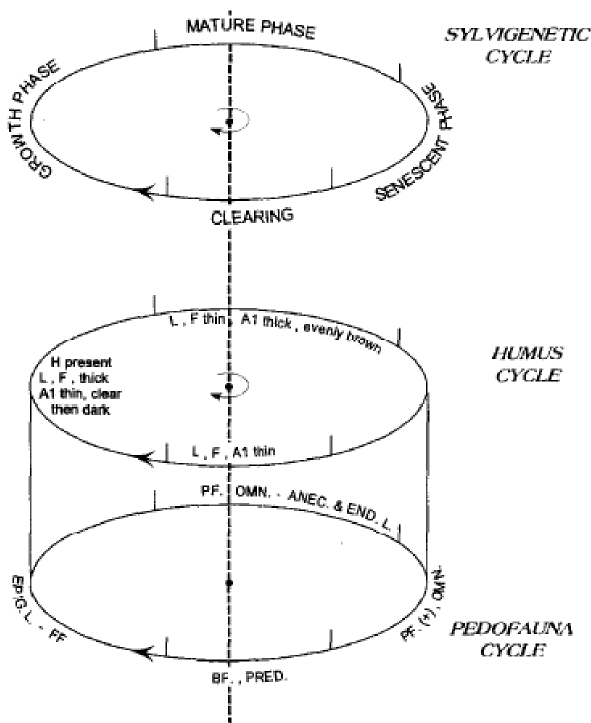


Figure 1.12. *Relationships between the sylvigenetic cycle and the edaphic cycle (humus forms and soil animal communities) in 'La Tillaie'.*
(BE, FE, PF., PRED., OMN., bacterial-, fungal-, and plant-feeders, predators and omnivores nematode trophic groups, respectively; ANEC., END., EPLL., anecic, endogeic and epigeic earthworm species, respectively) (Arpin et al. 1998).

Succession of humus forms along forest maturation was often been described using chronosequence analyses in temperate and mountain forests (Bernier and Ponge 1994; Ponge and Delhaye 1995; Aubert *et al.* 2004). Generally, mull humus form is associated with early developmental phase (regeneration), moder ones with intense trees growth phase, and mull recovers during maturity and senescence of trees. For instance, Arpin *et al.* (1998) observed thin OL and OF horizons, no white rot in litter and a thin light-colored A horizon in the clearing phase. The phase of intense growth and competition was characterized by thick OL and OF horizons, a thicker A horizon (colored at the surface and clear at the base) and an OH horizon. The mature phase was characterized by the presence of thin OL and OF horizons and a thick uniformly colored A horizon. Ponge (2003) explained these changes in humus forms by higher uptake of nutrients by trees during the phase of intense growth of trees for the build-up of wood inputs (Ponge 2003). This may lead to lower soil fertility affecting soil biological activity.

There is also a global trend of decreasing abundance of earthworms during the phase of intense growth of trees followed by progressive recovering as trees reach maturity then senesce (Bernier and Ponge 1994; Ponge 2003). Ponge (2003) considered this process as the driving force for the observed changes in humus forms. Furthermore, it was suggested that humus forms can be used as indicators of soil functioning since they indicate the rate at which nutrients are circulating within terrestrial ecosystems (Fig. 1.13) (Ponge 2003). Consequently, early developmental stages have been associated with fast OM turnover (translating into mull humus forms) while old mature stands have been frequently associated with lower OM recycling (expressed as moder occurrence) (Ponge 2003; Salmon *et al.* 2006). For instance, Arpin *et al.* (1998) supposed that the passage to the thin light-colored A horizon observed in clearings can be explained by a probable increase in the mineralization rate of OM in the A horizon.

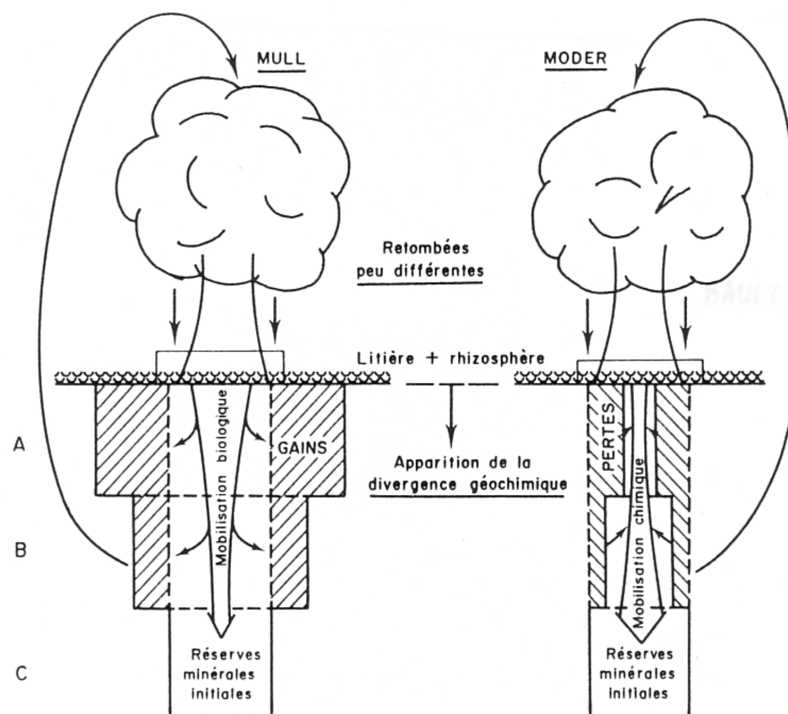


Figure 1.13. *Nutrient cycling under mull versus moder humus forms (Toutain 1974).*

In parallel, some studies indicated that changes in humus forms are associated with great changes in soil N processes (Bottner *et al.* 1998; Hirobe *et al.* 2003). For instance, among seven humus forms sampled in coniferous forest sites along a north-south climatic sequence in Western Europe, Bottner *et al.* (1998) observed the lowest potential net ^{15}N mineralization for the acid mor humus forms (Figure 1.14). In contrast, higher potential net nitrification was observed in mull humus forms (soils 6 and 7). Thus, humus form succession along forest maturation supposes strong changes in soil N mineralization pathways. Nevertheless, any works dealing with links between the morphology and the functional profiling of forest soils at the rotation scale were found up to now.

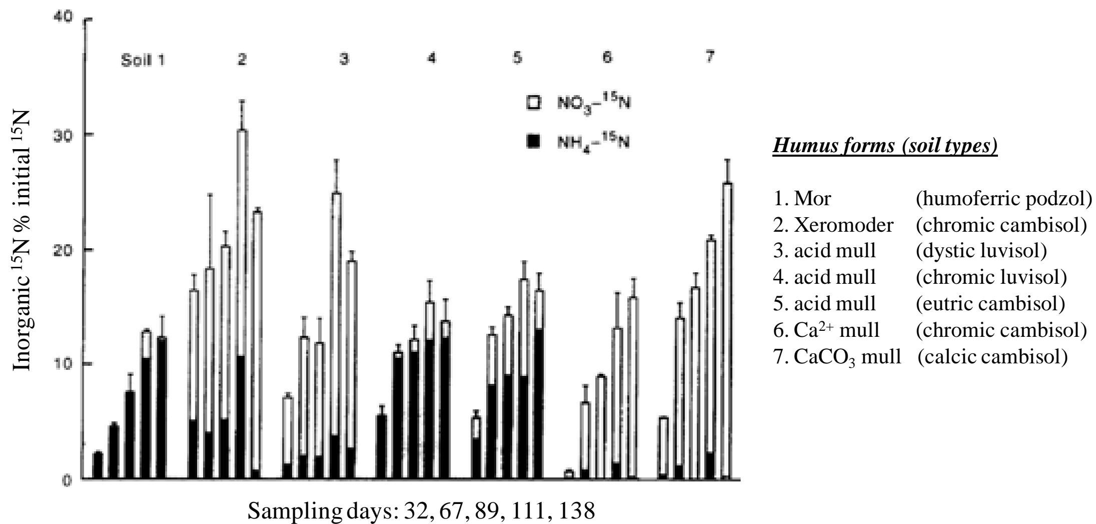


Figure 1.14. *Labelled inorganic N (NH_4^+-^{15}N and NO_3^--^{15}N) as % of initial total ^{15}N on five sampling occasions. Bar = standard deviation (Bottner et al. 1998).*

4.4. Changes in soil N cycle along forest maturation

Soil N fluxes variability along forest maturation has already been studied (Table 1.2). Thirteen studies were reported in this chapter. Discrepancies between studies are numerous. No significant and robust pattern of variation in soil N fluxes at the rotation scale emerges from this review. For instance, Welke and Hope (2005) observed lower N mineralization in the forest floor of young (10-15 years) compared to mid-aged (50-65 years) and old (>85 years) stands of pure and mixed Douglas-fir and paper birch. In contrast, Coté *et al.* did not found any changes in N mineralization in the forest floor between age classes (50 *versus* 124 years). Brais *et al.* (1995) and Paré and Bergeron (1996) found sharp declines in forest floor nitrification rates with forest maturation. White *et al.* (2004) studied soil N availability along 87-yr-old aspen-dominated post-logging fires chronosequence. From the 20-yr-old stand, both net N mineralization and net nitrification increased progressively in the first 10 centimeters above the OH layer. They also observed higher N mineralization and nitrification rates immediately following disturbance. For instance, Coté *et al.* (2000) did not observed changes in N mineralization within the forest floor between stand age contrary to Trap *et al.* (2009) and Fisk *et al.* (2002). However, an increase in soil N mineralization and a decrease in nitrification along stands maturation have been frequently observed.

4.5. Sources of discrepancies

Discrepancies in N patterns of variation at the rotation scale between studies could come from differences in (i) forest types (boreal or temperate), climate and edaphic conditions, species (beech, oak, hornbeam, spruce, aspen, sugar maple, etc.), methods used (ex situ aerobic incubation, ex situ anaerobic incubation, buried bag method, core incubation technique, ^{15}N isotopic technique, etc.), silvicultural phases selected (seedling, sapling, immature, growing, mature phases), soil layers or forest management (coppice stands, high stands, plantation).

Table 1.2. *Studies focused on soil N transformations changes along forest maturation or between two-contrasted silvicultural phases.*

References	Sites and species	Soil layers	Methods	Stand age (years old)	Soil N patterns with stand ageing
Brais et al. (1995)	Boreal forest in northwestern Quebec *Aspen, paper birch and conifers (balsam fir/white spruce)*	Organic	Exchange resin	Post fire succession 27, 47, 75, 121, 144, 168, 194, 231	Constant ammonium concentrations Highest values of nitrate at age 27, decreased between 27 and 47, and increased in older stand
Clinton et al. (2002)	Pure mountain beech forest, Central South Island, New Zealand *Mounatin beech*	Organic 0-10 cm	Anaerobic incubation technique (40°C for 7 d) In situ incubation using cores and ^{15}N	10, 25, 120, >150	Higher N inputs by litter fall in the yougnest stands Higher net N mineralization in the mature stands Lower N storage in the forest floor in the mature stands No differences in N availability in the forest floor No differences in net nitrification in the forest floor and the mineral soil
Côté et al. (2000)	Boreal forest in northwestern Quebec *Aspen, paper birch and conifers (balsam fir/white spruce)*	Organic 0-10 cm	long-term incubation (282 d)	50, 124	No differences in N mineralization in the forest floor Higher N mineralization in the mineral soil in mature stand
Fisk et al. (2002)	Northern hardwood forest in the southwestern Upper Michigan, US *Sugar maple*	0-15 cm	^{15}N pool dilution technique Buried bag method Porous-cup tension lysimeters	60-80, 200-300	Two times higher gross N mineralization in mature stands No differences in gross nitrification and in situ N mineralization Higher microbial immobilization of mineral N in mature stands Longer N residence time in microbial pool in younger stands No differences in N leaching
Griffiths and Swanson (2001)	H.J. Andrews Experimental Forest, Oregon, US *Douglas-fir*	0-10 cm	Potential denitrification (25°C for 1h) Waterlogged technique	5, 15, 40	No differences in mineralizable N
Idol et al. (2003)	Central region of the US, Indiana *Mix of deciduous hardwood species*	0-30 cm	Field incubation core technique	1-3, 12-14, 31-33, 80-100	Higher N mineralization, nitrification and N uptake rates in the mature stands Decrease of nitrification rates at the onset of the chronosequence
Inagaki et al. (2004)	Shikoku district, southern Japan *Pine, cypress, cedar and mixed deciduous hardwood forests*	0-5 cm	28-day laboratory incubation	19-39, 43-67	Higher N mineralization in the oldest stands
Jussy et al. (2000)	Temperate forest, north-east Massif Central, France *Douglas-fir*	0-15 cm	Aerobic laboratory incubation Field incubation core technique	20, 40, 60	Higher *ex situ* and *in situ* N mineralization in the mature stands No differences in net nitrification
Pedersen et al. (1999)	Blodgett Forest Research Station, US *Mixed-conifer forest*	0-10 cm	^{15}N isotopic pool dilution technique	14, >100	No significant differences in gross mineralization rates on a mass basis Lower gross nitrification rates in mature stand
Trap et al. (2009)	Temperate forest, central France *Oak/hornbeam forest*	Organic 0-5 cm	28-day laboratory incubation	20, 45, 70, 100	Higher net N mineralization in mature stands but no changes in net nitrification in the forest floor No differences in N mineralization but lower nitrification values in mature stands in mineral layer
Welke & Hope (2005)	Southern interior British Columbia *Pure and mixed Douglas-fir and paper birch*	Organic	Buried bag incubation method 5 weeks of incubation 4 sampling times	10-25, 50-65, >85	No consistent significant differences at any sampling time Higher N mineralization and ammonium content in mature stand (sampling time pooled)
White et al. (2004)	Northern Lower Michigan, US *Bigtooth aspen*	0-10 cm	In situ buried bag incubation	Post disturbances succession 0, 18, 44, 50, 62, 87	Increase of N mineralization and nitrification from 18-year-old stands
Zeller et al. (2007)	Temperate forest, central France *Beech*	0-5 cm	Laboratory incubation at 15°C ^{15}N isotopic pool dilution technique	25, 150	Lower net and gross N mineralization in old stand

For instance, Welke and Hope (2005) used the buried bag incubation method to estimate N mineralization in the forest floor of young (10-15 years), mid-aged (50-65 years) and old (>85 years) stands of pure and mixed Douglas-fir and paper birch. In contrast, Zeller *et al.* (2007) used the ^{15}N dilution technique to compared N fluxes in a 150-year-old coppice and a 25-year-old beech plantation. Inagaki *et al.* (2004) worked on the first 5 centimeters of the mineral layer while Idol *et al.* (2003) sampled the first 30 centimeters. All these parameters (forest types, climate and clay, soil layers, species, etc.) are well known to affect soil N cycling and may cause divergences in the literature about N cycle patterns with stand ageing. As discussed previously, it is important to note that, among all studies reported in this chapter, only three used stands repetitions (n=3) per silvicultural phases as true repetitions (Clinton *et al.* 2002; Fisk *et al.* 2002; Trap *et al.* 2009). All the others experimental designs included intra-stand plot or transect repetitions as true repetition. This type of experimental design may constitute a substantial source of inaccuracy and a major shortcoming. Conclusions about N patterns of variation may therefore be nuanced. Also, studies focusing on soil N cycle changes with forest stand ageing are usually based upon comparing two contrasted silvicultural phases or stand age (Coté *et al.* 2000; Fisk *et al.* 2002; Inagaki *et al.* 2004; Zeller *et al.* 2007). Forest cycle is achieved by several successive functional soil changes which cannot be revealed using such sampling design. It appears central to explore soil N cycle dynamics at the full forest rotation scale.

5. Plant mechanisms to limit N loss along forest maturation-Hypotheses

Trees are an integral part of ecosystems N cycling since they uptake N, produce organic N and release it into forest soils. Empirical studies over the last 30 years have documented important influences of plant species on nutrients cycling, particularly for N, which is considered as limiting factors for plant growth (Aerts 1997b; Knops *et al.* 2002; Lovett *et al.* 2004). Here, I present the main hypotheses of trees mechanisms most likely implicated in the control of soil N availability at the scale of the forest cycle.

5.1. N Resorption Efficiency (NRE)

"Nutrient resorption" corresponds to the process by which nutrients are mobilized from senescent leaves and transported to others plant tissues. Resorption of N from senescing leaves enables trees to re-use and conserve this nutrient (Aerts 1996; Aerts 1997b; Cote *et al.* 2002; Sariyildiz and Anderson 2005). N resorption from senescing leaves is an important adaptation of plants to infertile soils since it reduces N loss and increase N-use efficiency (NUE). This important feedback mechanism of N re-absorption from leaves before abscission can amount more than the half N content in leaf (Eckstein *et al.* 1999; Cote *et al.* 2002). Enoki and Kawaguchi (1999) examined the N resorption efficiency (percentage change of N content between green and senescent needles) in needles of *Pinus thunbergii* Parl. trees growing at 5 positions along a slope. N resorption efficiencies increased upslope from 43 to 77% with decreasing soil N availability. There are supports for the hypothesis that on the intra-specific level, mature trees show higher leaf N resorption efficiency compare to younger ones (Nordell and Karlsson 1995; Eckstein *et al.* 1999). Total N inputs into the forest floor may thus change according to the age of trees and affect differently soil N cycle. Unfortunately, this mechanism was poorly studied along forest maturation although it may provide new insights on trees control on soil N availability.

5.2. Litter quality

Trees species may affect soil N functioning by producing different litter quality and especially polymeric compounds (Scott and Binkley 1997; Schweitzer *et al.* 2004). It is well known that tree species produce different litter quality with great impacts on soil N functioning (Table 1.3a and b). Usually, early successional species such as birch (*Betula pendula*) produce litter with higher quality (high N content, lower lignin content) than late successional species such as Beech (*Fagus sylvatica*) or conifers (Wardle 2005; Hobbie *et al.* 2006). Secondary metabolites such as lignin are difficult to break down by decomposers retarding rates of decomposition and N mineralization (Satti *et al.* 2003). At present, the lignin/N ratio is the most frequently recommended indicator of the relationships between litter quality and soil N dynamics at individual sites. Besides inter-specific variability, within-species variability in woody litter quality have also been observed (Sanger *et al.* 1996; Sanger *et al.* 1998; Inagaki *et al.* 2004) and often related to differences in site quality (soil water and nutrient availability and climate conditions). For instance, Cordell *et al.* (1998) showed, using a common garden experiment, that differences in foliar N content among *Metrosideros polymorpha* Gaud. populations are due in large part to water and nutrient availability among sites.

Table 1.3a. *Litter nutrient for 14 species grown in a common garden experiment* (Hobbie *et al.* 2006).

Species	Nutrients (mg/g)											
	C/N		N		Ca		K		Mg		P	
Abies alba	41.2	(4.1)	12.5	(1.3)	12.4	(1.1)	2.2	(0.4)	0.8	(0.1)	1.18	(0.07)
Acer platanoides	68.9	(4.8)	6.5	(0.4)	19.0	(1.7)	6.6	(0.9)	1.6	(0.1)	1.29	(0.05)
Acer pseudo-platanus	49.1	(6.2)	9.4	(1.1)	21.8	(2.3)	6.9	(0.8)	1.6	(0.2)	1.16	(0.24)
Betula pendula	38.5	(1.1)	12.6	(0.2)	11.5	(0.4)	3.2	(0.6)	2.4	(0.1)	1.61	(0.02)
Carpinus betulus	42.7	(2.0)	11.0	(0.5)	9.2	(0.5)	3.9	(0.8)	1.5	(0.1)	1.85	(0.05)
Fagus sylvatica	55.9	(3.6)	8.4	(0.5)	12.9	(0.5)	4.2	(1.0)	1.1	(0.1)	1.41	(0.15)
Larix decidua	64.9	(10.1)	8.7	(1.3)	6.9	(0.7)	1.5	(0.3)	1.4	(0.1)	1.39	(0.11)
Picea abies	49.3	(2.5)	9.9	(0.4)	11.1	(0.5)	3.4	(0.2)	0.8	(0.0)	1.10	(0.12)
Pinus nigra	98.7	(13.9)	5.4	(0.9)	3.1	(0.5)	2.3	(0.1)	0.5	(0.1)	0.51	(0.09)
Pinus sylvestris	81.9	(15.9)	6.8	(1.4)	5.4	(0.5)	2.0	(0.2)	0.5	(0.0)	0.62	(0.13)
Pseudotsuga menziesii	72.0	(3.0)	7.2	(0.3)	9.1	(0.7)	2.9	(0.2)	1.1	(0.0)	1.15	(0.11)
Quercus robur	37.7	(5.1)	12.7	(0.6)	12.0	(0.5)	5.0	(0.7)	1.8	(0.1)	1.70	(0.09)
Quercus rubra	68.7	(5.5)	7.1	(0.5)	11.8	(0.6)	3.1	(0.6)	1.4	(0.1)	1.38	(0.05)
Tilia cordata	37.0	(1.3)	12.2	(0.1)	18.8	(0.8)	4.0	(1.1)	1.8	(0.0)	1.47	(0.08)

(SE)

5.3. Mycorhizal fungi – positive N feedbacks

Mycorhizal fungi, which differ widely in function and in associated host plant, could mediate the influence of plant species on soil N cycling. The development of mycorhizal fungi increases the ability of roots to absorb nutrients such as organic N from the soil. The degree to which this ability is enhanced depends on the mycorhizal colonization. Colonization by mycorhizal fungi can increase the nutrient absorbing surface area of a plant by a factor of 60 (Simard *et al.* 2002; Simard and Durall 2004). Knops *et al.* (2002) considered that the majority of the N contained in litter is incorporated into the soil organic matter (SOM) pool (Fig. 1.15a). In this model, N from plant litter must pass through the SOM pool before it is available to plants.

Table 1.3b. *Litter C fractions for 14 species grown in a common garden experiment* (Hobbie *et al.* 2006).

Species	C fractions (mg/g ash-free dry mass)									
	Solubles		Cellulose		Hemicellulose		Lignin		Ash	
Abies alba	370.1	(36.5)	199.9	(5.9)	123.4	(2.9)	304.6	(27.6)	3.4	(0.7)
Acer platanoides	530.6	(30.5)	180.2	(14.3)	160.8	(9.8)	125.9	(6.2)	16.1	(4.6)
Acer pseudo-platanus	524.2	(5.9)	184.3	(9.8)	122.7	(2.7)	166.8	(7.9)	16.7	(1.8)
Betula pendula	278.0	(31.9)	186.8	(1.9)	124.4	(6.2)	408.7	(39.7)	57.1	(22.0)
Carpinus betulus	496.2	(20.1)	200.8	(6.8)	155.3	(5.1)	143.6	(16.0)	26.8	(2.9)
Fagus sylvatica	381.5	(26.3)	231.9	(12.9)	138.8	(8.1)	245.7	(18.9)	13.2	(3.3)
Larix decidua	365.5	(21.3)	222.0	(9.3)	92.6	(5.1)	317.4	(18.0)	3.7	(0.8)
Picea abies	414.6	(15.6)	231.3	(12.3)	138.1	(2.4)	214.1	(6.0)	4.8	(1.3)
Pinus nigra	289.7	(7.2)	316.0	(3.3)	153.8	(6.6)	238.7	(11.3)	2.9	(0.4)
Pinus sylvestris	389.3	(7.1)	298.8	(7.2)	133.2	(5.6)	176.6	(7.2)	2.6	(0.4)
Pseudotsuga menziesii	455.6	(9.0)	182.2	(5.3)	112.3	(3.3)	247.6	(6.6)	4.7	(0.7)
Quercus robur	388.5	(13.0)	222.3	(6.2)	153.7	(5.6)	233.5	(15.0)	14.4	(2.4)
Quercus rubra	417.8	(6.4)	232.2	(1.6)	149.6	(3.2)	198.4	(1.0)	9.5	(1.2)
Tilia cordata	270.9	(46.4)	193.6	(11.3)	115.5	(4.1)	417.1	(41.0)	94.0	(31.1)

(SE)

Chapman *et al.* (2006) considered that mycorhizal fungi can alter N cycling and contribute to plant-litter feedbacks by efficiently accessing mineral N, and by actively releasing N from OM (Zeller *et al.* 2000) (Fig. 1.15b). Also, the microbial bottleneck proposed by Knops *et al.* (2002) is potentially bypassed by the ability of trees and their associated mycorhizal fungi to exploit organic N (Nasholm *et al.* 1998; Schimel and Bennett 2004; Nasholm *et al.* 2009) (Fig. 1.15b). Indeed, mycorhizal fungi may facilitate the uptake of simple organic molecules such as amino acids (Hodge *et al.* 2000).

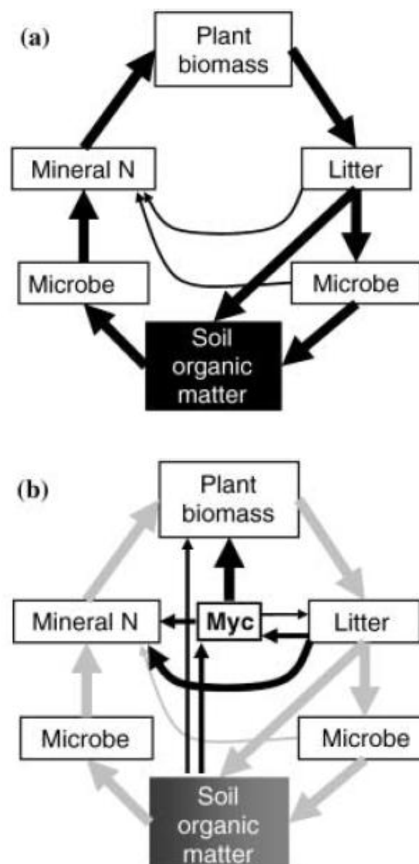


Figure 1.15. *Two representations of the N cycle. In each case, arrows represent N fluxes, thickness is approximately proportional to the magnitude of the flux.*
(a) The N cycle, as described by Knops et al. (2002)
(b) Chapman et al. (2006) representation including several superimposed fluxes (in bold) that create a tighter, plant-oriented loop that allows for plant litter-mediated feedback.
Myc, mycorrhizas. (Chapman et al. 2006).

Organic N uptake allows plants to access directly the N contained within their own litter. Wardle (2005) and Aerts (2002) discussed on the assumption that the quality of litter and the saprotrophy of mycorhizal symbionts can function together promoting positive N feedback. Indeed, several studies provide evidence of an intricate N feedback where plant litter chemistry influences the cycle of N to maximize N acquisition by the host's mycorhizal roots while hindering microbial N acquisition (Aerts 2002; Neff *et al.* 2003; Schimel and Bennett 2004; Wurzburger and Hendrick 2009).

Such positive N feedback has been proposed in ecosystems where plants produce litter with high levels of phenolic compounds, which are effective at tightly binding N in protein (Jones and Hartley 1999; Wurzburger and Hendrick 2009). Northup *et al.* (1995) suggested that ectomycorhizal fungi can effectively take up the N that is bound to phenolics in plant litter. The promotion along forest ageing of soil fungal-based energy channel by producing different litter quality may favor root colonization by mycorhizal fungi leading hence to positive N feedbacks. If such N feedback occurs it should derive a competitive advantage of trees over microbial species along trees ageing. This idea required further scrutiny and validation in different forest ecosystems.

6. Conclusion

The perception of the functioning of soil nitrogen (N) cycling in forest ecosystem has undergone considerable shifts during these last ten years (Schimel and Bennett 2004; Chapman *et al.* 2006; van der Heijden *et al.* 2008).

-(1) The critical point in the N cycle is the depolymerization of complex N molecules to easily available monomers such as amino acids or nucleic acids (Schimel and Bennett 2004).
-(2) It was recognized that trees species may take up organic monomers (dissolved organic N) and not exclusively mineral N (Nasholm *et al.* 1998; Nasholm *et al.* 2009).
-(3) Trees actively compete for N with microbes and are not a priori inferior in this competition (Schimel and Bennett 2004).
-(4) Trees may actively control N processes in soils by microbes (Paavolainen *et al.* 1998; Chapman *et al.* 2006; van der Heijden *et al.* 2008). This control occurs via diverse pathways including litter inputs and quality, roots exudates and turnover, mycorhizal species, uptake of nutrients and water (Jones *et al.* 1997; Schimel and Bennett 2004).

Long-term changes in soil N cycle along forest maturation have received little attention to date (Coté *et al.* 2000; Zeller *et al.* 2007; Trap *et al.* 2009) while humus forms changes are a sign of shifts in soil organic matter turnover along forest ageing. It is thus a serious shortcoming since trees N supplies, microbial N requirements and the interactions between trees and soil microbes, competitive and/or beneficial, are likely to change with forest maturation and to influence differently soil N fluxes. No robust pattern of soil N cycling emerges from our review. This may come from different methods and/or experimental design. For instance, studies focusing on soil N cycle changes with forest stand ageing are usually based upon comparing two contrasted silvicultural phases or stand age (Coté *et al.* 2000; Fisk *et al.* 2002; Inagaki *et al.* 2004; Zeller *et al.* 2007). Silvicultural and/or sylvigenetic cycle are achieved by several successive functional soil changes which cannot be revealed using such sampling design. The study of long-term changes in soil N cycle in forest ecosystems may provide new insights in our knowledge of soil N cycle and on controlling ecological factors.

AXE I

LES PATRONS DE VARIATIONS DU CYCLE INTERNE DE L'AZOTE LE LONG D'UNE CHRONOSEQUENCE DE 130 ANS DE HÊTRES

INCUBATION *EX SITU* – MAI 2007

CHAPITRE 2
Approche *ex situ*

I. Présentation du chapitre 2

La problématique de cette étude repose sur le constat suivant : au cours de la maturation des peuplements forestiers, la morphologie de l'épisolum humifère évolue depuis les mull sous les jeunes peuplements jusqu'à des formes d'humus de type MODER sous les peuplements matures. Or les formes d'humus seraient indicatrices du fonctionnement de l'épisolum humifère en termes de recyclage de la matière organique. Ce constat suppose que le turnover de la matière organique et des éléments majeurs au sein de l'épisolum humifère ralentit le long de la maturation des peuplements forestiers. L'objectif de cette étude était de tester cette hypothèse en caractérisant simultanément les formes d'humus et la production d'azote minéral au sein de l'épisolum humifère, le long d'une chronoséquence de hêtraie pure de 130 ans. Sur le terrain, 36 variables macro-morphologiques ont été déterminées et les différents horizons de l'épisolum humifère ont été échantillonnés. Nous avons utilisé la méthode d'incubation au laboratoire durant 28 jours décrite par Hart *et al.* (1994) pour caractériser le cycle interne de l'azote. Nous avons également couplé la méthode d'incubation *ex situ* avec l'utilisation d'un inhibiteur sélectif, l'acétylène (inhibiteur de la nitrification autotrophe) et d'un biocide, le captan (fongicide) afin d'identifier les microorganismes responsables de l'ammonification et de la nitrification. Enfin, les patrons de co-variations entre les formes d'humus et le cycle de l'azote ont été appréhendés à l'aide d'analyses statistiques.

II. Article 1

Changes in humus forms and soil N pathways along a 130-yr-old pure beech forest chronosequence

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Abstract

At the rotation scale, forest soil functioning has been mostly assessed through physical (structure, bulk density) and chemical (pH, CEC and majors nutrients contents) properties of humus forms. In this study, we investigated changes in organic and organo-mineral net N mineralization pathways along a pure beech even-aged high forest chronosequence composing by four stages (15, 65, 95 and 130 years old) on loamy acidic soil (Luvisol). We used a selective inhibitor (acetylene) and a fungicide (captan), to quantify the respective contributions of autotrophic, heterotrophic bacteria and fungi to soil ammonification and nitrification. We analyzed the data in order to highlight correlations patterns between soil N mineralization pathways and humus morphology. We sampled OL layer (unmodified leaf fragments), FH layer (mixture of coarse plant fragments with fine organic matter) and A layer. The data showed that within the organic layers, net N mineralization was two times higher in matures stands than in younger ones. In contrast, in the A layer, net N mineralization did not change significantly. Net nitrification decreased sharply within the OL and the A layers after 65 years of stand ageing. It was mainly localized in the FH layer. The relative contributions to ammonification of bacteria and fungi remained constant along the chronosequence in both the OL and the A layers. However, in the FH layer, fungal contribution to ammonification increased under the 95 and 130 years old stands (*i.e.* from 100% bacterial at the onset of the chronosequence to 30% fungal and 70% bacterial under matures stands). Both autotrophic and heterotrophic nitrifications were detected in the OL layer of 15 years old stands, but all of the nitrifying activity in the FH and the A layers was autotrophic. We observed strong correlations between morphological variables (*e.g.*. the percentage of fragments in OLv, the thickness of OLv and OF layers) and soil N transformations. Morphological variables potentially indicators of N mineralization pathways may not necessary constitute the most important criteria responsible for the segregation of humus forms along the chronosequence. Further research developments should aim at calibrating a humus-based ecological index of N mineralization pathways.

Keywords

Potential net ammonification, potential net nitrification, bacterial ammonification, fungal ammonification, autotrophic nitrification, fungal nitrification, humus forms, chronosequence, *Fagus sylvatica*, loamy acidic soil.

1. Introduction

Nitrogen (N) cycle in forest soils can be described as an internal cycle including mineralization (*i.e.* convertion of organic N to mineral N), nitrification, immobilization by microorganisms, root uptake, and litter turnover (Hart et al. 1994; Schimel and Bennett 2004). Mineral N production has long been recognized as a central soil process to tree N supply (Kaye and Hart 1997; Vitousek et al. 1997; Schimel and Bennett 2004). It includes ammonification, *i.e.* the biotic conversion of organic N to ammonium, and nitrification, *i.e.* the biotic conversion of ammonia to nitrate (Hart et al. 1994; Priha and Smolander 1999).

Ammonification and nitrification processes are performed by many groups of organisms including bacteria, fungi and archaea (De Boer and Kowalchuk 2001; Nicol and Schleper 2006; Boyle et al. 2008; Hayatsu et al. 2008). Nitrate production from ammonium oxidation is performed by autotrophic and heterotrophic organisms including bacteria, fungi and archaea. In contrast, the production of nitrate from organic N is carried out by heterotrophic organisms (bacteria and fungi) (De Boer and Kowalchuk 2001; Leininger et al. 2006; Islam et al. 2007; Hayatsu et al. 2008).

Usually, two nitrification pathways were distinguished, *i.e.* the autotrophic and the heterotrophic pathways. Autotrophic nitrification is carried out by ammonia-oxidizing bacteria or AOB (*Nitrosomonas*, *Nitrosospira* and *Nitrosococcus*) and nitrite-oxidizing bacteria or NOB (*Nitrobacter*, *Nitrospina*, *Nitrococcus* and *Nitrospira*). Heterotrophic nitrification is carried out by a wide phylogenetic range of bacteria (heterotrophic bacterial nitrification) and fungi (fungal nitrifrication) that can oxidize ammonia or reduced N from organic compounds to nitrate (De Boer and Kowalchuk 2001). Thanks to the use of selective inhibitors and biocides in soil N studies, both heterotrophic nitrification, bacterial or fungal (Lang and Jagnow 1986; Brierley and Wood 2001), and autotrophic nitrification (Ste-Marie and Paré 1999; Laverman et al. 2000) have already been detected in forest soils. However, in contrary to autotrophic, heterotrophic nitrification is considered to play a significant role in acidic forest soils (Brierley and Wood 2001).

Although N has been considered as the growth limiting factor in forests ecosystems (Hayatsu et al. 2008), the long-term changes in soil N transformations and it relationships with humus forms along forest maturation have been rarely considered (Jussy et al. 2000). Usually, studies focusing on N cycle changes with forest stand ageing are based upon a broad comparison between two contrasted silvicultural phases or stand ages (Pedersen et al. 1999; Coté et al. 2000; Fisk et al. 2002; Inagaki et al. 2004; Zeller et al. 2007). Yet, that kind of sampling design is by far too simplistic to highlight the whole complexity of the functional changes that may occur in soil during a complete silvicultural and/or sylvigenetic cycle. It is a serious shortcoming since trees N requirements, microbial communities and trees-microbes interactions either competitive and/or mutually beneficial, are likely to vary along forest maturation and to influence soil N cycle (Brais et al. 1995).

At the scale of the cyclic forest dynamics, soil functioning has been mostly assessed through the morphological properties of humus forms (Bernier and Ponge 1994; Ponge and Delhaye 1995; Jabiol et al. 2009). Successional patterns of humus forms have often been described along empirical forest chronosequence in both managed (Aubert et al. 2004) and semi-natural systems (Bernier and Ponge 1994; Ponge and Delhaye 1995). Early developmental stages have been often associated with mull (thin litter horizon, absence of humified horizon, presence of earthworm casts and thick

organo-mineral horizon) while moders (thick organic layers with humified horizon, higher proportion of litter fragments and mesofauna faeces and thin organo-mineral soil layer) have been frequently observed under old mature stands on acidic forest soils (Ponge 2003; Salmon et al. 2006). Bottner *et al.* (1998) showed significantly different soil N transformations between seven humus forms sampled under coniferous forests across a climatic sequence in Western Europe. This statement is supported by Hirobe *et al.* (2003) who show different influences of mull and moder on the N functioning in acidic soils planted with *Cryptomeria japonica* in Japan.

We can hence hypothezise that the shift from mull toward moder occurring along forest maturation is paired with substantial changes in both soil N transformations and in the nature of N pathways (Ponge and Delhaye 1995; Aubert et al. 2004). Namely, mull in younger stands should be paired with high soil N mineralization rates mainly performed by bacteria while the appearance of moder in older stands should favor lower soil N fluxes achieved by fungi (Ponge and Delhaye 1995; Gobat et al. 2004). In this study, we assessed the dynamics and co-variation patterns of soil N mineralization and humus macro-morphology along a 130-year chronosequence of pure beech forest. We used an original experimental approach to assess the respective contributions of bacteria (autotrophs *versus* heterotrophs) and fungi to ammonification and nitrification in both organic and organo-mineral horizons.

2. Materials and methods

2.1. Site description and sampling design

The study was carried out in the Eawy forest (France, Upper Normandy region, 7200 ha). The climate is temperate oceanic with a mean annual temperature of +10°C and a mean annual pluviometry of 800 mm (Hedde 2006). A space-for-time substitution procedure was used to empirically reconstitute a forest chronosequence (Pickett 1989). Sixteen pure beech (*Fagus sylvatica* L.) stands were selected within the Eawy forest (Table 2.1). All of them were managed as even-aged forest by the French Forestry Service (ONF). Site historic was fully described by hedde (2006). The selection included four silvicultural phases of different ages: 13-18 years (SP15), 65-66 years (SP65), 91-103 years (SP95) and 121-135 years (SP130). Each phase comprised 4 true replicate stands. All stands were situated on a flat topographic situation (plateau). Soil was an endogleyic dystric Luvisol (FAO 2006) developed on more than 80 cm of loess (lamellated silts) lying on clay with flints (Lautridou 1985; Laignel et al. 1998). Understory vegetation was defined as a characteristic *Endymio-Fagetum* according to the phytosociological classification (Durin et al. 1967). In each stand center, a 16 m^2 square plot was delimited away from vehicle tracks and tree trunks to avoid any acidification due to organic matter accumulation (Beniamino et al. 1991).

2.2 Organic and organo-mineral horizons morphology

Macro-morphological descriptions of organic and organo-mineral layers were previously done within frames (25 cm x 25 cm) at three corners of the central plot according to the French nomenclature (Jabiol et al. 2007) in May 2007. A total of 36 macro-morphological variables were described in the field on the basis of variation visible to the naked eye (Table 2.2). We distinguished mull (mainly dysmull) and moder (hemimoder + eumoder + dysmoder) humus forms on the base of morphological characters (Jabiol et al. 2007). A total of 48 humus profiles were described (3 descriptions per stand × 16 stands).

Table 2.1. *Main characteristics of stands used to reconstitute the 130-yr-old pure beech forest chronosequence on loamy soils (Upper Normandy, France).*

Silvicultural phases	**SP15**				**SP65**				**SP95**				**SP130**			
Stands	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Age in 2009 (years)	15	20	15	15	67	67	67	68	105	93	103	93	137	137	123	123
Last year cut	2004	2003	2003	2003	2003	2003	2003	2004	2003	2004	2002	2004	2002	2003	2004	2004
Area (ha)	14.52	8.03	4.57	4.45	10.84	9.46	9.24	12.1	4.52	16.48	18.06	13.47	3.56	13.81	16.25	18.7
Basal area (m²)	15	21	22	17	26	30	27	30	20	29	30	22	20	23	20	24
% Beech (G/ha)	100	100	100	100	100	100	100	90	90	100	100	100	90	90	100	100
Humus forms[a]	Du	Du	H	Du	E	E	E	H	Do	E	E	H	E	Do	E	E
Vertical sequence[b]																
OLn	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
OLv	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
OF	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
OH					x / (x) <1cm	x / (x) <1cm	x / (x) <1cm		x >1cm	x / (x) <1cm	x / (x) <1cm		x / (x) <1cm	x >1cm	x / (x) <1cm	x / (x) <1cm
A structure[c]	BM	BM	J	BM	J	J	J	J	J	J	J	J	J	J	J	J
Topsoil pH[d]	4.47	3.83	3.56	3.99	3.87	3.83	3.91	4.24	3.80	3.85	3.83	3.72	3.83	3.97	3.73	3.91
Topsoil C/N[d]	15.1	16.6	17.8	15.7	16.3	15.4	16.9	15.0	15.5	15.4	18.5	15.9	15.4	15.8	15.0	14.8
Topsoil P (g kg)[d]	0.13	0.23	0.18	0.16	0.18	0.12	0.10	0.20	0.18	0.21	0.22	0.23	0.48	0.12	0.12	0.15
Topsoil CEC (cmol+ kg)[d]	5.21	6.19	7.43	6.95	5.69	5.66	4.99	5.25	5.74	5.56	7.09	5.60	6.17	5.71	5.28	5.75

[a] With Du : Dysmull; H : Hemimoder; E : Eumoder; Do : Dysmoder (Jabiol et al. 2007)

[b] With OLn : unmodified leaf less than one year old, OLv : unmodified leaf more than one year old, OF : coarse plant fragment with fine organic matter (FOM), OH : more than 70% FOM, A: organic-mineral horizon according to Jabiol et al. (2007). x : continuous; (x) : discontinuous

[c] BM : Biomacrostructured; J : Juxtaposition A (A with massive or single-grain structure but no biological or chemical structure)

[d] Data are means (n=3)

We differentiated:

(1) **the OL horizon** consisting of almost unmodified leaves and woody fragments. Most of the original plant structures are easily discernible. Leaves may be recently fallen and unmodified (OLn) or bleached and slightly fragmented (OLv);

(2) **the OF horizon** consisting of a mixture of coarse plant debris with fine organic matter (humus). This horizon is characterized by an accumulation of partly decomposed organic matter derived mainly from leaves and woody materials, which are fragmented and mixed with invertebrate faeces. The proportion of organic fine substances ranges from 10 to 70 % by volume;

(3) **the OH horizon**. This horizon is an organic horizon characterized by an accumulation of decomposed organic matter. The original plant structures and materials are not discernible. Organic fine substance (invertebrate faeces) accounts for more than 70 % by volume. The OH horizons differs from the OF horizon by more advanced humification due to the action of soil organisms;

(4) **the A horizon** or **organo-mineral horizon** different in depth and structure among humus forms.

2.3. Soil layers sampling

We sampled the OL, OF, OH and A layer in May 2007 within frames (25 cm x 25 cm) in the same three corners of the central plot. Because the OH layer was sometimes discontinuous and not enough abundant, we decided to mix OF and OH layers in a single pool called FH layers. The sum of organic layers and organo-mineral horizon correspond to the “humic epipedon” (Jabiol et al. 2009). A total of 144 samples (3 subsamples x 3 soil layers x 4 silvicultural phases x 4 stands for each phases) were collected. Samples were stored at 4°C for the transport (Forster 1995b). In the laboratory, leaves were roughly cut, brushwood, stones and large roots were removed and the A layer was sieved at 2 mm.

2.4. Soil incubations

Aerobic incubations were conducted for each sample by introducting 15 g of OL, 15 g of FH and 35 g of A in sealed tight glass beakers (500 ml) that were placed for 28 days in the dark at 28°C according to Hart et al (1994). The A moisture was adjusted at 85% of its maximum water holding capacity and the organic material was adjusted to 75% of fresh weight before the incubation (Pansu and Gautheyrou 2003). Beakers were frequently opened and aerated to avoid anoxic conditions. In an attempt to discriminate bacterial from fungal potential net ammonification and autotrophic from heterotrophic (bacterial and/or fungal) potential net nitrification, three incubation treatments were used. The first treatment corresponded to control (treatment C) without biocide nor selective inhibitor addition. The second treatment was performed with acetylene addition (treatment A). Acetylene is the most commonly applied specific inhibitor of autotrophic ammonia oxidation and is very useful to distinguish autotrophic to heterotrophic nitrification (Hynes and Knowles 1978; Bédard and Knowles 1989; De Boer and Kowalchuk 2001). Heterotrophic nitrification is not affected by acetylene at low concentrations (2% v/v) (De Boer and Kowalchuk 2001). The third and last treatment consisted in combining 2% acetylene with a fungicide (treatment AF) in order to inhibit both chemolitho-autotrophic nitrification and fungal activity simultaneously.

Table 2.2. *Morphological variables codes and modalities.*

Soil layers[a]	Variables	Codes	Modalities	Rank 1[b]	Rank 2[c]
OLn	Leaves fragment	OLnfg	Percentage	31	14
	Skeletonized leaves	OLnsk	Percentage	13	30
	Macrofauna faeces	OLnMfae	Nomber per square meters	5	35
OLv	Maximal thickness	OLvMt	Centimeters	16	5
	Minimal thickness	OLvmt	Centimeters	20	3
	Leaves fragments	OLvfg	Percentage	32	28
	Skeletonized leaves	Olvsk	Percentage	19	1
	Macrofauna faeces	OLvMfae	Nomber per square meters	21	25
	Bleach leaves	OLvBl	Percentage	33	6
	Brown leaves	OLvBr	Percentage	4	16
	Recovery	OLvrec	Percentage	28	24
	Compacted leaves	OLvCom	From 1 to 4 (1: no compact, 2: slightly compact, 3: moderately compact, 4: strongly compact)	17	21
	Mycelium	OLvmy	From 0 to 3 (0: absent, 1: rare, 2: moderately present, 3: abundant)	11	32
	Living roots	OLro	From 0 to 3 (0: absent, 1: rare, 2: moderately present, 3: abundant)	35	23
OF	Maximal thickness	OFMt	Centimeters	8	18
	Minimal thickness	OFmt	Centimeters	14	11
	Leaves fragment	OFfg	Pourcentage	30	8
	Fine organic matter	OFfog	Pourcentage	23	26
	Macrofauna faeces	OFMfae	Nomber per square meters	2	33
	Bleach leaves	OFBl	Pourcentage	10	13
	Recovery	OFrec	Pourcentage	36	27
	Compacted leaves	OFCom	From 1 to 4 (1: no compact, 2: slightly compact, 3: moderately compact, 4: strongly compact)	27	10
	Mycelium	OFmy	From 0 to 3 (0: absent, 1: rare, 2: moderately present, 3: abundant)	29	2
	Living roots	OFro	From 0 to 3 (0: absent, 1: rare, 2: moderately present, 3: abundant)	26	32
OH	Maximal thickness	OHMt	Centimeters	7	7
	Minimal thickness	OHmt	Centimeters	15	12
	Fine organic matter from fauna faeces	OHffog	Percentage	2	22
	Vegetable fine organic matter	OHvfog	Percentage	3	20
	Recovery	OHrec	Percentage	6	4
	Mycelium	OHmy	From 0 to 3 (0: absent, 1: rare, 2: moderately present, 3: abundant)	25	17
	Living roots	OHro	From 0 to 3 (0: absent, 1: rare, 2: moderately present, 3: abundant)	1	19
A	Maximal thickness	AMt	Centimeters	14	9
	Minimal thickness	Amt	Centimeters	22	15
	Agregate size	Aas	Millimeters	9	36
	Structure	Ast	From 1 to 4 (1: without aggregate, 2: slightly aggregate, 3: moderately aggregate, 4: strongly aggregate)	18	29
	Living roots	Aro	From 0 to 3 (0: absent, 1: rare, 2: moderately present, 3: abundant)	34	34

[a] According to Jabiol et al. 2007. OLn: New. OLv: slightly altered.

[b] The rank of the variable according to its R^2 value on the first axis of the PCA1.

[c] The rank of the variable according to its R^2 value on the second axis of the PCA1.

We used captan (N-(trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide, IUPAC) which is a common nonspecific fungicide with limited non-target effects on soil fungi relative to other fungicides (Ingham *et al.* 1991). At low concentrations (1 g kg^{-1}), captan have few effects on bacterial biomass and activity (Martinez-Toledo 1997; Bailey et al. 2003; Piotrowska-Seget et al. 2008; Trap et al. 2009).

In both treatments A and AF, acetylene was generated by adding water to calcium carbide in scintillation flasks. Final acetylene concentration reached 2% (v/v). Acetylene concentration was checked at the beginning and the end of the incubation experiment using Gas Chromatograph equipped with a flame ionization detector (Girdel 30 GC, Spherosil XOB 75 column, France). Captan is a broad-spectrum contact fungicide that has little effect on soil bacteria (Bailey et al. 2002). Captan was applied by a fine spraying while soil mixing (Zhao et al. 2005). Two applications of captan were made, the first before the incubation and the second 14 days after incubation began. Final captan concentrations reached 125 mg kg^{-1} for the A material in order to approximate application rates usually used in field experimentations, and 320 mg kg^{-1} in the organic material to maximize the inhibition (Bailey et al. 2002; Zhao et al. 2005; Trap et al. 2009).

2.5. Inorganic N extraction and quantification

At the beginning and at the end of incubation, an aliquot of 5g (organic material) or 10g (A layer) of each sample was placed in beakers with K_2SO_4 (0.2 M) solution (200 or 100ml for organic and A material, respectively) and shacked for 1 hour at 100 rev min^{-1} (Alef 1995; Forster 1995a). The obtained extractions were filtered through Schleicher & Schuell 0790 ½ filter papers and frozen for analyzed mineral N pools. Filters were pre-leached with 0.2 M K_2SO_4 in order to avoid any ammonium and/or nitrate contamination. Concentrations of NH_4^+-N and NO_3^--N were determined colorimetrically (AA3, BRAN+LUEBBE, Norderstedt, Germany). An aliquot each sample was dried at 105°C for 24 hours for A layer and 65°C for 48 hours for organic material to obtain the gravimetric water content. An other aliquots of each soil layer were air-dried and total carbon and nitrogen were measured by gas chromatography with a CHN pyrolysis micro-analyzer (Flash 2000 Series, CHNS/O Analysers Thermo Scientific, France).

2.6. Calculations

Soil N fluxes were assessed using following equations (Robertson et al. 1999):

Potential net ammonification = $[(NH_4^+-N)_f - (NH_4^+-N)_i]/T_d$
Potential net nitrification = $[(NO_3^--N)_f - (NO_3^--N)_i]/T_d$
N mineralization rates = $[(NH_4^+-N + NO_3^--N)_f - (NH_4^+-N + NO_3^--N)_i]/\text{Total N}$

Where i and f indicate mineral N concentrations before and after aerobic incubation, respectively, and T_d indicates incubation time in days. A negative value indicates microbial net immobilization (Hart et al. 1994). Potential net ammonification and nitrification were expressed as µg N g^{-1} C d^{-1} (Hart et al. 1994; Persson et al. 2000). N mineralization rates were expressed as ‰ of total N mineralized after 28 days of incubation. Soil N fluxes were assessed in each soil layers. The nature of ammonification and nitrification pathways (bacterial or fungal) was secondly assessed as follows.

-Bacterial ammonification = AF / A x 100
-Fungal ammonification = (A - AF) / A x 100
-Autotrophic nitrification = (C-A) / C x 100
-Heterotrophic nitrification = A / C x 100
-Bacterial heterotrophic nitrification = AF / C x 100
-Fungal nitrification = (A - AF) / C x 100

Where C, A and AF indicate values of ammonification and nitrification for control (C), acetylene treatment (A), and acetylene plus fungicide treatment (AF), respectively. Ammonification and nitrification pathways were expressed as absolute percentage of contribution to control ammonification and nitrification, respectively. When the percentage of contribution was negative or higher than 100%, we set to 0% or 100% of contribution, respectively.

2.6. Statistical analysis

Means and standard errors were calculated per silvicultural phase for each soil layer both for N and morphological variables. Comparisons of means between silvicultural phases and soil layers were performed using analyses of variances (ANOVA) and Tukey HSD post hoc tests. Beforehand, data normality and variances homogeneity were checked using the Wilk-Shapiro and the Bartlett test, respectively. When necessary, logarithmic transformations were applied and, if normality and/or homoscedasticity conditions were still refuted, non-parametric tests (Kruskal-Wallis rank sum test and Multiple comparison tests after Kruskal-Wallis test) were preferred. Comparisons of means between pathways of both ammonification and nitrification were performed using Wilcoxon rank sum test after arcsin transformation of percentage data to correct non-normality and heterogeneity of variance (Ahrens et al. 1990). A Principal Component Analysis (PCA) was performed on the humus form data matrix (16 rows corresponding to stands and 36 columns corresponding to morphological variables). The PCA illustrated which morphological variables discriminated humus forms along the chronosequence. Codes of morphological variables are listed in Table 2.2. We used linear correlations to describe co-variation patterns between macro-morphological variables and N mineralization pathways along the forest chronosequence. Levels of significance for linear correlations were adjusted and corrected with the Truncated Product Method for combining P-values (Zaykin et al. 2002). All tests were performed with R (2008) and statistical significance was set at $P = 0.05$.

3. Results

3.1. Changes in soil C-to-N ratio and N pools along the chronosequence

In the OL layer, the C-to-N ratio was maximal in SP65 (28.2) and minimal in SP95 (21.6). C-to-N ratio did not change between silvicultural phases in both the FH and the A layer. Total N was maximal in SP95 with about 25.7 g kg^{-1} and minimal SP65 (16.5 g kg^{-1}) in the OL layer (Table 2.3). SP15 and SP130 exhibited intermediate values. In both the FH and the A layers, total N did not change significantly between silvicultural phases. Initial ammonium and nitrate pools did not change significantly between silvicultural phases whatever the soil layers. However, ammonium content tended to be higher in SP130 (about 172 mg N kg^{-1}) in the OL layer and in SP15 (about 266 mg N kg^{-1}) in the FH layer. Nitrate content tended to be higher in SP95 whatever the soil layers (Table 2.3).

Table 2.3. *C-to-N ratio and N pools within the OL, the FH and the A layers according to silvicultural phases.*

Horizons Silvicultural phases	C-to-N ratio			Total N (g kg^{-1})			NH_4^+ (mg N kg^{-1})			NO_3^- (mg N kg^{-1})		
OL layer												
SP15	22.7	(2.3)	**B**	18.4	(2.4)	**AB**	112.6	(58.8)	A	47.0	(8.4)	A
SP65	28.2	(1.3)	**A**	16.5	(2.1)	**B**	126.5	(29.6)	A	51.9	(8.5)	A
SP95	21.6	(2.8)	**B**	25.7	(5.2)	**A**	165.9	(32.5)	A	63.3	(10.3)	A
SP130	25.2	(1.3)	**AB**	17.6	(1.4)	**B**	171.9	(26.9)	A	52.1	(6.2)	A
FH layer												
SP15	20.8	(4.1)	A	17.8	(4.1)	A	266.1	(41.8)	A	58.2	(30.5)	A
SP65	25.1	(1.9)	A	16.6	(3.0)	A	250.2	(61.7)	A	65.1	(40.4)	A
SP95	23.5	(1.5)	A	20.4	(5.7)	A	244.1	(61.9)	A	101.1	(48.9)	A
SP130	22.5	(1.6)	A	16.3	(0.6)	A	218.1	(33.2)	A	97.1	(54.2)	A
A layer												
SP15	16.3	(0.2)	A	3.3	(0.3)	A	12.7	(5.6)	A	11.2	(11.1)	A
SP65	15.9	(0.3)	A	2.6	(0.4)	A	14.5	(12.1)	A	20.9	(6.8)	A
SP95	16.3	(0.4)	A	3.0	(0.1)	A	9.2	(3.1)	A	29.6	(8.0)	A
SP130	15.3	(0.8)	A	3.0	(0.2)	A	13.6	(3.8)	A	25.0	(6.7)	A

Data are means (SD). Letters (A and B) refer to significant differences between silvicultural phases according to one-way ANOVA and Tukey HSD test (P=0.05 level, n=4).

3.2. Changes in mineral N production along the chronosequence

Both potential net ammonification and N mineralization rates increased gradually along the chronosequence (*i.e.* about 50% increases) within the OL and the FH layers (Table 2.4). In contrast, no significant change in potential net ammonification and N mineralization rates was detected in the A layer. The maximal potential net ammonium was observed in SP130 within the OL layer with a production of 249.5 µg N g^{-1}-C d^{-1}. Potential net ammonification and N mineralization rates were significantly higher in the organic layers compared to the A layer, and were also higher in the OL layer compared to the FH layer in SP15 and SP130. Potential net nitrification did not differ significantly between silvicultural phases within the FH layer and the A layer (Table 2.4), even if it tended to be higher in SP15 (23.6 and 12.1 µg N g^{-1} C d^{-1}, respectively). Within both the OL layer and the A layers, potential net nitrification was positive only for SP15 (*i.e.* 2.1 and 12.2 µg N g^{-1} C d^{-1}, respectively), while negative values suggested N immobilization in older silvicultural phases. Except in SP15, potential net nitrification was significantly higher in the FH layer and N immobilization was significantly higher in the OL layer (Table 2.4).

3.3. Changes in ammonification and nitrification pathways along the chronosequence

Fungal ammonification was significantly higher (70 to 85%) than bacterial ammonification (15 to 30%) in the OL layer whatever the silvicultural phase (Figure 2.1). In contrast, potential net ammonification was exclusively bacterial in SP15 and SP65 (100%) in FH layers. In SP95, we found a positive fungal ammonification (about 30%) that was however significantly lower than bacterial ammonification (about 70%) within FH layers.

Table 2.4. *Potential net N mineralization pathways expressed as µg N g⁻¹ C d⁻¹ within the OL, the FH and the A layers according to silvicultural phases.*

Soil N transformations	Silvicultural Phases															
Soil layers	SP15				SP65				SP95				SP130			
Potential net ammonification (µg N g^{-1} C d^{-1})																
OL layer	123.8	(22.3)	**C**	x	152.3	(30.4)	**BC**	x	177.5	(16.9)	**AB**	x	249.5	(20.9)	**A**	**x**
FH layer	57.6	(12.4)	**B**	y	98.6	(30.8)	**AB**	x	145.5	(41.2)	**A**	x	144.2	(25.0)	**A**	**y**
A layer	16.1	(6.6)	A	z	33.1	(18.6)	A	y	12.6	(7.7)	A	y	22.7	(24.8)	A	**z**
Potential net nitrification (µg N g^{-1} C d^{-1})																
OL layer	2.1	(2.5)	**A**	x	-3.8	(0.8)	**B**	**y**	-4.4	(0.7)	**B**	**y**	-4.0	(0.7)	**B**	**y**
FH layer	23.6	(17.8)	A	x	10.6	(9.9)	A	**x**	17.4	(8.6)	A	**x**	11.7	(6.9)	A	**x**
A layer	12.1	(24.3)	A	x	-9.0	(4.7)	A	**y**	-19.0	(16.8)	A	**y**	-14.9	(3.4)	A	**y**
N mineralization rates (‰)																
OL layer	7.9	(1.5)	**B**	x	11.5	(2.3)	**B**	x	9.7	(2.3)	**B**	x	17.3	(0.5)	**A**	**x**
FH layer	4.5	(1.0)	**B**	y	8.4	(2.9)	**AB**	x	10.0	(3.3)	**A**	x	9.8	(1.1)	**A**	**y**
A layer	1.2	(0.9)	A	**z**	1.1	(0.7)	A	y	0.2	(0.4)	A	y	0.5	(0.8)	A	**z**

Data are means (SD). Letters refer to significant differences between silvicultural phases (A, B and C) or between horizons (x, y and z) according to one-way ANOVA and Tukey HSD test (P=0.05 level, n=4).

In SP130, potential net ammonification in this layer was evenly mediated by bacterial and fungal activities. Potential net ammonification in the A layer was exclusively related to bacterial activity. The results obtained in acetylene treatments, either with or without captan addition, show that potential net nitrification was achieved either by autotrophic, fungi or by both types of microorganisms along the chronosequence (Fig. 2.2). Potential net nitrification was both autotrophic (55%) and fungal (44%) in the OL layer, and almost completely autotrophic in the FH layer (from 93% to 100%). A potential net nitrification was exclusively autotrophic in SP15.

3.4. Humus forms changes along the chronosequence

The first two axes of the Principal Component Analysis (PCA) explained 43 % of the total inertia of humus morphological data (Fig. 2.3). The first axis of the PCA (relative inertia = 29.8%) opposed the SP15 with negative coordinates to the three others phases (Fig. 2.3.a). This axis opposed OLn and macrofauna-based variables (negative scores) such as the number of earthworm casts in organic layers or the structure of the A horizon, to OLv, OF and OH variables (positive scores) (Fig. 2.3.c). The first axis was interpreted as the changes in humus morphology during the shift mull-moder (Fig. 2.3.a'). The second axis of the PCA (relative inertia = 12.7%) opposed the SP65 in negative coordinates to SP95 and SP130 in positives coordinates (Fig. 2.3.a). The second axis opposed OLv-based variables (OLvmt, OLvBl, OLvCom) against OH-based variables (OHMt, OHmt, OHffog, OHvfog and OHro) (Fig. 2.3.c).

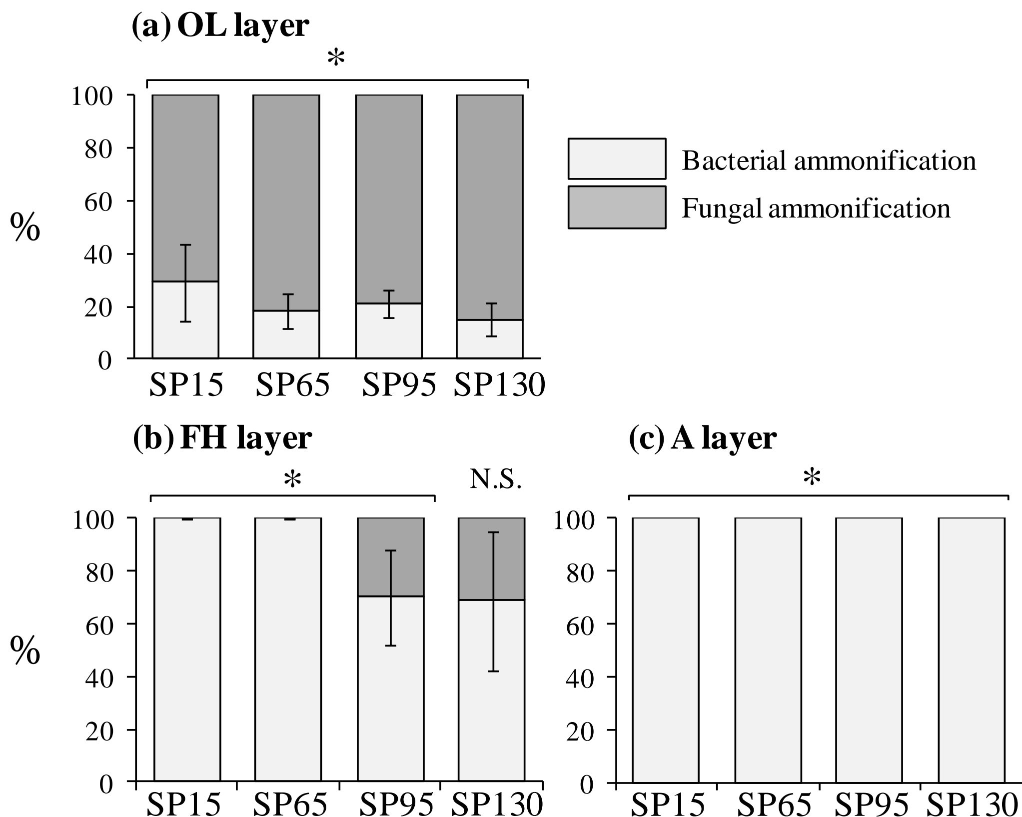

Figure 2.1. *Net ammonification pathways, i.e. bacterial and fungal, within the OL (a), the FH (b) and the A layers (c) expressed as % of contribution in total net ammonium production according to silvicultural phases. Vertical bars correspond to standard deviation. "*" indicates significant differences at P < 0.05 between bacterial and fungal contributions while "N.S." refers to non significant (Wilcoxon rank sum test, n=4).*

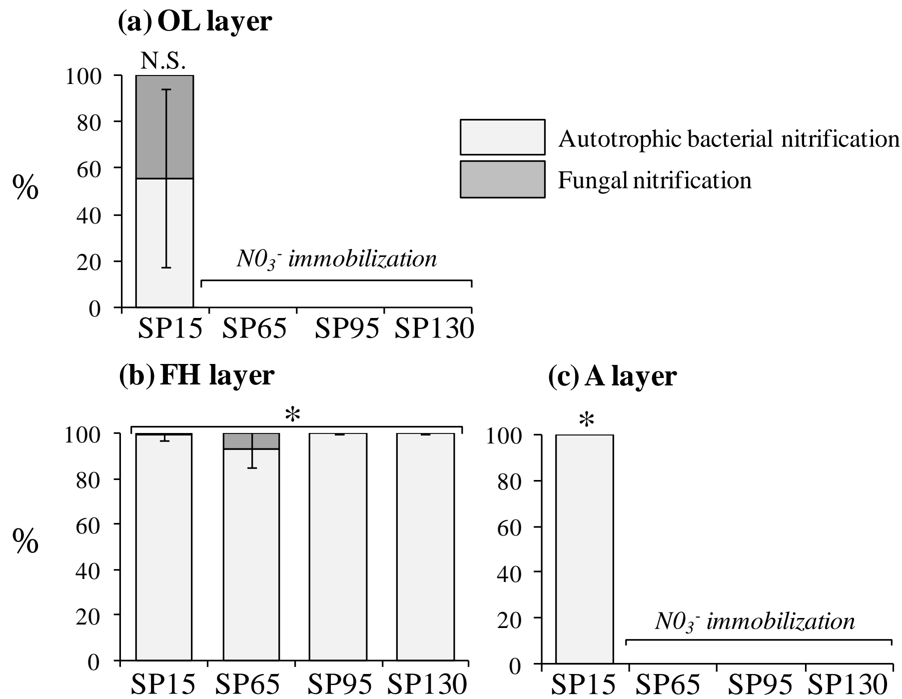

Figure 2.2. *Net nitrification pathways, i.e. autotrophic bacterial and fungal, within the OL (a), the FH (b) and the A layers (c) expressed as % of contribution in total net nitrate production according to silvicultual phases. Vertical bars correspond to standard deviation. "*" indicates significant differences at P < 0.05 between bacterial and fungal contributions while "N.S." refers to non significant (Wilcoxon rank sum test, n=4).*

3.5. N mineralization pathways and humus forms changes

Four humus forms variables were significantly correlated with N mineralization pathways (Table 2.5). The others macro-morphological variables were not significantly related with N variables. The percentage of leaves fragments in OLv (OLvfg) was for instance positively linked to both autotrophic and potential net nitrification in the FH layer (R^2=0.69, p<0.01 and R^2=0.69, p<0.01, respectively). In contrast, the percentage of fragments in OLv was negatively corelated with autotrophic nitrification in the OL layer (R^2=0.81, p<0.001). Bacterial ammonification in the OL layer decreased as OLv cover and maximal thickness increased (R^2=0.63, p=0.015 and R^2=0.58, p<0.05, respectively). Fungal ammonification in the FH layer increased with OF thickness (R^2=0.63, p<0.05) (Table 2.5).

Each macro-morphological variable was ranked from 1 to 33 according to its R squared correlation coefficient with the two first axes of the PCA (33 ranks per PCA axis). On each axis, the first rank corresponded to the highest R squared, *i.e.* "living roots in OH" for the first axis and "OLv cover" for the second one of the PCA (Table 2.2). Conversely, the 33th rank corresponded to the lower R squared, *i.e.* "OF cover" for the first axis and "A aggregate size" for the second one of the PCA (Table 2.2). This ranking thus reflected the relative power of each morphological variable in discriminating the silvicultural phases on the PCA axes. The morphological variables significantly correlated with N variables were positioned on average at the ranks 21 and 18 on the first and the second PCA axes, respectively. The morphological variables with the highest ranks did not showed significant correlations with N variables, except for OLv cover which was highly correlated with the second PCA axis.

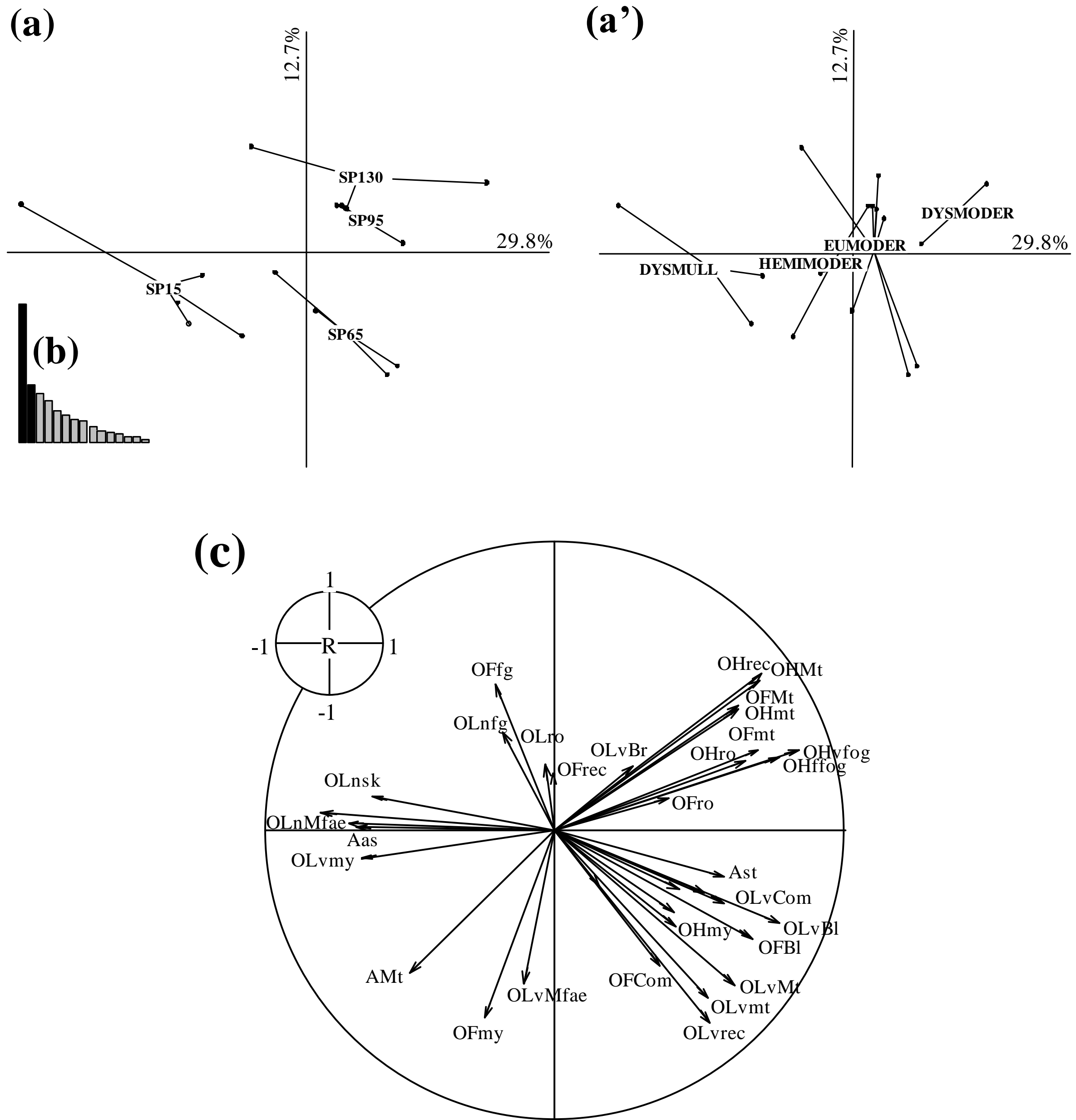

Figure 2.3. *Principal Component Analysis performed on humus forms data set. Age class ordination* ***(a)*** *and humus forms ordination* ***(a')*** *represented by ellipse of dispersion, i.e. 13-18 years (SP15), 65-66 years (SP65), 91-103 years (SP95) and 121-135 years (SP130); each black point corresponds to a stand. Eigenvalue diagram (b). Correlations circle* ***(c)****; only labels of the more relevant variables are indicated; variable codes are given in Table 2.2.*

4. Discussion

4.1. Changes in soil N pathways along the forest chronosequence

Our results show that soil N mineralization pathways changed greatly along the pure beech chronosequence. They also highlight significant differences between soil layers, both in mineral N production and in the contribution of bacteria and fungi to N transformations. Even if discrepancies between studies exist in the literature, this pattern corroborates several previous works (Welke and Hope 2005; Zeller et al. 2007; Trap et al. 2009).

Table 2.5. *Significant linear correlations between N variables and humus forms variables.*

N variables	Humus forms variables			Correlations		
	Names	R^2 rank PCA-1[a]	R^2 rank PCA-2[b]	Slope	R^2	*P* value
Autotrophic nitrification in the OL layer	Leaves fragments in OLv	32	28	-	0.81	0.00
Autotrophic nitrification in the FH layer	Leaves fragments in OLv	32	28	+	0.69	0.00
Potential net nitrification in the FH layer	Leaves fragments in OLv	32	28	+	0.69	0.00
Bacterial ammonificiation in the OL layer	OLv cover (%)	19	1	-	0.63	0.01
Fungal ammonification in the OL layer	OF maximal thickness	8	18	+	0.63	0.03
Bacterial ammonification in the OL layer	OLv maximal thickness	16	5	-	0.58	0.04
	Mean	***23***	***18***			

[a] The rank of the variable according to its R squared value on the first axis of the PCA

[b] The rank of the variable according to its R squared value on the second axis of the PCA

The temporal patterns observed in our study highlight the existence of two main functional changes in mineral N production during forest ageing. The first one corresponds to the gradual increase in potential net ammonification in the organic layers with stand ageing. The second one is characterized by the sharp decrease in net nitrification (from 2.06 to -3.85 µg N g^{-1} C d^{-1}) between the first (15 years old) and the second (65 years old) silvicultural phases. These results agree with Welke and Hope (2005) who also observed lower N mineralization in the forest floor of young (10-15 years) compared to mid-aged (50-65 years) and old (>85 years) stands of pure and mixed douglas-fir and paper birch. The decrease of net nitrification along stand development was also observed by other authors (Brais et al. 1995; Bauhus et al. 1997; Trap et al. 2009). For instance, Zeller *et al.* (2007) showed lower net nitrate production in old stands compared to young stands in a pure beech forest in north-east France.

We found that changes in mineral N production were clearly paired with substantial variations in the relative contribution of bacteria and fungi in soil N mineralization pathways along the chronosequence. For instance, fungal contribution in the FH layer increased after 65 years of stand ageing. This may be related to the production by fungi of extracellular enzymes such as oxidases, peroxidases or polyphenols-oxidases, that are efficient to degrade complex molecules such as lignin (Gobat et al. 2004). Organic N input by litter could be thus more rapidly mineralized in the presence of fungal enzymes. Hence, the higher potential ammonium production in mature stands (90 and 130 years old) compared to younger stands could be due to the presence of fungal ammonification in the FH layer. This hypothesis is supported by the observed variation pattern in mineral N production between soil layers. Indeed, potential net ammonification was higher in the OL layer where fungal activity dominated, intermediate in the FH layer where both fungal and bacterial activities were measured, and lower in the A where bacterial activity dominated.

We observed high fungal ammonification in the OL layer (about 80% fungal ammonification) while captan has apparently no effect on soil fungi in the A layers result, according to the calculation procedure, to 0% fungal ammonification. It appears doubtful that fungi did not participate in ammonium production. However, in a parallel study (see chapter 5), we investigated soil microbial community structure using DNA fingerprints. We found that fungal biomass was 5-times higher in the OL layer and 5-times lower in the A layer than bacterial one. Those results support fungal and bacterial contributions in net ammonification. It is possible that the use of captan maximized the contribution of fungi and bacteria leading to extreme percentages of contribution (100% and 0%).

However, our results suggest that ammonification is a soil layer-specific process, the efficiency of which is probably controlled by fungal activity. Higher net ammonification observed in OL layer may be due to higher gross fungal ammonification rates. On the other hand, lower ammonium immobilization by fungi could also enhance ammonium content in soil. As discussed by Kooijamn *et al.* (2009), high efficiency of potential net N mineralization by fungi in mature stands may also be due to their low N requirement. Indeed, C-to-N ratio of fungi biomass (between 7 and 25) is often higher than for bacteria (between 5 and 7) (Lavelle and Spain 2001). Therefore, gross N mineralization could be higher in younger stands than in mature ones, but net N mineralization was finally reduced by high microbial N immobilization. These results suggest a strong influence of fungi-to-bacteria biomass ratio on soil N transformations and especially on ammonification between soil layers and along forest chronosequences (Hogberg et al. 2007). In contrast to ammonification, the nature of nitrification did not

seem to change significantly along the chronosequence. This concurs with the study of Pedersen *et al.* (1999) who showed that heterotrophic net nitrification was the main process of nitrate production whatever stand age in organic and mineral acid soils of a mixed conifer forest in California.

Conversely, Trap *et al.* (2009) showed a shift from autotrophic to heterotrophic nitrifier activity in the forest floor of a mixed oak-hornbeam chronosequence of north-western France. In the present study, net nitrification was mainly autotrophic in both organic and mineral layers despite the acidic context, except in the L layer where 50% of net nitrification was fungal in the younger stands. The autotrophic nature of the net nitrification pathways despite the acidic conditions suggests the presence of acid tolerant autotrophic bacteria (De Boer and Kowalchuk 2001).

The decrease in autotrophic net nitrification along the chronosequence can be explained by (i) a decrease in ammonia availability (Booth et al. 2005), (ii) an inhibition of ammonia monooxigenase of autotrophic bacteria by allelopathic compounds or by competition with fungi for ammonium (De Boer and Kowalchuk 2001; Strauss and Lamberti 2002; Zeller et al. 2007) and (iii) an decrease of autotrophic bacteria in mature stands. Conversely to what is currently acknowledged (Hart et al. 1997; Booth et al. 2005), the availability of ammonia may not be the main driving factor since ammonification was higher in mature than in young stands.

A possible inhibition of ammonia monooxigenase of autotrophic bacteria by allelopathic compounds may occur. Allelopathic inhibition of nitrification could come from both litter (Northup et al. 1995; Persson et al. 2000) and microbial degradation products as suggested by Zeller *et al.* (2007). This hypothesis is supported by Kanerva *et al.* (2008), who found higher concentrations of both phenolic compounds and terpenes in OL than in FH layers of silver birch, Norway spruce and Scots pine stands in northern Finland. In our study, autotrophic net nitrification was mainly located in the FH layer and decreased sharply in the OL layer. Fungal degradation products from the OL layer may be transfered in the OL, FH and A layers, leading to autotrophic net nitrification inhibition. The absence of acid tolerant autotrophic bacteria in mature stands could also explain the decrease of net nitrification along the forest chronosequence. Further works based on microbial structure (microbial C-to-N ratio) and function along the chronosequence, as well as on allelopathic compounds production by litter and microbes should be done in order to evaluate theses hypotheses.

4.2. Humus forms: potential indicators of soil N changes?

In our study, humus forms shifted from dysmull in younger stands towards moders in older stands. The PCA showed that the macro-morphological variables discriminated clearly the four silvicultural phases and appeared robust as temporal indicators of forest functioning at the rotation scale. The use of humus forms as indicator of forest ecosystem functioning has been proposed by several authors (Brêthes et al. 1995; Ponge 2003; Jabiol et al. 2009). Mull humus is usually associated to high soil fertility and rapid turnover of nutrients (Ponge 2003), and we therefore did not expected to observe higher N mineralization rates in moder compared to mull. In our study, FH thickness was for instance correlated with high fungal net ammonification. This may be explained by two different mechanisms. First, the development of OF and OH layers in mature stands could provide large pools of dissolved organic C and N from fine organic matter (Park et al. 2002). Dissolved organic matter may be rapidly mineralized under optimal conditions (high temperature and moisture) leading to higher net ammonification in the mature stands. The presence of fungi in OL could

enhance net ammonification, probably due to the production of exoenzymes that efficiently depolymerize and mineralize organic N. Second, the high net N mineralization rates found in moder could be due to low fungal N requirements by C unit compared to bacteria (Baath and Anderson 2003; Kooijman et al. 2009). The presence of fungi, which generally dominate in moder (Ponge 2003; Gobat et al. 2004) may favor lower microbial N immobilization leading to higher net mineral N production.

Among the morphological variables, the percentage of fragments in OLv appeared as an important indicator of autotrophic net nitrification in the FH layer. The development of the OLv layer is mainly due to high fungal activity by leaves bleaching (Jabiol et al. 2009) but the percentage of organic fragments in this layer may directly due to faunal activity. Soil macroinvertebrates are for instance known to provide favorable conditions for autotrophic bacteria growth (Lavelle and Spain 2001). For instance, earthworms grazing affect fungal population and favor N release at the microsite scale and casting may provide mineral N by leaching promoting bacteria growth (Decaens et al. 1999; Marhan and Scheu 2003). Finally, the presence of earthworms in mull may lead to higher autotrophic bacteria biomass in the organic layer (Schimel and Bennett 2004) and thus probably enhance locally autotrophic net nitrification. The characterization of soil microbial biomass and structure may provide complementary indications required to validate this hypothesis.

Our results finally allowed us sorting the morphological variables in two groups. The first one includes the most powerfull morphological variables to discriminate silvicultural phases on the PCA and which explained the shift from mull towards moder humus forms (higher R^2 rank). The second group gathers morphological variables significantly correlated with soil N mineralization pathways at the rotation scale. The first group includes macrofauna-based variables such as the number of earthworm casts and variables of the organo-mineral horizon such as aggregation level. The second one includes the percentage of fragments in OLv, the maximal thickness of the OLv and the OF layers. Our results thus highlight that morphological variables identified as the best candidate indicators of N mineralization pathways may not constitute necessarily the best morphological criteria to discriminate the main kinds of humus forms. Rather, they suggest the potential use of selected macro-morphological variables as indicators of both soil N mineralization rates and the nature of N pathways. It appeared necessary to explore this question and provide datasets from contrasted pedo-climatic situations that may be used to calibrate humus-based ecological indices of soil N cycle.

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III. Synthèse du chapitre 2

Changements morphologiques

Le vieillissement des peuplements purs de hêtres s'accompagne d'une accumulation de matériel organique qui se traduit sous la forme d'un horizon d'humification OH et d'un passage d'un horizon A faiblement biomacrostructuré à un horizon A de juxtaposition. La forme d'humus de type **dysmull** est présente uniquement sous le stade de 15 ans. L'**eumoder** est présent dès 65 ans jusqu' à 130 ans alors que l'**hémimoder** est présent uniquement sous les peuplements de 65 et 95 ans. Enfin, la forme la moins active observée est le **dysmoder** sous les peuplements les plus agés, *i.e.* 95 et 130 ans.

Changements fonctionnels

Le développement des formes d'humus de type moder le long de la chronoséquence s'accompagne de changements fonctionnels importants en termes de production d'azote minéral (Fig. 2.4).
(1) La production nette d'ammonium au sein des horizons holorganiques (OL et FH) augmente le long de la chronoséquence. La disponibilité en azote total ne semble pas être un facteur déterminant des patrons de variations observés. En effet, le taux de minéralisation de l'azote augmente de 54% entre le stade le plus jeune (20 ans) et le stade le plus âgé (130 ans) au sein des horizons holorganiques.
(2). La nitrification nette potentielle chute le long de la chronoséquence. Seul le stade de 20 ans présente une production nette de nitrate positive. A partir de 65 ans, nous observons une immobilisation.
(3) La contribution des bactéries dans le processus d'ammonification est importante dans les horizons FH en début de chronoséquence et au sein de l'horizon organo-minéral A. L'ammonification fongique est omniprésente dans l'horizon OL et apparaît dans les horizons FH à partir de 95 ans. En revanche, la contribution fongique dans la production d'ammonium au sein de l'horizon A est nulle.
(4) La nitrification potentielle est essentiellement autotrophique et localisée dans les horizons FH.

Ces résultats suggèrent une régulation en faveur des processus en amont du cycle (*i.e.* ammonification) au sein des horizons organiques. L'augmentation du taux de minéralisation favorise la production nette d'ammonium alors que la chute de la nitrification nette limite les pertes en ammonium. L'augmentation de la contribution fongique semble être un facteur important, responsable en partie des patrons de variations de l'azote.

Relations formes d'humus – cycle de l'azote

Certaines variables morphologiques (*i.e.* le pourcentage de fragments organiques dans l'OLv, le pourcentage de recouvrement de l'OLv, l'épaisseur de l'OLv et de l'OF) présentent un potentiel indicateur de production *ex situ* d'ammonium et de nitrate. Les variables morphologiques spécifiques à l'horizon OLv sont très représentées et pourraient constituer des indicateurs robustes de production *ex situ* d'azote minéral.

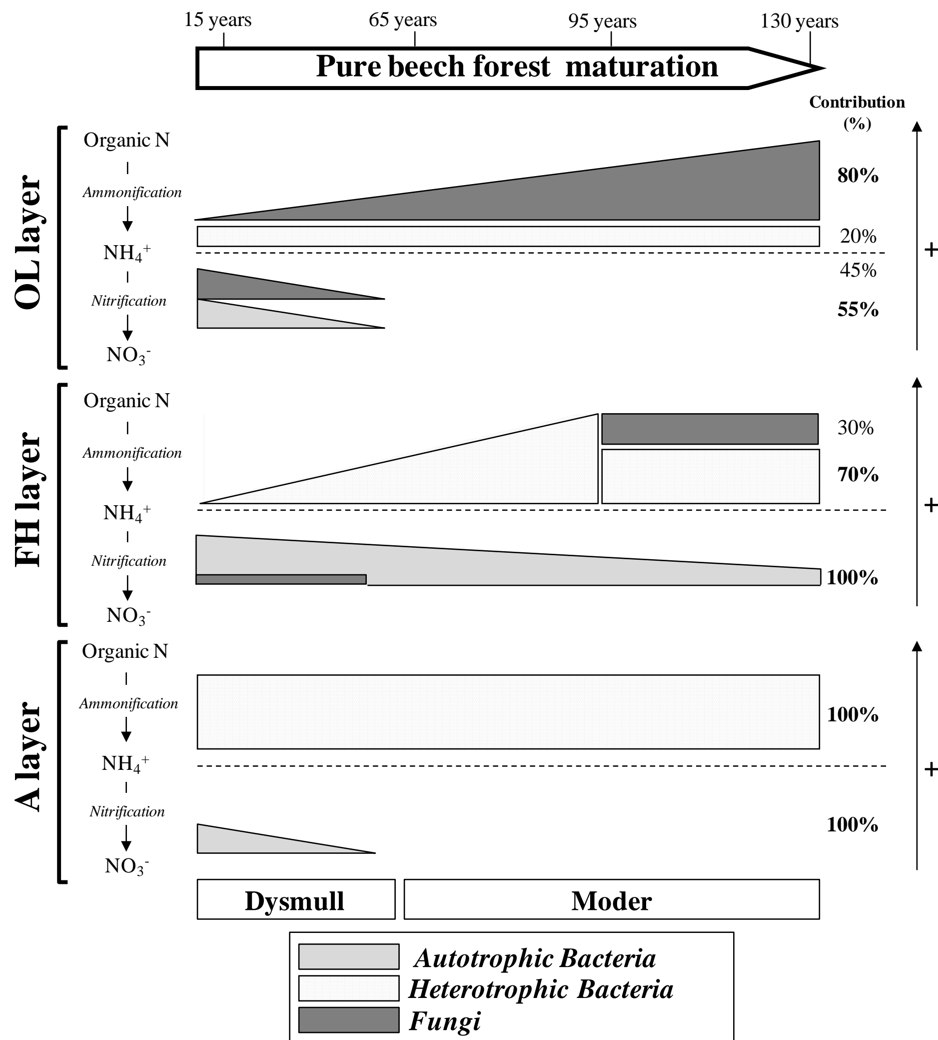

Figure 2.4. *Changes in potential net N mineralization and nitrification pathways and humus forms along a 130-years-old pure beech chronosequence. Acetylene and captan permitted assessment of bacterial (dotted) versus fungal (dark grey) ammonification and autotrophic (light grey) versus fungal (dark grey) nitrification.*

CHAPITRE 3
Approche *in situ*

I. Présentation du chapitre 3

L'objectif de cette étude est de caractériser la morphologie et la dynamique *in situ* de l'azote au sein de l'épisolum humifère le long d'une chronoséquence de hêtraie pure de 130 ans. Sur le terrain, 36 variables macro-morphologiques de l'épisolum humifère ont été déterminées. Le cycle de l'azote dans le sol (minéralisation, nitrification, dénitrification, lessivage) a été caractérisé sur le terrain par incubation de carottes intactes d'épisolum humifère, incluant les horizons organiques (OL, OF et OH) et l'horizon organo-minéral. Nous avons également couplé la méthode d'incubation *in situ* avec l'utilisation d'un inhibiteur de la nitrification autotrophe, (*i.e.* l'acétylène) afin d'identifier les microorganismes responsables de la nitrification. Des résines échangeuses d'ions ont été utilisées pour caractériser le lessivage des nitrates et de l'ammonium le long de la chronoséquence avec ou sans prélèvement racinaire. Des analyses statistiques ont été utilisées pour tester les patrons de covariations entre les deux matrices obtenues (cycle de l'azote dans le sol *versus* formes d'humus).

II. Article 2

Changes in humus forms and soil mineral N production, leaching and root uptake along a 130-yr-old pure beech (*Fagus sylvatica* L.) forest chronosequence

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Abstract

Forest ecosystem development is usually paired with great changes in belowground subsystem properties such as humus forms and soil N cycle. In this study, we assessed *in situ* variations in N pools and fluxes (*i.e.* N mineralization, autotrophic *versus* heterotrophic nitrification, denitrification, leaching and root uptake) within OL, FH (a mixture of OF and OH) and A (organo-mineral) layers in a set of stands that represented a 130-yr-old chronosequence of pure beech (*Fagus sylvatica* L.) forest. We also assessed, using linear regressions, co-variations patterns between humus forms and belowground N cycle. Annual *in situ* net N mineralization in OL and A layers was maximal in the oldest stands while it was constant within the FH layers along the chronosequence. The contribution of ammonification in mineral N production increased with stand ageing. In the OL layer, annual net nitrification was maximal in the youngest stands, namely 40% of total mineral N production. It was minimal in the oldest stands to reach only 10% of total mineral N production. We observed the same trend in the FH layers. Nitrification was absent in the A layer. The use of acetylene revealed that annual net nitrification was mainly achieved by autotrophic bacteria whatever soil layers and stand age, except in OL layer of the oldest stands, where about 35% of nitrate production is achieved by heterotrophic microorganisms. Annual *in situ* denitrification was maximal in the yougnest stands and minimal in the oldest ones. The use of exchange resins showed the maximal values in ammonium leaching in the oldest stands while nitrate leaching was stable across the chronosequence. We observed an increase in the ratio ammonium/nitrate uptake by roots along the chronosequence. Humus forms shifted from dysmull in younger stands towards dysmoder in older stands but only nitrification process and nitrate pools were correlated with morphological variables. The shift mull-moder occurring along the chronosequence indicates lower *in situ* net nitrification and nitrate pools in the organic layers. However, the morphological variables investigated in this present study predict poorly *in situ* ammonium-based variables trends along the chronosequence.

Keywords

In situ N mineralization, net ammonification, autotrophic *versus* heterotrophic nitrification, anionic and cationic exchange resins, N leaching, *in situ* denitrification, humus forms, 130-yr-old chronosequence, *Fagus sylvatica*, loamy acidic soil.

1. Introduction

European forest ecosystems covered in 2005 about 193 million hectares, namely an increase of about 7% since 1980 (FAO 2007). In France, managed forest ecosystems represent in 2006 more than the quarter of the national area (IFN 2007) and has increased by 73000 ha per year on average this last decade. With regard to the increasing firewood demand due to renewable character of this energy, forest is becoming again an important economic sector. Understanding forest ecosystem functioning in order to improve forest health, seedlings regeneration, and sustainable wood production thus appears as a central issue for economical and ecological sustainable forest management.

Forest ecosystem development is usually paired with great changes in belowground system functioning (Ponge and Delhaye 1995; Aubert et al. 2004; Trap et al. 2009). For instance, successional patterns of humus forms have often been described along empirical forest chronosequence in both managed (Aubert et al. 2004) and semi natural systems (Bernier and Ponge 1994a; Ponge and Delhaye 1995). Early developmental stages have been often associated with mull (thin litter horizon, absence of humified horizon, presence of earthworm casts and thick organo-mineral horizon) while moders (thick organic layers with humified horizon, higher proportion of litter fragments and mesofauna faeces and thin organo-mineral soil layer) have been frequently observed under old mature stands on acidic forest soils (Ponge 2003; Salmon et al. 2006). Since humus forms are considered as great indicators of soil organic matter turnover, we can hence hypothesize that the shift from mull toward moder occurring along forest maturation lead to substantial changes in soil biogeochemical functioning (Ponge and Delhaye 1995; Aubert et al. 2004).

The nitrogen (N) cycle is one of the most important biogeochemical cycles in forest ecosystems. Indeed, regarding on trees requirements toward available inorganic N forms (ammonium *versus* nitrate) and quantity, N is often considered as a limiting nutrient for forest ecosystem productivity (Duchaufour 1989; Vitousek et al. 1997). To date, however, there have only been a few field characterizations of soil N cycling in forest ecosystems with regards to their temporal dynamics (Jussy et al. 2000; Idol et al. 2003). Usually, studies focusing on N cycle changes with forest stand ageing are based upon a broad comparison between two contrasted silvicultural phases or stand ages (Pedersen et al. 1999; Coté et al. 2000; Fisk et al. 2002; Inagaki et al. 2004; Zeller et al. 2007) or limited to mineral soil layers (Jussy et al. 2000; Griffiths and Swanson 2001). That kind of sampling design is by far too simplistic to highlight the whole complexity of the functional changes that may occur in soil during a complete silvicultural and/or sylvigenetic cycle. It is a serious shortcoming since trees N requirements, microbial communities and trees-microbes interactions either competitive and/or mutually beneficial, are likely to vary in both organic and organo-mineral soil layers along forest maturation and thus to influence soil N dynamic (Brais et al. 1995).

In this study, we have assessed *in situ* variations in N pools and fluxes within both the organic and organo-mineral layers in a set of stands that represented a 130 years old chronosequence of pure beech (*Fagus sylvatica* L.) forest on loamy soils. More specifically, the aims were to measure *in situ* variations in soil N mineralization, autotrophic *versus* heterotrophic nitrification, denitrification and leaching along the chronosequence, and to assess using linear regressions, co-variations patterns between humus forms and N cycle within both organic and organo-mineral layers.

2. Materials and methods

2.1. Site description

The study was carried out in the Eawy forest (France, Upper Normandy region, 7200 ha). The climate is temperate oceanic with a mean annual temperature of +10°C and a mean annual pluviometry of 800 mm (Hedde 2006). A space-for-time substitution procedure was used to empirically reconstitute a forest chronosequence (Pickett 1989). Sixteen pure beech (*Fagus sylvatica* L.) stands were selected within the Eawy forest (Table 2.1). All of them were managed as even-aged forest by the French Forestry Service (ONF). The selection included four silvicultural phases of different ages: 13-18 years (SP15), 65-66 years (SP65), 91-103 years (SP95) and 121-135 years (SP130). Each phase comprised 4 true replicate stands. All stands were situated on a flat topographic situation (plateau). Soil was an endogleyic dystric Luvisol (FAO 2006) developed on more than 80 cm of loess (lamellated silts) lying on clay with flints (Lautridou 1985; Laignel et al. 1998). Understory vegetation was defined as a characteristic *Endymio-Fagetum* according to the phytosociological classification (Durin et al. 1967). In each stand center, a 16 m^2 square plot was delimited away from vehicle tracks and tree trunks to avoid any acidification due to organic matter accumulation (Beniamino et al. 1991).

2.2. Organic and organo-mineral horizons morphology

Macro-morphological descriptions of organic and organo-mineral layers were previously done within frames (25 cm x 25 cm) at three corners of the central plot according to the French nomenclature (Jabiol et al. 2007) in May 2007 (Table 2.1, page 45). A total of 36 macro-morphological variables were described in the field on the basis of variation visible to the naked eye (Table 2.2, page 47). We distinguished mull (mainly dysmull) and moder (hemimoder + eumoder + dysmoder) humus forms on the basis of morphological characters (Jabiol et al. 2007). A total of 48 humus profiles were described (3 descriptions per stand × 16 stands).

We differentiated:

(1) **the OL horizon** consisting of almost unmodified leaves and woody fragments. Most of the original plant structures are easily discernible. Leaves may be recently fallen and unmodified (OLn) or bleached and slightly fragmented (OLv);

(2) **the OF horizon** consisting of a mixture of coarse plant debris with fine organic matter (humus). This horizon is characterized by an accumulation of partly decomposed organic matter derived mainly from leaves and woody materials, which are fragmented and mixed with invertebrate faces. The proportion of organic fine substances ranges from 10 to 70 % by volume;

(3) **the OH horizon**. This horizon is an organic horizon characterized by an accumulation of decomposed organic matter. The original plant structures and materials are not discernible. Organic fine substances (invertebrate faces) account for more than 70 % by volume. The OH horizon differs from the OF by more advanced humification due to the action of soil organisms;

(4) **the A horizon** or **organo-mineral horizon** characterized by high or low clay-humus complexes abundance.

2.3. In situ incubation

2.3.1. Experimental design

In order to assess patterns of *in situ* variations in soil N transformations along the pure beech chronosequence, we monitored monthly soil N fluxes on the field during one year. The experiment was carried out between October 2007 and September 2008. N fluxes were assessed in the OL, OF, OH layers and in the first five centimeters above the OH layer called "A layer". Because the OH layer was sometimes discontinuous and not enough abundant, we decided to analyze N pools in a mixture of the OF and OH layers called "FH layers".

We conducted four different series of undisturbed soil cores incubation in the field (Fig. 3.1). The first treatment was used to characterize *in situ* N mineralization and nitrification by jar incubation (Hatch et al. 2000). The second one permitted the assessment of nitrification pathways (autotrophic *versus* heterotrophic). The third treatment was used to assess *in situ* denitrification. The last one was used to assess mineral N leaching with or without root uptake by exchange resins incubation and to estimate N uptake by trees (Fig. 3.1). All soil cores were enclosed in jars for field incubation (treatments 1, 2 and 3) except treatment 4 for which we used unenclosed PCV cylinder. Incubations were performed during 7 days.

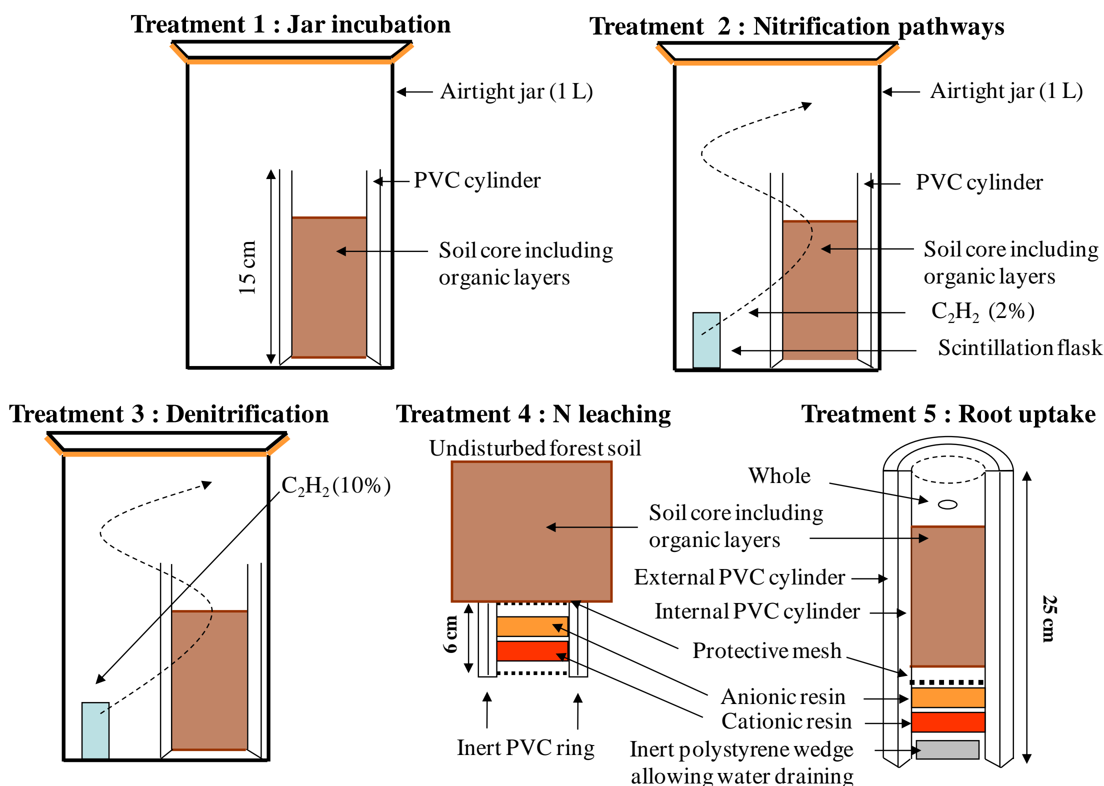

Figure 3.1. *Field experimental design including four different treatments to assess in situ soil N cycling.*

2.3.2. Soil layers sampling

Each month, three intact soil cores were sampled within the central plot using a double PVC cylinder system (7.5 cm inner diameter, 15 cm long) (Fig. 3.2). The double PVC cylinder system includes an internal PVC cylinder open in the side allowing us to remove carefully the mineral horizon with a knife and an external tube used to hold the

internal one into the soil (Fig 3.2). Soil cores included the OL, OF, OH and A layers. Intact soil cores were then used for the treatments 1, 2 and 3. We sampled a fourth intact soil core for the treatment 4 with a longer double PVC cylinder system (7.5 cm inner diameter, 25 cm long) in order to place exchange resins inside the cylinder at the bottom (Fig. 3.2). Each month, a non-incubated soil core was also sampled in the central plot in each stand at the beginning and at the end of the incubation to determine initial and final soil mineral N and gravimetric water contents. Samples were stored at 4°C during transportation and in the laboratory before mineral N analyses (Forster 1995b).

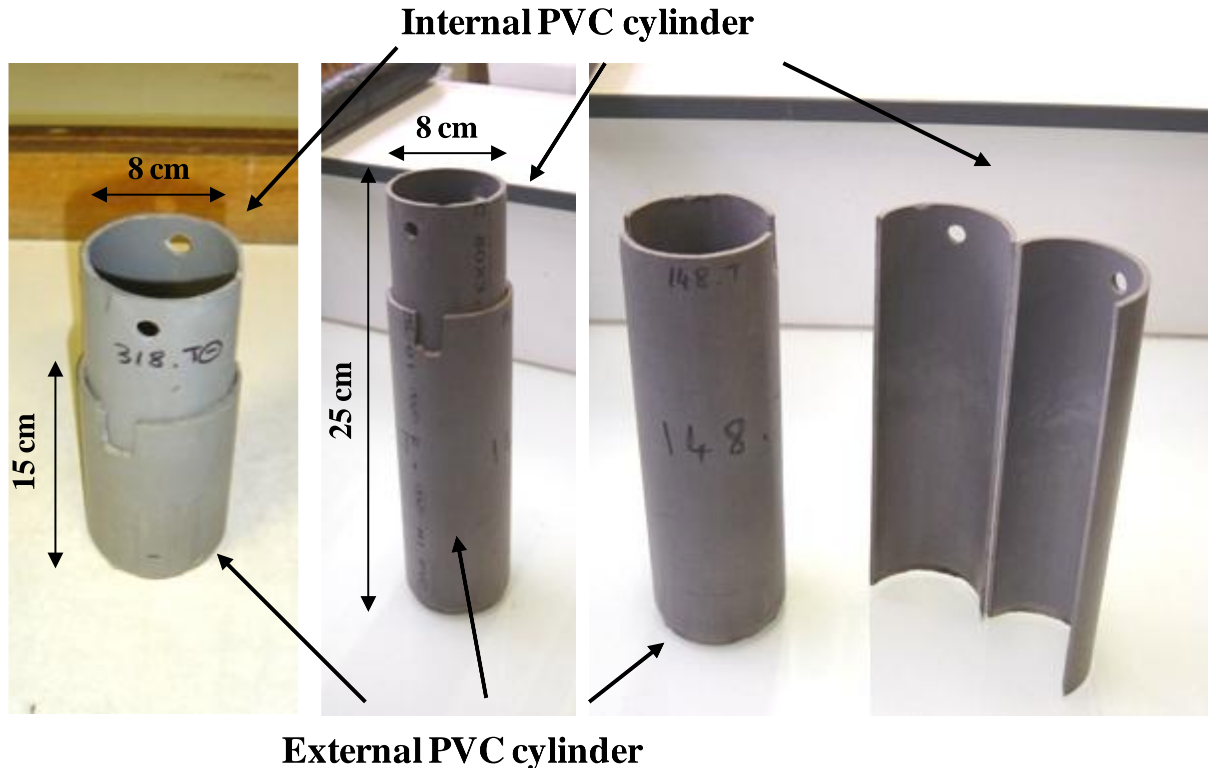

Figure 3.2. *Photography of the double PVC cylinder system used to sample intact soil core (15 cm long) and to measure N leaching in the treatment 4 (25 cm long).*

2.3.3. Net N mineralization by jar incubation (treatment 1)

Net N mineralization pathways (*i.e.* ammonification and nitrification) were determined using jar incubation. An undisturbed soil core was introduced in a hermetically sealed 1L glass jar immediately after sampling. The soil core was introduced with the internal PVC cylinder in order to avoid soil layers disruption. After 7 days of incubation, the soil core was removed, transported and stored in the laboratory at 4°C before mineral N extraction.

2.3.4. Autotrophic versus heterotrophic nitrification (treatment 2)

In an attempt to discriminate autotrophic from heterotrophic net nitrification, we conducted a jar incubation with 2% acetylene (v/v) (Hatch et al. 2000). Acetylene is the most specific inhibitor applied to inhibit autotrophic ammonia oxidation and to assess heterotrophic nitrification (Hynes and Knowles 1978; Bédard and Knowles 1989; De Boer and Kowalchuk 2001). Heterotrophic nitrification is not affected by acetylene at low concentrations (2% v/v) (De Boer and Kowalchuk 2001). Alike the treatment 1, an intact soil core was also introduced in a hermetically sealed 1L glass jar with 2% (v/v)

acetylene. The soil core was introduced with the internal PVC cylinder in order to avoid soil layers disruption. Acetylene was generated by adding water to calcium carbide in scintillation flasks. Acetylene concentration was checked at the beginning and the end of the incubation experiment using Gas Chromatograph equipped with a flame ionization detector (Girdel 30 GC, Spherosil XOB 75 column, France). After 7 days of incubation, the soil core was removed, transported and stored in the laboratory at 4°C before mineral N extraction.

2.3.5. Denitrification (treatment 3)

The treatment 3 was similar to the second one but acetylene concentration in the jar reached 10% (v/v) in order to inhibit nitrous oxide reductase activity (Jarvis et al. 2001). Acetylene inhibition has been widely used to measure denitrification since it prevents the reduction of N_2O to N_2. Acetylene was also generated by adding water to calcium carbide in scintillation flasks and its concentration was checked at the beginning and the end of the incubation experiment using Gas Chromatograph equipped with a flame ionization detector (Girdel 30 GC, Spherosil XOB 75 column, France). After 7 days of incubation, a 5-ml gas sample was removed from the airtight jar and inject in a venoject tube. This yube was stored in the laboratory at room temperature before N_2O analysis. In order to assess soil water content at the end of the jar incubation, we sampled the soil core from the airtight jar, transported and stored in the laboratory at 4°C.

2.3.6. N leaching (treatment 4)

Mineral N leaching was assessed using the exchange resins (treatment 4) (Hatch et al. 2000; Jussy et al. 2000). N leaching with root uptake was assessed using two nylon bags containing 10g of anion exchange resin (DOWEX 21 K, 20±50 mesh, Cl^--saturated) to quantify nitrate leaching in the first bag and 10g cation exchange resin (IRN 77, 16±40 mesh, Na^+-saturated) to quantify ammonium leaching in the cylinders in the second bag (Jussy et al. 2000). Nylon bags were placed into an inert PVC ring (two bags per PVC ring) closed by a nylon mesh (0.175µm) (Fig. 3.1). PVC ring with resins was then carefully placed below the A layer without disturbing soil layers or cutting roots as far as possible. Any mineral N that leached directly from the soil through the PVC ring was captured by the resin during the incubation period of 7 days. After 7 days, we sampled the PVC box with resins.

Resins utilization procedure was detailed by Jussy *et al..* (2000) and Jussy *et al..* (2004). Anionic exchange resin was saturated with Cl^- before incubation by a slow percolation of ultrapure water followed by 1M NaCl (1 L: 100 g of resin). Cationic resin was saturated with Na^+ by a slow percolation of ultrapure water followed by 1M HCl (1 L: 100 g of resin) and 1M NaOH (1 L: 100 g of resin) with a final pH between 6 and 7. After 7 days of incubation, nylon bags with anionic or cationic resins were sampled transported in the laboratory in a plastic bag. In the laboratory, resins were rinsed with ultrapure water to remove soil particles and adhering organic residues (Giblin *et al..* 1994). Rinsing with ultrapure water had no effect on N desorption from both anionic and cationic resins. Bags were open-topped and air-dried. Nitrate and ammonium were extracted by 1M NaCl (4 g: 40mL dwt:v) after manual shaking and batch contact (1 h). Extractions were then filtered through Schleicher & Schuell 0790 ½ filter papers and frozen for analyzing mineral N pools. Filters were pre-leached with 0.2 M K_2SO_4 in order to avoid any ammonium and/or nitrate contamination. Background levels of N were

absent in both resin types before incubation, as determined by non-incubated control resin bags prepared at the same time as the incubated bags, stored in the laboratory during field incubation, air-dried and analyzed with the incubated resin bags.

2.3.7. N uptake by trees (treatment 5)

Root uptake was suppressed in the case of the unenclosed PVC cylinder (treatment 5) and consequently mineral N contents and N leaching were different inside and outside the cylinder. We can thus estimate roots N uptake by comparing soil N content inside and outside the cylinders. N leaching without root uptake was assessed by placing anionic and cationic resins inside the unclosed PVC cylinder below the A layer. The internal PVC cylinder can be open in the side allowing us to remove with precision the mineral horizon with a knife and placed at the bottom two superimposed nylon bags containing the resins (Fig. 3.1) (Jussy et al. 2000). The remaining space was filled with an inert polystyrene wedge (5 cm thick and 7 cm in diameter). The internal PCV tube was then carefully replaced in the external PCV cylinder into the soil. The inert polystyrene wedge helped to maintain contact with the surrounding soil, but prevented any direct exchange of mineral N from sources outside the soil core. Resins were sampled after 7 days of incubation and mineral N was extracted as described previously.

2.4. Mineral N and moisture quantification

In the laboratory, brushwood, stones and large roots were removed and the A materials were sieved (2 mm). An aliquot of 5g (organic material) or 10g (organo-mineral material) of each fresh sample was placed in beakers with K_2SO_4 (0.2 M) solution (200 or 100ml for organic and organo-mineral material, respectively) and shook for 1 hour at 100 rev min^{-1} (Alef 1995; Forster 1995a). The obtained extractions were filtered through Schleicher & Schuell 0790 ½ filter papers and frozen for analyzed mineral N pools. Filters were pre-leached with 0.2 M K_2SO_4 in order to avoid any ammonium and/or nitrate contamination. Concentrations of NH_4^+-N and NO_3^--N were determined colorimetrically (AA3, BRAN+LUEBBE, Norderstedt, Germany). Soil gravimetric water content was determined by drying organo-mineral material at 105°C for 24 hours and organic material at 65°C for 48 hours. 5-ml gas samples were removed from the forest floor in each stand at the beginning of the incubation and from the airtight jar (treatment 3) after 7-day of field incubation and inject in a venoject tube. The venoject tubes were analyzed for N_2O on a hand injection GC equipped with an electron capture detector (Centre d'Ecologie Fonctionnelle et Evolutive, Montpellier, France).

2.5. Calculations

Soil N fluxes were assessed using following equations (Robertson et al. 1999; Hatch et al. 2000; Jussy et al. 2000; Persson et al. 2000; Idol et al. 2003).

$T0_iX$ = initial mineral N (X = NO_3-N, NH_4^+-N or N_2O-N) content at the beginning of the field incubation period.

$T0_fX$ = final mineral N (X = NO_3^--N or NH_4^+-N or N_2O-N) content of the soil outside the PVC cylinders at the end of the incubation period.

$T1_fX$ = final mineral N (X = NO_3^--N or NH_4^+-N) content of the soil inside the PVC cylinders at the end of the jar incubation (treatment 1) period.

$T2_fNO_3^-$-N = final NO_3^--N content inside the PVC cylinders at the end of the jar incubation with 2% v/v acetylene (treatment 2) period.

$T3_fN_2O$-N = final N_2O-N content inside the PVC cylinders at the end of the jar incubation (treatment 3) period.

$T4_fX$ = final mineral N (X = NO_3^--N or NH_4^+-N) in the resins placed in the PVC ring above the A layers (treatment 4) at the end of the incubation period.

$T5_fX$ = final mineral N (X = NO_3^--N or NH_4^+-N) content of the soil inside the unenclosed PVC cylinders (treatment 4) at the end of the incubation period.

Net ammonification = $T1_fNH_4^+$-N – $T0_iNH_4^+$-N

Net nitrification = $T1_fNO_3^-$-N – $T0_iNO_3^-$-N

Net N mineralization = Net ammonification + Net nitrification

Net heterotrophic nitrification = $T2_fNO_3^-$-N – $T0_iNO_3^-$-N

Net autotrophic nitrification = Net nitrification – Net heterotrophic nitrification

Percentage of nitrification = Net nitrification / Net N mineralization × 100

Net denitrification = $T3_fN_2O$-N – $T0_iN_2O$-N

N leaching = $T4_fX$ (final NO_3^--N or NH_4^+-N content in the resins)

NH_4^+ uptake by roots = $T5_fNH_4^+$-N – ($T0_fNH_4^+$-N + NH_4^+-N leaching inside the PVC cylinder) – NH_4^+-N leaching outside the PVC cylinder

NO_3^- uptake by roots = $T0_fNO_3^-$-N – ($T0_fNO_3^-$-N + NO_3^--N leaching inside the PVC cylinder) – NO_3^--N leaching outside the PVC cylinder

A negative value indicates microbial net immobilization (Hart et al. 1994). Each N fluxes was calculated monthly and computed to obtain annual budgets for these fluxes. Net ammonification, nitrification and root uptake were expressed as g-N kg^{-1} dry matter yr^{-1} (Hart et al. 1994; Persson et al. 2000). Net denitrification and N leaching was expressed as kg N ha^{-1} yr^{-1} (Persson and Wirén 1995). For hectare-based N fluxes calculations, the bulk density of both the organic layers (SP15 = 14.7, SP65 = 16.4, SP95 = 15.1, SP130 = 16.7 t ha^{-1}) and the A layer (SP15 = 587.5, SP65 = 661.2, SP95 = 601.2, SP130 = 521.2 t ha^{-1}) was determined using soil cores in each stand. The bulk density of the organic layers was determined using intact soil cores each month during the sampling period (n=12). The A bulk density was done in July 2008 using 12 replications of intact soil cores per silvicultural phase. We used equations developed by Jussy *et al.*. (2004) to take into account the desorption efficiency of ammonium and nitrate from the

resins. They found empirical relationships between desorbed nitrogen (D) and adsorbed nitrogen (A), *i.e.* for anionic resins: $A = 2.363 \times D^{0.945}$ ($n = 30$, $r^2 = 0.976$) and for cationic resin: $A = 1.88 \times D - 2.141$ ($n = 13$, $r^2 = 0.997$). We corrected our values using these equations.

2.6. Statistical analysis

Statistics were done using the R software (ADE4 and Statistics packages; R Development Core Team, 2008). Statistical significance was set at $P < 0.05$. Comparisons of means between silvicultural phases were done using Kruskal-Wallis rank sum test. Comparisons of means between nitrification pathways were performed using Wilcoxon rank sum test after arcsin transformation of percentage data to correct non-normality and heterogeneity of variance (Ahrens et al. 1990). We investigated the relationships between humus forms and soil N cycle using linear regressions. Levels of significance for linear correlations were adjusted and corrected with the Truncated Product Method for combining P-values (Zaykin et al. 2002).

3. Results

3.1. Mineral N pools

In both the OL and A layers, annual mean ammonium content did not change significantly between silvicultural phases but tended to be higher in SP15 (Fig. 3.3). Within the FH layers, ammonium content was significantly higher in SP65 and SP95 (about 60 µg-N g^{-1}) compared to SP130 (about 35 µg-N g^{-1}). SP15 exhibited intermediate value. In the OL layer, SP15 exhibited the highest nitrate content (about 11 µg-N g^{-1}) while SP95 showed the lowest one (about 7 µg-N g^{-1}). In the FH layer, nitrate content did not differ between the silvicultural phases, except in SP130 where it was significantly lower. We observed the same trend within the A layer. Irrespective of the age of stands, the FH layers showed the highest values of both ammonium and nitrate pools while the A layers showed the lowest one. We did not observe significant differences in soil N_2O pools between the silvicultural phases (mean 23 g-N ha^{-1}) (Fig. 3.4).

3.2. N mineralization pathways by jar incubation

Annual *in situ* net N mineralization in the OL layer was maximal in SP130 (about 4.1 g-N kg^{-1} dry matter yr^{-1}) and minimal in SP65 (about 2.6 g-N kg^{-1} dry matter yr^{-1}) (Fig. 3.5). SP15 and SP95 showed intermediate values. In the FH layers, we did not observed significant differences between silvicultural phases but net N mineralization tended to be lower in SP95. In the A layer, net N mineralization was higher in SP130 (about 3 g-N kg^{-1} dry matter yr^{-1}) while the three others phases exhibited lower values (about 0.2 g-N kg^{-1} dry matter yr^{-1}). Net ammonification estimated by jar incubation was significantly higher in SP130 (more than 4 g-N kg^{-1} dry matter yr^{-1}) compared to SP15 (about 2 g-N kg^{-1} dry matter yr^{-1}), namely about 50% increases (Fig. 3.6). In the FH layer, annual net ammonification did not change significantly between silvicultural phases (mean 2.5 g-N kg^{-1} dry matter yr^{-1}).

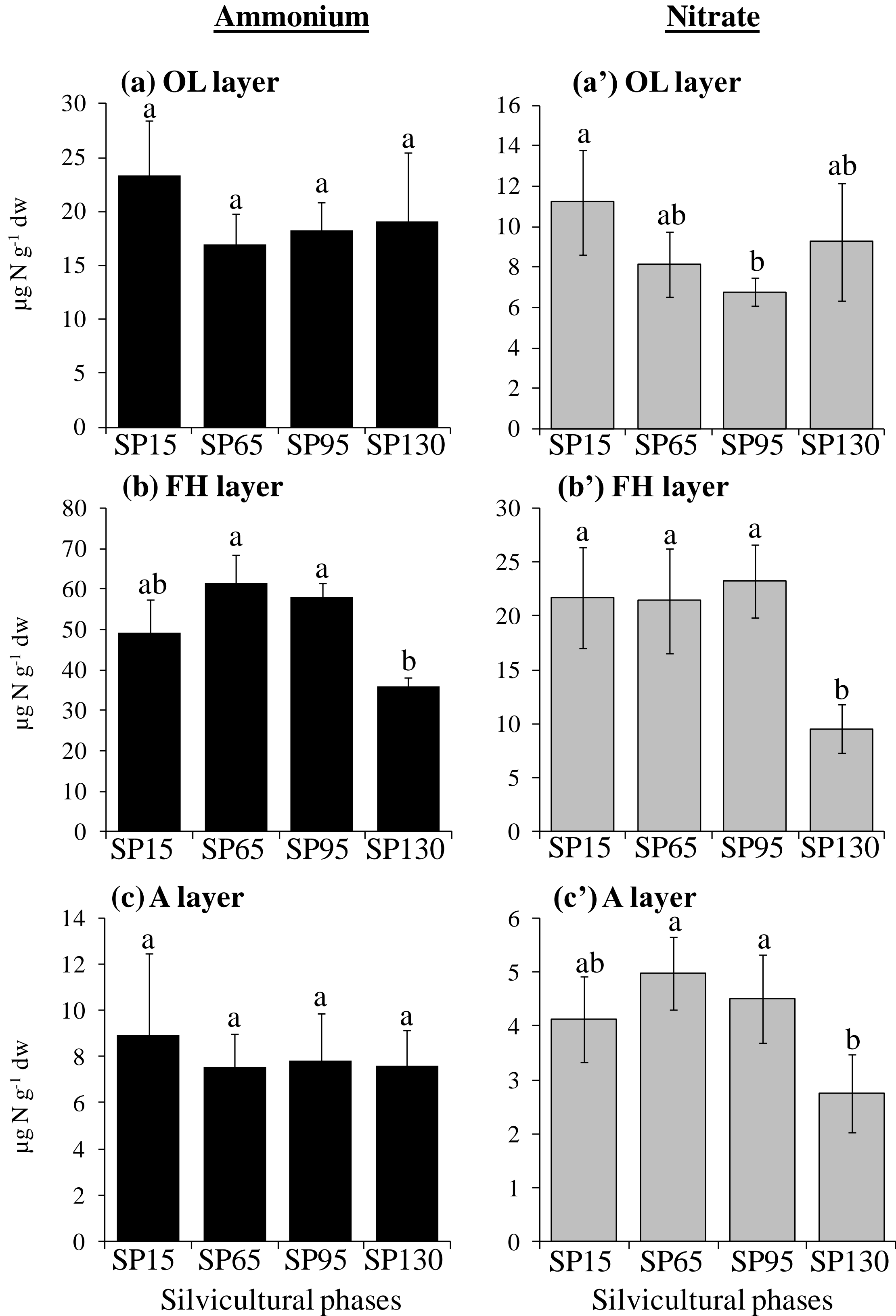

Figure 3.3. *Field mineral N contents (annual mean) within the OL (a=ammonium, a'=nitrate), FH (b=ammonium, b'=nitrate) and A layers (c=ammonium, c'=nitrate) expressed as mg N kg*$^{-1}$ *dw (dry weight) according to silvicultural phases. Bars are standard deviation and different letters indicate significant differences according to Kruskal-Wallis test at P < 0.05 (n=4).*

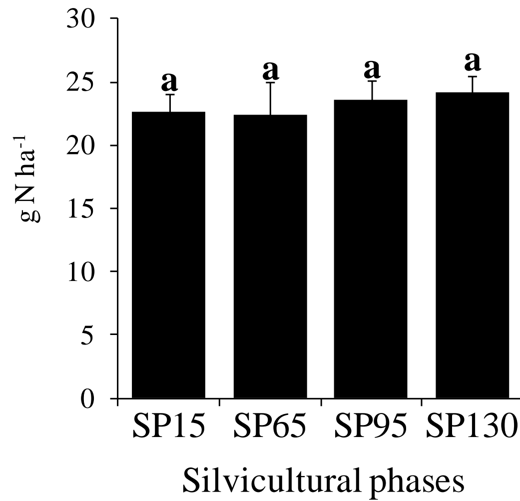

Figure 3.4. *In situ soil N_2O pools (annual mean) expressed as g N ha^{-1} according to silvicultural phases. Bars are standard deviation and different letters indicate significant differences according to Kruskal-Wallis test at P < 0.05 (n=4).*

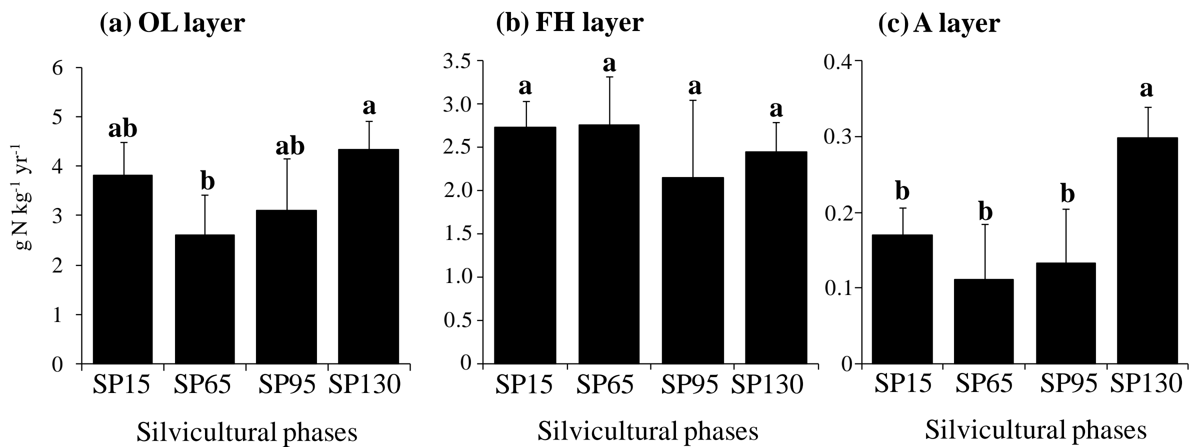

Figure 3.5. *In situ net N mineralization (annual mean) within the OL (a), FH (b) and A layers (c) expressed as g N kg^{-1} dry weight yr^{-1} according to silvicultural phases. Bars are standard deviation and different letters indicate significant differences according to Kruskal-Wallis test at P < 0.05 (n=4).*

In the A layer, SP130 exhibited the highest value (about 300 mg N kg^{-1} dry matter yr^{-1}) while SP15 showed the lowest one (about 50 mg N kg^{-1} dry matter yr^{-1}). SP15 and SP65 showed intermediates values. In the OL layer, annual net nitrification was maximal in SP15 and corresponded to 40% of total mineral N production (1.7 g-N kg^{-1} dry matter yr^{-1}). It was minimal in SP130 and percentage of nitrification attained only 10% (0.4 g-N kg^{-1} dry matter yr^{-1}) (Fig. 3.6). SP65 and SP95 exhibited intermediates values (28 and 32% of total mineral N production, respectively). Net nitrification and percentage of nitrification showed the same pattern in the FH layers. The maximal value was observed in SP15 with about 1 g-N kg^{-1} dry matter yr^{-1} (42%) while SP130 showed net nitrate immobilization (negatives values). In the A layer, we observed net nitrate immobilization in all silvicultural phases without differences along the chronosequence (Fig. 3.6).

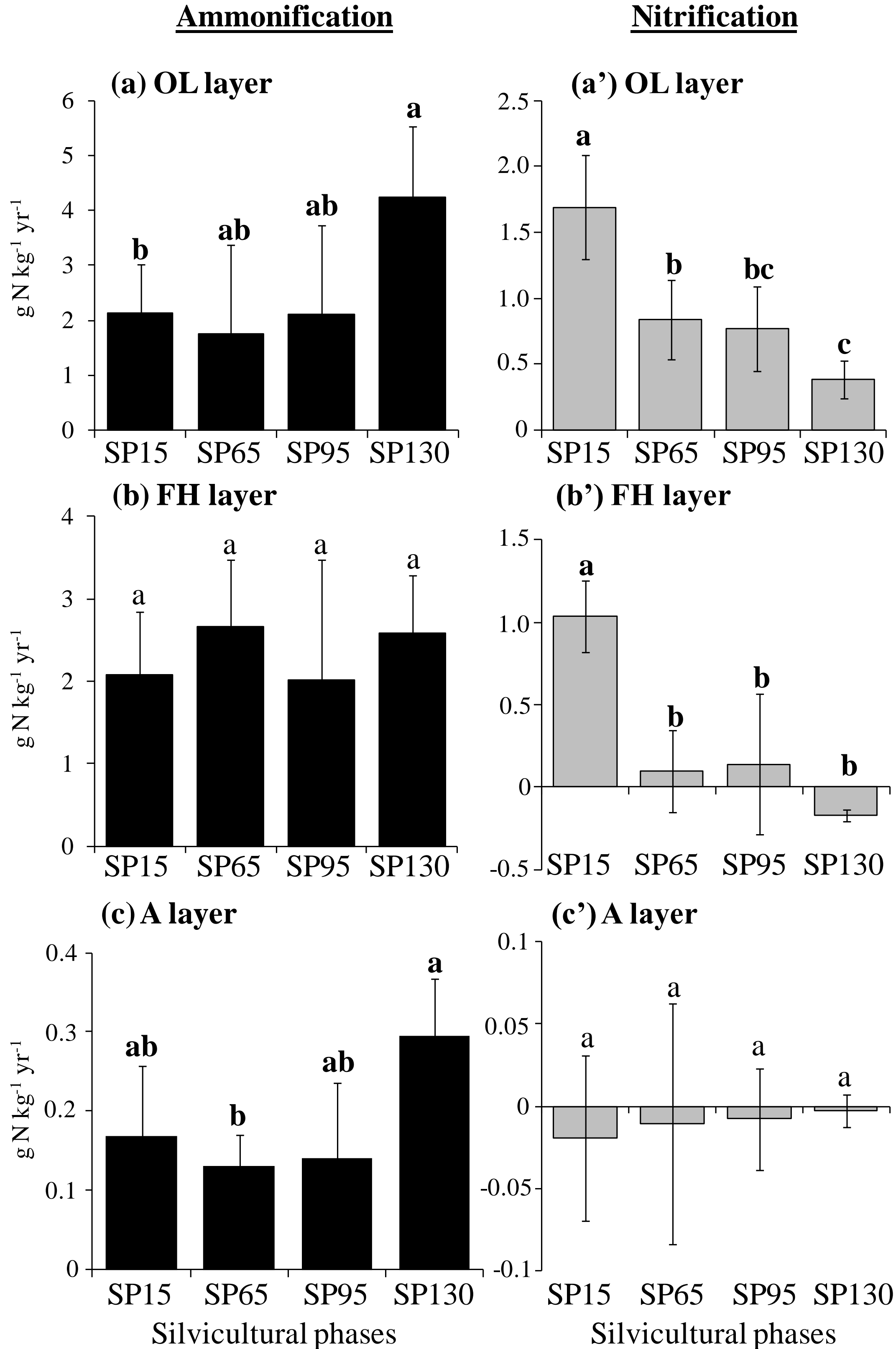

Figure 3.6. *In situ net ammonification and nitrification (annual mean) within the OL (a=ammonification, a'=nitrification), FH (b=ammonification, b'=nitrification) and A layers (c=ammonification, c'=nitrification) expressed as g N kg-1 dry weight yr-1 according to silvicultural phases. Bars are standard deviation and different letters indicate significant differences according to Kruskal-Wallis test at P < 0.05 (n=4).*

3.3. Autotrophic versus heterotrophic nitrification

The use of acetylene revealed that annual net nitrification was mainly achieved by autotrophic bacteria in the OL layer in SP15, SP65 and SP95 (more than 95% of contribution in net nitrate production) (Fig. 3.7). In contrast, in SP130, about 35% of nitrate production is achieved by heterotrophic microorganisms (bacteria and/or fungi). In the FH layer, annual net nitrification was mainly autotrophic (more than 97%), irrespective of silvicultural phases. However, we measured a weak heterotrophic contribution in net nitrification process in SP65 (about 3%). We observed the same trend in the A layer (Fig. 3.7).

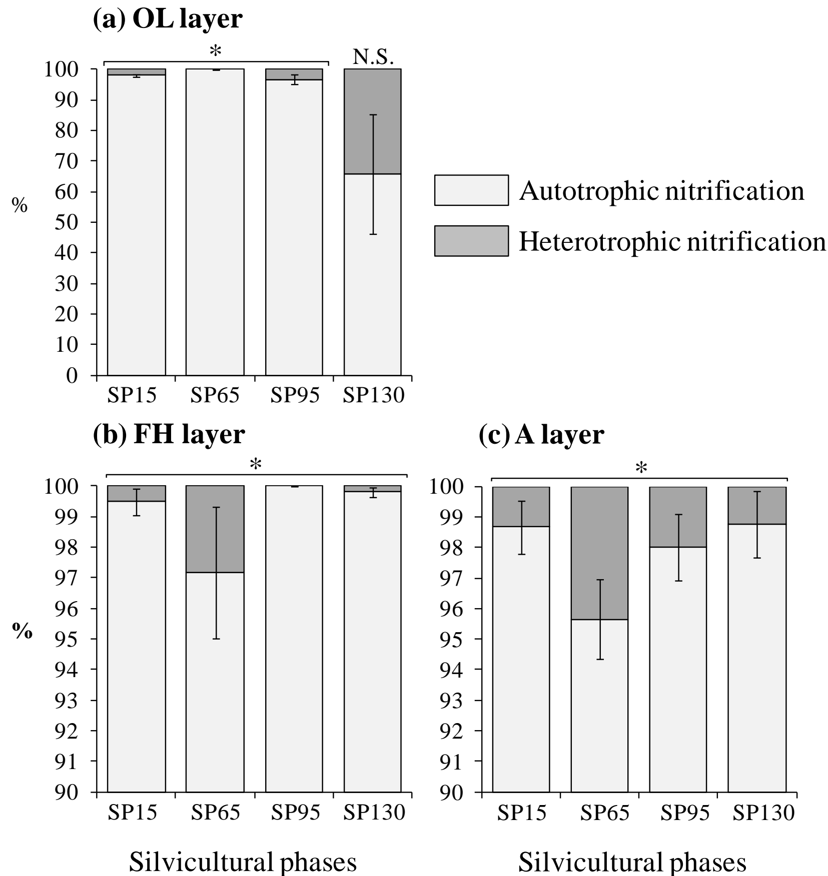

Figure 3.7. *Field net nitrification pathways, i.e. autotrophic bacterial and fungal, within the OL (a), the FH (b) and the A layers (c) expressed as % of contribution in total net nitrate production according to silvicultural phases. Bars are standard deviation. "*" indicates significant differences at $P < 0.05$ between autotrophic and heterotrophic pathways while "*N.S.*" refers to non significant (Wilcoxon rank sum test, n=4).*

3.4. Denitrification and N leaching

Annual *in situ* denitrification was maximal in SP15 (about 1.6 kg N ha^{-1} yr^{-1}) and minimal in SP130 (about 0.4 kg N ha^{-1} yr^{-1}) (Fig. 3.8). SP65 and SP95 showed intermediate values. The use of exchange resins outside the PVC cylinder allowed us to characterize mineral N leaching (with root uptake). SP135 exhibited the maximal value in ammonium leaching with about 3 kg N ha^{-1} yr^{-1} while SP95 showed the lowest one with about 1.5 kg N ha^{-1} yr^{-1}. SP65 and SP95 exhibited intermediate values. Ammonium leaching in SP15 was significantly higher than in SP95. In contrast to ammonium, nitrate leaching did not differ between silvicultural phases (about 10 kg N ha^{-1} yr^{-1}).

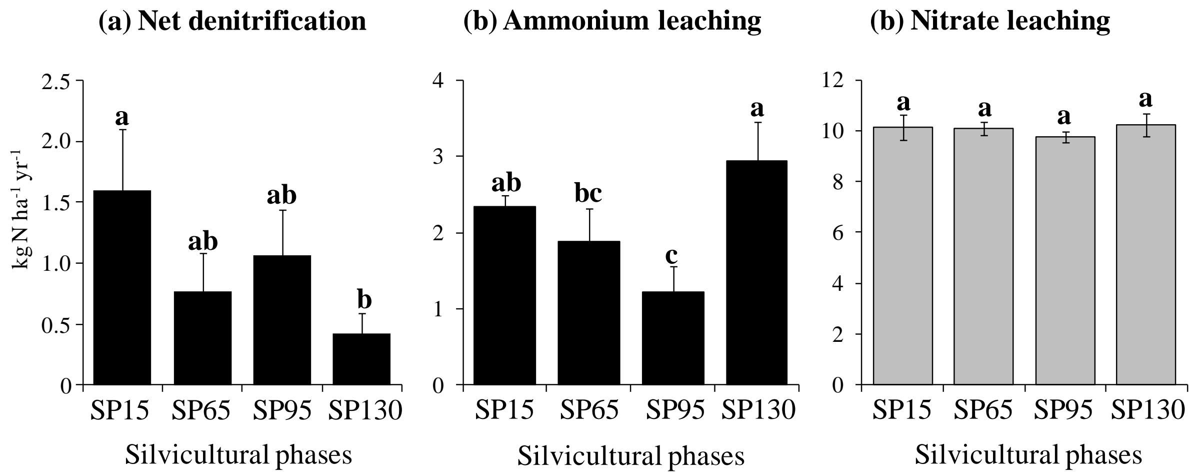

Figure 3.8. *(a) Field net denitrification and (b) mineral N leaching (with root uptake) expressed as kg N ha^{-1} yr^{-1} according to silvicultural phases. Bars are standard errors and letters (a, b and c) indicate significant differences according to Kruskal-Wallis test at P < 0.05 (n=4).*

3.5. N uptake by roots

Ammonium uptake by roots was higher in SP130 (about 115 kg N ha^{-1} yr^{-1}) and lower in SP15 (about 70 kg N ha^{-1} yr^{-1}) (Fig. 3.9). SP65 and SP95 exhibited intermediate values. The highest nitrate uptake was observed in SP95 (about 55 kg N ha^{-1} yr^{-1}) while SP130 exhibited the lowest value (about 45 kg N ha^{-1} yr^{-1}). SP15 and SP65 exhibited intermediate values. Total mineral N uptake was maximal in SP95 (about 160 kg N ha^{-1} yr^{-1}) and minimal in SP15 (about 120 kg N ha^{-1} yr^{-1}). SP15 and SP65 exhibited intermediate values.

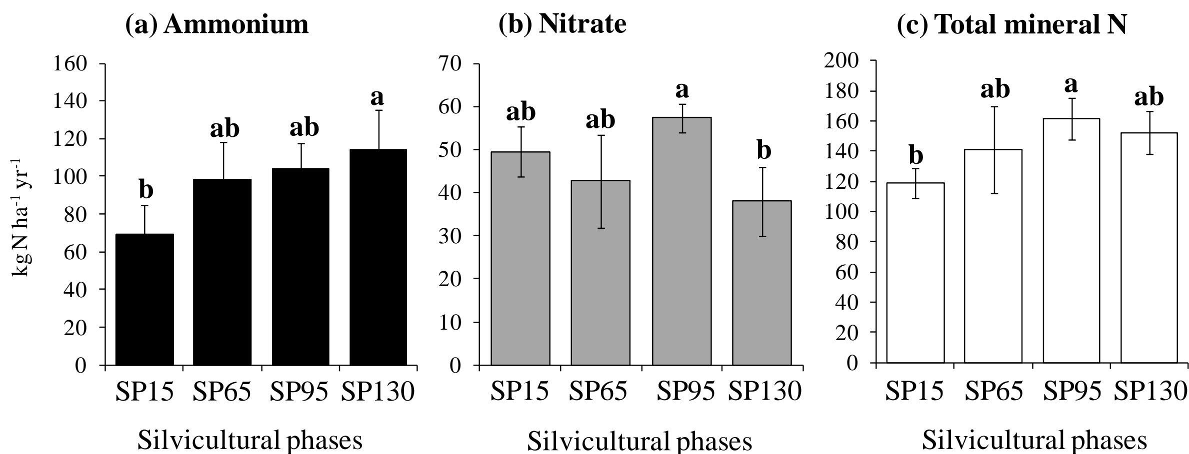

Figure 3.9. *(a) Ammonium, (b) nitrate and total mineral N uptake by roots expressed as kg N ha^{-1} yr^{-1} according to silvicultural phases. Bars are standard errors and letters (a and b) indicate significant differences according to Kruskal-Wallis test at P < 0.05 (n=4).*

3.6. Humus forms and soil N cycle co-variation patterns

Only two N variables were significantly correlated with morphological variables after adjusting and correcting levels of significance with the Truncated Product Method (Table 3.1). Net nitrification in the OL layer was positively correlated with the structure of the A layer (R^2=0.69, p<0.01) while net nitrification in the FH layers was negatively

correlated with the percentage of fine vegetal organic matter in the FH layer (R^2=0.63, p<0.05). *In situ* nitrate pool in OL was negatively correlated with the percentage of bleached leaves in OLv (R^2=0.67, p<0.05) and the thickness of the OF layer (R^2=0.66, p<0.05) and positively correlated with the number of earthworm casts in OLn (R^2=0.68, p<0.05).

Table 3.1. *Significant linear correlations between in situ N cycle and morphological variables.*

Soil layers	N variables	Morphological variables[a]	R^2	Slope	*P* value
OL layer	Net nitrification	A structure	0.69	+	**
	in situ nitrate pool	Percentage of bleached leaves in OLv	0.67	-	*
	in situ nitrate pool	OF maximal thickness	0.66	-	*
	in situ nitrate pool	Number of earthworm casts in OLn	0.68	+	*
FH layer	Net nitrification	Percentage of fine vegetale organic matter in OF	0.63	-	*

[a]Morphological variables are listed in the table 2.2 (chapter 2)
* $P < 0.05$
** $P < 0.01$

4. Discussion

We observed great changes in annual *in situ* mineral N production, leaching and uptake along the pure beech chronosequence, but two main shifts within N cycling emerged from this study. The first one corresponded to an increase in net ammonification in the OL and A layers in the mature stands, paired with higher ammonium leaching and ammonium uptake by roots. The second one corresponded to a decrease in net nitrification in OL and FH layers paired with lower nitrate uptake by roots and denitrification.

4.1. Ammonification increased along the chronosequence

The first main shift is characterized by a sharp increase in net ammonification in the OL and A layers. Those results are consistent with Idol *et al.*. (2003) who observed higher values in N mineralization in a 80-100-year-old upland hardwood forest stands compared to regeneration stands. It is possible that the development of the fragmentation (OF) and humification (OH) layers promote net ammonium production by providing large pools of particulate and dissolved organic matter. The OH horizon was considered by Park *et al.*. (2002) as the main source of dissolved organic carbon and nitrogen. In SP130, the abundance of fine organic matter characterizing OH horizon could facilitate high level of ammonification activity. Organic fecal pellets found in the OH horizon may serve as incubators for microbial activity and enhance ammonium production (Lavelle 1996; Lavelle and Spain 2001).

Another explanation lies on the promotion of fungi populations along the beech chronosequence and their role in ammonium production. In a parallel study (chapter 5), we observed higher fungal biomass in mature stands from the same chronosequence within the FH layers. Firstly, fungi are able to produce high levels of exoenzymes that efficiently depolymerize and mineralize organic N (Gobat et al. 2004). Second, fungal N requirements by C unit is very low compare to bacteria (Baath and Anderson 2003; Kooijman et al. 2009). The presence of fungi, which generally dominate in moder (Ponge 2003; Gobat et al. 2004) may favor lower microbial N immobilization leading to higher net ammonium production. This hypothesis may also explain why we observed heterotrophic nitrification in mature stands in the OL layers. It was already shown that

acidic soils can support the activity of heterotrophic nitrifiers (Lang and Jagnow 1986; Brierley and Wood 2001; Trap et al. 2009). Heterotrophic nitrifiers include bacteria and fungi that oxidize both organic and inorganic ammonia while utilizing organic carbon as an energy source. Finally, high fungal biomass may favor heterotrophic nitrification in the mature stands. The increase in ammonification is paired with higher ammonium uptake by roots and leaching. The explanation for these results is straightforward. Trees demand for N should increase during their phase of intense growth for the build-up of wood. Furthermore, it is well know that beech species uptake preferentially ammonium to nitrate (Gessler et al. 1998). Higher net ammonification in older stands may increase ammonium availability leading to higher ammonium uptake by roots and leaching.

4.2. Net nitrification decreased along the chronosequence

The second main shift is the sharp decrease in net nitrification occurring at the onset of the chronosequence. The decrease of net nitrification along stand development was also observed by other authors (Bauhus et al. 1997; Idol et al. 2003; Trap et al. 2009). For instance, Zeller *et al..* (2007) showed lower net nitrate production in old stands compared to young stands in a pure beech forest in north-east France. Brais *et al..* (1995) and Paré and Bergeron (1996) also found sharp declines in forest floor nitrification rates with increasing age in boreal mixedwoods.

Net nitrification was mainly achieved by autotrophic bacteria since nitrate production was reduced in presence of acetylene. The presence of autotrophic nitrification in acidic forest soils was already observed (Sainte-Marie and Paré 1999; Laverman et al. 2000) and its inhibition was often related to ammonium availability reduction and changes in soil properties such as pH or organic matter quality (De Boer et al. 1996; McCarty 1999; Sainte-Marie and Paré 1999; Brierley and Wood 2001; De Boer and Kowalchuk 2001; Strauss and Lamberti 2002; Montano et al. 2007). In this present study, we did not observe lower ammonium contents in OL, FH and A layers after 15 years of beech stands ageing. In addition, ammonium losses by leaching decreased at the onset of the chronosequence while ammonium production was stable. Those results suggest that autotrophic nitrification rates were not limited by the supply of ammonium. Autotrophic nitrification occurrence has been also explained by the presence of pH-neutral micro-sites (De Boer and Kowalchuk 2001). Nevertheless, we did not observed significant changes in pH or CEC in the first 5 centimeters above the OH layer along the beech chronosequence (table 2.1). It thus appears unlikely that autotrophic nitrification was inhibited by changes in soil nutrients availability or by soil acidification. Unfortunately, we cannot conclude and further investigations are required to identify the mechanisms implicated in the inhibition of nitrification.

Despite lower net nitrate production, nitrate leaching did not change between silvicultural phases while *in situ* denitrification decreased along the chronosequence. This indicates that nitrate leaching is not influenced by net nitrification but probably by N dry and wet N deposition. In contrast, denitrification is probably partly controlled by nitrification.

4.3. The weight of organic layers in field mineral N production

Changes in N cycle along forest maturation within the organic layers at different litter decay stages (*i.e.* unmodified, fragmentation litter or humus layer) are poorly documented with regards to the amounts of literature focusing on mineral horizons (Coté et al. 2000; Jussy et al. 2000; Idol et al. 2003; Inagaki et al. 2004; Zeller et al. 2007).

Furthermore, the amount and the structure of the organic materials within the forest floor change considerably during forest development, hence affecting soil N dynamics. Our study showed that the OL and FH layers contributed significantly to total mineral N production compared to organo-mineral layer and their contribution varied along the chronosequence. This may be related to the high active microbial biomass within the organic layers compared to the organo-mineral one (Graystone and Prescott 2005; Hannam et al. 2007; Trap et al. 2009). Higher microbial biomass usually leads to higher N turnover since all soil N transformations are performed by microorganisms. However, it is important to precise that organic layers contribution in mineral N production is reduced when calculating soil N fluxes in hectare-based unit, *i.e.* the contribution of the A layer in total mineral N production increase to a great extent compared to the organic layer due to higher depth and bulk density (*i.e.* about 500-700 t ha^{-1}) compared to the organic layers (*i.e.* between 14 and 16 t ha^{-1}).

4.4. Humus forms: potential indicators of in situ mineral N production?

In our study, humus forms shifted from dysmull in younger stands towards dysmoders in older stands (see table 2.1). Our results confirm that some morphological variables can be used to predict soil N mineralization pathways, especially nitrate-based variables. We showed that mull-type variables (*i.e.* variables which increase in mull) such as the number of earthworm cast in OLn and the structure of the organo-mineral layer increase with increasing values of net nitrification and nitrate pools in the OL layer. In contrast, moder-type variables (*i.e.* variables which increase in moder) such as the OF thickness, the abundance of fine organic matter in OF and the percentage of bleached leaves in OLv, decrease with decreasing values of net nitrification and nitrate pools in both the OL and the OF layers. Those results are in accordance with Bottner *et al.*. (1998) who observed, among seven humus forms sampled in coniferous forest sites along a north-south climatic sequence in Western Europe, the highest potential net nitrification values in mull humus forms and the lowest one in an acidic mor. Hirobe *et al.* (2003) found similar results in a *Cryptomeria japonica* plantation in Japan. They measured rapid net nitrification in mull soil while net nitrification in moder soil was relatively low and sometimes negative. The development of OF and OH layers in mature stands could favor fungal community and limit by competition autotrophic bacterial growth (Strauss and Lamberti 2002; Aubert et al. 2005) while higher earthworms grazing in mull may affect fungal population and favor N release at the microsite scale favoring autotrophic nitrification. Also, casting may provide mineral N by leaching, promoting hence bacterial growth (Decaens et al. 1999; Marhan and Scheu 2003).

In contrast to nitrate, ammonium-based variables were poorly correlated with humus forms and it appears difficult to predict net ammonium production with morphological variables. We found contrasting results in a parallel study where soil N pathways variations along the same chronosequence were characterized by aerobic incubation in the laboratory (see chapter 2). We found significant linear correlations between morphological variables and ammonium-based variables. For instance, the thickness of the FH layer was positively correlated with net ammonification. Aerobic laboratory incubation under optimal conditions provides estimates of potential N mineralization with low variability within groups. In contrast, the *in situ* conditions vary considerably and *in situ* investigations usually present high variability in soil N variables within silvicultural phases. High variability in N transformations may certainly hide significant correlations between N and morphological variables. However, we can conclude without doubt that the shift mull-mode*r* along the chronosequence is

synonymous to lower *in situ* net nitrification and nitrate pools in the organic layers even if morphological variables investigated in this present study predicted poorly *in situ* ammonium-based variables trends along the chronosequence.

4.5. Positive N feedbacks

Our results show that beech forest maturation leads to substantial shifts in both soil N pools and fluxes. More accurately, we observed an increase in the contribution of net ammonification in total net mineral N production and of ammonium in total mineral N uptake by trees along the pure beech chronosequence. Finally, those results suppose that forest maturation favor upper-N process in the cycle (such as N depolymerization and ammonification) but inhibited lower-N processes (such as nitrification and denitrification). We wonder if these changes in soil N fluxes maximize N acquisition by beech trees and consequently forest productivity. Indeed, it is well know that *F. sylvatica* is a strong ectomycorhizal species (Simard et al. 2002; Smith and Read 2008), efficient in taking up both organic and mineral N and especially ammonium (Gessler et al. 1998; Wallenda and Read 1999). It is possible that the increase in net ammonification and the decrease in net nitrification along beech forest ageing limit ammonium loss and favor ammonium accumulation in both the organic and organo-mineral layers. It would thus lead to a clear competitive advantage of beech trees over microbial species (bacteria and saprotrophic fungi) in mineral N acquisition. Further investigations, with comparable methods to reduce the error in the data, have to be performed in order to identify what factors are implicated in the control of soil N cycle along forest maturation.

Acknowledgements

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III. Synthèse du chapitre 3

La caractérisation *in situ* du cycle de l'azote expose des patrons de variations temporels et spatiaux significatifs. Les résultats montrent une augmentation de l'ammonification nette et une diminution de la nitrification nette le long de la chronoséquence. Au sein des horizons organiques, la nitrification nette est présente uniquement à 15 ans. Elle est absente au sein de l'horizon organo-mineral, quel que soit l'âge du peuplement. Le lessivage des ions nitrate ne varie pas le long de la chronoséquence alors que celui des ions ammonium diminue de 65 à 95 ans. La dénitrification *in situ* diminue le long de la chronoséquence. La contribution de l'ammonificiation dans la production totale d'azote minéral augmente graduellement le long de la chronoséquence. Le prélèvement d'azote ammoniacal par le peuplement, ainsi que le ratio ammonium/nitrate prélevé, augmentent le long de la chronoséquence. Ces résultats suggèrent un contrôle du cycle de l'azote en faveur des processus en amont du cycle (*i.e.* apport d'azote, production d'ammonium). Au contraire, les processus en aval du cycle (*i.e.* nitrification, denitrification) semblent être inhibés au fur et à mesure que le peuplement vieillit. Nous avons également observé des corrélations significatives entre les variables morphologiques et la nitrification nette ou la teneur en nitrate au sein des horizons organiques. Certaines variables morphologiques (*i.e.* l'épaisseur de l'OF, le nombre de turricules de vers de terre, la structure de l'horizon A ou le pourcentage de feuilles blanchies au sein de l'horizon OLv) présentent un potentiel indicateur de production *in situ* d'azote minéral.

PARTIE II

Les facteurs de contrôle des formes d'humus et du cycle de l'azote le long d'une chronoséquence de hêtraies pures

PANIERS A LITIERE - FORÊT D'EAWY - PARCELLE 265 - NOVEMBRE 2007

CHAPITRE 4
Production et décomposition de la litière

I. Présentation du chapitre 4

La formation d'un horizon d'humification le long de la maturation des peuplements forestiers dépend de deux processus : la production et la vitesse de décomposition de la litière. L'apport annuel croissant de litière peut aboutir après plusieurs années, à une accumulation de débris organiques transformés, dans le cas où la vitesse de décomposition de la litière resterait constante le long de la maturation des peuplements. Une autre hypothèse, non exclusive, repose sur une diminution de la vitesse de décomposition de la litière au cours du vieillissement des peuplements forestiers. L'objectif de cette étude est de caractériser la production et la vitesse de décomposition de la litière de hêtre le long d'une chronoséquence de 130 ans de hêtraies pures. Pour cela, des paniers à litière ont été placés dans chaque parcelle et la production de litière a été suivie pendant un an. La technique de « litterbag » a été utilisée afin de caractériser les vitesses de décomposition des litières.

II. Article 3

Does moder development along pure beech (*Fagus sylvatica* L.) stands ageing come from changes in litter production or decomposition rates?

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Abstract

Development of forest usually goes with organic material accumulation within the forest floor, leading to a shift from mull humus forms under young stands to *moder* ones under older stands. It has been suggested that an increase in nutrient uptake by trees during their intense growth phase leads to a topsoil acidification, a decrease in earthworms density and thus a decrease in litter turnover. The question of the paper was to ask whether this shift mull-moder occurring along forest ageing is due to higher leaf litter production and/or lower decay rates. For that purpose, we investigated changes in macro-morphological properties of humus forms, leaf litter production and litter decay rates along a 130 years old pure beech (*Fagus sylvatica* L.) forest chronosequence. We also aimed at assessing topsoil pH and nutrient availability along the chronosequence. Annual litter production did not change significantly along the chronosequence but tended to increase in stands older than 15 years old. In contrast, litter decay rates decreased significantly during the phase of intense growth of trees. Even in absence of significant patterns of variation, litter production was significantly correlated with the thickness of the OF layer. However, litter decay was significantly correlated with the OH maximal thickness suggesting that the appearance of the humification layer was mainly due to the decrease in litter decay rates. We did not found significant changes in the main properties of the organo-mineral horizon suggesting that soil nutrients availability may not affect litter dynamics along the chronosequence. Complementary studies focusing on the quality of beech leaves along stand ageing should provide insights about the cause of humus forms and litter dynamics changes at the rotation scale.

Keywords

Litter production, litter decomposition rates, humus forms, soil nutrient availability, 130-yr-old chronosequence, *Fagus sylvatica*, loamy acidic soil.

1. Introduction

Successional patterns of humus forms have often been described along both temperate managed (Aubert et al. 2004, Chauvat et al. 2007) and semi-natural forest maturation (Bernier and Ponge 1994, Ponge and Delhaye 1995). Early successional stages have been associated with fast organic matter turnover (translated into mull humus form) while lower organic matter (OM) recycling (expressed as moder occurrence) have been frequently observed under older forest on acidic soils (Ponge 2003, Salmon et al. 2006). More precisely, the appearance of moder with stands ageing consists in development of both fragmentation and humification litter layers, including high proportions of fine organic matter from fauna faeces.

Accumulation of organic materials on the forest floor along the forest development takes place until litter production do not equal litter decomposition. The gradually higher amount of organic matter falling each year along stands ageing may not be fully assimilated and incorporated within mineral horizons by soil organisms. Only soluble and non-lignified cell carbohydrates are totally assimilated and transformed during the early phases of leaf litter decomposition (Lavelle and Spain 2001, Berg and Laskowski 2006c, a, Salmon et al. 2006). Each year, more and more recalcitrant compounds (lignified carbohydrates) may accumulate within the forest floor and promote the formation of fragmented and humified horizons (Lebret et al. 2001, Salmon et al. 2006).

Besides litter production, changes in litter decay rates may also lead to the appearance of a humification horizon. Ponge (2003) and Ponge *et al.* (1998) assumed that higher uptake of nutrients by trees during their phase of intense growth, for the build-up of wood, may lead to lower nutrient availability in the soil thereby affecting soil biological activity, especially earthworms activity (Salmon et al. 2006). Indeed, there is also a global trend of decreasing abundance of earthworms during the phase of intense growth of trees (Ponge 2003). This may lead to a decrease in litter decay rates promoting the accumulation of fragmentation and humification litter layers. Nevertheless, Hedde *et al.* (2007) did not found any significant changes in macrofauna species turnover along a 200 years old pure beech chronosequence in France despite clear changes in humus forms suggesting that others factors than earthworms occurrence may be implicated.

The question of the paper was thus to ask whether the shift mull-moder occurring along beech forest ageing is due to changes in leaf litter production and/or decay rates. For that purpose, we investigated changes in macro-morphological properties of humus forms, leaf litter production and litter decay rates along a 130-yr-old pure beech (*Fagus sylvatica* L.) forest chronosequence in the same site used by Hedde *et al.* (2007). The co-variation patterns of litter dynamics (production and decomposition) and humus macro-morphology were investigated using multiple regression analysis. We also aimed at assessing soil nutrient availability along the pure beech forest chronosequence in order to validate in a managed context the hypothesis developed by Ponge (2003) about semi-natural systems.

2. Materials and methods

2.1. Study site

The study site was located in the Eawy state forest (France, Upper Normandy, 01°18’ E; 49°44’ N; 7200 ha). The climate is temperate oceanic with a mean annual

temperature of +10°C and a mean annual precipitation of 800 mm. A space-for-time substitution procedure (Pickett 1989) was used to empirically reconstitute an even-aged forest chronosequence: sixteen pure beech stands were selected to represent four silvicultural phases (SP) of different ages: 13-18 years (SP15), 65-66 years (SP65), 91-103 years (SP95) and 121-135 years (SP130) (see Table 2.1). Each phase was comprised of four replicated stands. All stands were managed as even-aged forest by the French Forestry Service (ONF). All of them were in a flat (plateau) topographic situation (205 m a.s.l.). The soil was an endogleyic dystric Luvisol (FAO 2006) developed on more than 80 cm of loess (lamellated silt) lying on clay with flints (Lautridou 1985, Laignel et al. 1998). The understory vegetation was defined as a characteristic *Endymio-Fagetum* according to phytosociological classification (Durin et al. 1967). At the centre of each stand, a 16 m^2 square plot was delimited away from vehicle tracks and tree trunks to avoid any acidification due to local organic matter accumulation (Beniamino et al. 1991).

2.3. Organic and organo-mineral horizons morphology

Macro morphological descriptions of organic and organo-mineral layers were previously done within frames (25 cm x 25 cm) at three corners of the central plot according to the French nomenclature (Jabiol et al. 2007) in May 2007 (Table 2.1, page 45). A total of 36 macro-morphological variables were described in the field on the basis of variation visible to the naked eye (Table 2.2, page 47). We distinguished mull (mainly dysmull) and moder (hemimoder + eumoder + dysmoder) humus forms on the basis of morphological characters (Table 4.1) (Jabiol et al. 2007).

Table 4.1. *Main properties of the humus forms occurring along the chronosequence according to Brêthes et al. (1995; 1998); Jabiol et al. (2007); Gobat et al., (1998) and correspondence with Green et al. (1993) classification.*

Properties	Humus forms			
	Mull	Moder		
	Dysmull	Hemimoder	Eumoder	Dysmoder
OLn	×	×	×	×
OLv	×	×	×	×
OF	×	×	×	×
OH			×/(×) <1cm	× >1cm
O/A transition	Discontinuity between O and A	O – A progressive transition		
A structure	Biomacrostructured	juxtaposition A$^{\$}$		
A pH	<5	<4.5	<4.5	<4.5
A C/N ratio	15-20	20-25	20-25	≤25
Soil fauna main groups	Anecic earthworms White rot fungi	Macro-arthropods	Mesofauna and insecta larvae	
Bacterial activity	+ ←			−
Fungal activity	−			→ +
Green et al (1993)	Lepto-moder	Leptomoder / mormoder		

× : continuous horizon; (×) discontinuous horizon

$^{\$}$juxtaposition A : A with massive or single-grain structure but no biological or chemical structure; humified organic matter is juxtaposed to mineral matter

A total of 48 humus profiles were described (3 descriptions per stand × 16 stands). We differentiated:

(1) **the OL horizon** consisting of almost unmodified leaf and woody fragments. Most of the original plant structures are easily discernible. Leaves may be recently fallen and unmodified (OLn) or bleached and slightly fragmented (OLv);

(2) **the OF horizon** consisting of a mixture of coarse plant debris with fine organic matter (humus). This horizon is characterized by an accumulation of partly decomposed organic matter derived mainly from leaves and woody materials, which are fragmented and mixed with invertebrate faeces. The proportion of organic fine substances ranges from 10 to 70 % by volume;

(3) **the OH horizon**, which is an organic horizon characterized by an accumulation of decomposed organic matter. The original plant structures and materials are not discernible. Organic fine substances (invertebrate faeces) account for more than 70 % by volume. The OH horizons differs from the OF horizon by more advanced humification due to the action of soil organisms;

(4) **the A horizon** or **organo-mineral horizon** different in depth and structure among humus forms.

2.3. Chemical properties of the A horizon

Four subsamples of the A horizon were collected in May 2007 within frames (25 x 25 cm) located in each corner of the central square plots. In the laboratory, the samples were sieved (2 mm) and air-dried. In dry samples of the A horizon, concentrations of total C and N were measured by gas chromatography with a CHN pyrolysis micro-analyser. C-to-N ratio, pH_{water} and pH_{KCl} (Baize 2000), contents in available P (Duchaufour and Bonneau 1959), and Cation Exchange Capacity (CEC) were also determined in the samples as well as total elements (Ca, Mg, K, Mn, Na, Al, Fe) by the cobaltihexamine exchangeable method (Ciesielski and Sterckeman 1997).

2.4. Litter production

Litterfall collectors, $1m^2$, 30cm deep and 1m height, were built in order to characterize the annual litter production. Three collectors were placed in each stand along a 40m transect at 0, 20 and 40m. Litter was sampled every month from October 2007 to October 2008. A total of 48 collectors were installed (16 stands x 3 collectors per stand). Litterfall samples were oven-dried at 65°C for 48h. Dry samples were sorted and litter components were classified into categories then weighed. Litter categories were leaves, wood fragments and reproductive organs. The wood category included dead wood and bark. Reproductive organs included beech mast and male flowers. Litterfall samples were stored at room temperature.

2.5. Litter decay

We used the litterbag method (Bocock and Gilbert 1957, Verhoef 1995). Litterbags (15 x 20cm) were made from nylon net with 0.175mm mesh. They were filled with 10g of dried leaves. This amount of litter corresponds to the mean annual litter production in French beech forests according to Lebret and al. (2001). Four different litterbag types were prepared, consisting of litter from four tree age classes (15, 65, 95 and 130 years). In each stand, 6 litterbags filled with the corresponding age class were

inserted in the forest floor between OL and OF horizons. A total of 96 litter bags (6 litterbags x 16 stands) were thus placed in the field on December 2007. For each silvicultural phase, 4 replicate bags (one per stand) were removed 1 month, 3 months, 5 months, 7 months, 10 months and 12 months after the beginning of the experiment. Litter bags were packed in plastic bags and transported to the laboratory. The content was then oven dried at 60°C until constant weight and weighed to determine litter dry mass.

2.6. Data treatment and statistical analyses

The decay rate coefficient (k) estimates the disappearance of litter on an annual basis, using the following negative exponential decay function:

$$X_t/X_0=e^{-kt}$$

where X_0 is the original mass of litter and X_t is the mass remaining at time t (Olson 1963). The k value was used to assess the turnover time of litter ($1/k$) (Olson 1963). Means and standard errors were calculated by silvicultural phases for all variables (4 true replications of stands by age class). Comparisons of means between silvicultural phases were done by one way ANOVA and Tukey HSD post-hoc tests. The normality of data and the homogeneity of variances were previously checked using Wilk-Shapiro and Bartlett tests, respectively. We performed stepwise multiple regressions with backwards elimination to assess the soil morphological variables that was significantly link to both litter production and litter decay, after screening potential independent variables for significant autocorrelation (R^2> 0.90). We also investigated the relationships between OH-based variables and litter production or decay rates using linear correlations. All tests were computed with the R freeware (R Development Core Team 2008) and statistical significance was set at P < 0.05.

3. Results

3.1. Litter production along the chronosequence

Leaf production did not vary between silvicultural phases (Table 4.1). It was minimal in SP15 (2.08 t.ha^{-1}) and maximal in SP65 (2.58 t.ha^{-1}). The production of reproductive organs was higher in SP130 and lower in SP65. Wood production did not change between silvicultural phases. Total litter production was higher in SP130 (2.82 t ha^{-1}) and lower in SP15 (2.27 t ha^{-1}). SP65 and SP95 showed intermediate values. Leaf was the most abundant component of litterfall in all silvicultural phases. Wood was the second one except for SP130 where reproductive organs were most abundant (Table 4.1).

Table 4.2. *Litter production (expressed as t ha^{-1} yr^{-1}) according to silvicultural phases.*

Variables	**Silvicultural Phases**											
	SP15			SP65			SP95			SP130		
Leaves	2.08	(0.25)	a	2.58	(0.41)	a	2.42	(0.41)	a	2.57	(0.36)	a
Reproductive organs	0.05	(0.03)	**ab**	0.04	(0.01)	**b**	0.11	(0.06)	**ab**	0.13	(0.07)	**a**
Wood	0.09	(0.04)	a	0.05	(0.02)	a	0.15	(0.09)	a	0.11	(0.08)	a
Total	2.27	(0.20)	a	2.69	(0.42)	a	2.68	(0.51)	a	2.82	(0.45)	a

Data are means (SD). Letters (a and b) refer to significant differences between silvicultural phases according to one-way ANOVA and Tukey HSD test (P < 0.05 level, n=4)

3.2. Litter decomposition along the chronosequence

After 12 months of litterbag experiment, SP95 and SP130 exhibited a higher remaining mass percentage (68 and 65%, respectively) compared to the youngest stands (53%) (Table 4.2). SP65 showed intermediate values (59.99%). The decay rate coefficient (k) was higher in SP15 (0.050) compared to SP95 (0.032). SP65 and SP130 showed intermediate values of k (0.043% and 0.036%, respectively). The turnover time ($1/k$) was lower in SP15 compared to the older stands (SP95 and SP130), with about 20 months, 31 and 28 months, respectively (Table 4.2).

Table 4.3. *Litter decomposition parameters according to silvicultural phases. The decomposition constant k was calculated from* $(X_t/X_0)=e^{-kt}$*, where* X_0 *is the original amount of litter and* X_t *is the amount of litter remaining at time t.*

Variables	Silvicultural Phases SP15	SP65	SP95	SP130
Final remaining mass (% initial mass)	52.86 (3.38) **b**	59.99 (6.03) **ab**	67.65 (2.80) **a**	65.04 (4.69) **a**
k value	0.050 (0.008) **a**	0.043 (0.008) **ab**	0.032 (0.003) **b**	0.036 (0.006) **ab**
$1/k$ (months)	19.98 (2.62) **b**	23.98 (5.09) **ab**	30.91 (3.12) **a**	28.34 (4.47) **a**
R^2	0.91***	0.91***	0.95***	0.93***

Data are means (SD). Letters (a and b) refer to significant differences between silvicultural phases according to one-way ANOVA and Tukey HSD test ($P < 0.05$ level, n=4). ***$P < 0.001$

3.3. Chemical properties of the A horizon

We did not observe any significant changes in chemical properties in the A horizon, except for Mg pools (Table 4.3). Soil Mg content was higher in SP15 compared to SP65 and SP130. CEC, Ca and Na contents as well as pH_{water} and pH_{KCl} decreased marginally along the chronosequence, while P, H and Al contents and ΔpH tended to increase.

Table 4.4. *Main properties of the organo mineral horizon (A) according to silvicultural phases.*

A properties	Silvicultural Phases SP15	SP65	SP95	SP130
Total C (g kg^{-1})	54.42 (24.92) a	41.81 (4.63) a	50.38 (14.80) a	45.92 (8.93) a
Total N (g kg^{-1})	3.26 (1.29) a	2.62 (0.28) a	3.04 (0.71) a	2.97 (0.52) a
C/N (g kg^{-1})	16.31 (1.16) a	15.92 (0.88) a	16.37 (1.46) a	15.29 (0.41) a
P (g kg^{-1})	0.17 (0.04) a	0.15 (0.05) a	0.21 (0.02) a	0.22 (0.17) a
CEC (cmol+ kg^{-1})	6.45 (0.97) a	5.40 (0.34) a	6.00 (0.73) a	5.73 (0.36) a
H (cmol+ kg^{-1})	0.90 (0.30) a	0.90 (0.05) a	1.14 (0.13) a	1.10 (0.15) a
Ca (cmol+ kg^{1})	1.44 (1.05) a	0.54 (0.25) a	0.72 (0.39) a	0.39 (0.15) a
Mg (cmol+ kg^{-1})	0.37 (0.07) **a**	0.21 (0.03) **b**	0.27 (0.07) **ab**	0.22 (0.04) **b**
Na (cmol+.kg^{-1})	0.09 (0.05) a	0.06 (0.01) a	0.07 (0.01) a	0.07 (0.01) a
K (cmol+ kg^{-1})	0.21 (0.04) a	0.18 (0.03) a	0.26 (0.06) a	0.21 (0.05) a
Fer (cmol+ kg^{-1})	0.07 (0.06) a	0.07 (0.04) a	0.05 (0.02) a	0.08 (0.03) a
Mn (cmol+ kg^{-1})	0.19 (0.10) a	0.21 (0.10) a	0.23 (0.07) a	0.19 (0.05) a
Al (cmol+ kg^{-1})	3.79 (1.52) a	3.91 (0.49) a	4.14 (0.37) a	4.11 (0.32) a
pH_{water}	3.97 (0.38) a	3.97 (0.19) a	3.80 (0.06) a	3.86 (0.10) a
pH_{KCl}	3.33 (0.25) a	3.20 (0.06) a	3.09 (0.04) a	3.10 (0.09) a
ΔpH	0.64 (0.14) a	0.77 (0.14) a	0.71 (0.06) a	0.76 (0.12) a

Data are means (SE). Letters (a and b) refer to significant differences between silvlicultural phases according to Kruskal-Wallis rank sum test and Multiple comparison tests after Kruskal-Wallis test (P=0.05 level, n=4)

3.3. Humus forms and litter dynamics co-variation patterns

Litter production was positively related to the percentage of leaves fragments in OLn and bleaches leaves in OLv, the OF maximal thickness, the abundance of live roots and negatively related to the number of earthworms casts in the OLv (P < 0.01, R^2 = 0.89) (Table 4.4.). In contrast, litter decay rate (k) was negatively related to the maximal thickness of the OF and OH layers, the percentage of skeletonized and brown leaves in the OLv layer (P < 0.05, R^2 = 0.62) (Table 4.4.).

Table 4.5. *Stepwise multiple regressions with backwards elimination of litter production or decay with soil morphological variables.*

Soil morphological variables	Litter production	Litter decay rate (*k*)
Leaves fragments in OLn	X(+)	
Earthworms casts in OLn	X(-)*	
Bleach leaves in OLv	X(+)**	
Live roots in OLv	X(+)*	
Skeletonized leaves in OLv		X(-)
Brown leaves in OLv		X(-)
OF maximal thickness	X(+)*	X(-)**
OH maximal thickness		X(-)**
P value	**	*
R^2	**0.89**	**0.62**

An "X" indicates that the variable was included in the model from the multiple regression analysis. The sign of the relationship between significant soil morphological variables and litter production or decay rates is indicated between brackets. * P < 0.05; ** P < 0.01.

4. Discussion

4.1. Litter dynamics and humus forms co-variation patterns along the chronosequence

Our results showed that litterfall production did not change significantly along the pure beech chronosequence. The absence of significant variation agree with Starr *et al.* (2005) who did not find any correlation between stand age and litterfall production in Scots pine stands in Finland. Nevertheless, even insignificant, a shift in litterfall production occurred at the onset of the chronosequence (SP15 *versus* SP65). This tendency was also observed by Lebret *et al.* (2001). In their study, beech litter production varied from 1.15 t ha^{-1} yr^{-1} in a thicket stand (10-yr-old) to 3.13 t ha^{-1} yr^{-1} in a mature stand (147-yr-old) from the Fougères forest in northwestern France.

Besides climate and soil conditions, changes in litter production were often related to stand structure such as relative basal area, breast height diameter or mean height and thinning intensity (Ranger et al. 1995, Lebret et al. 2001, Saarsalmi et al. 2007). In this present study, we did not find significant relationships between stand structure variables and litter production. Probable higher foliar biomass per tree in mature stand may compensate the decrease in tree density along the chronosequence leading to constant litter production.

In contrast, our results showed that litter decomposition changes greatly along the chronosequence. The litter decay rates during the intense tree growth phase (SP65 and SP95) were significantly lower than for the young and old phases (SP15 and SP130). These changes in litter decay may be often related to changes in the activity of soil organisms (Berg and Laskowski 2006b). Ponge (2003) explained that anecic earthworms populations are particularly sensitive to tree development due to soil acidification (Ponge and Delhaye 1995, Salmon et al. 2006). The abundance of anecic earthworms tends to decrease during the phase of intense growth of trees followed by progressive recovering as trees reach maturity then senesce (Bernier and Ponge 1994, Salmon et al. 2006). However, Hedde *et al.* (2007) did not found any significant changes in macrofauna species turnover along a 200 years old pure beech chronosequence from the same site used in this present study, *i.e.* the Eawy forest in France. The authors discussed that the lack of change in species composition along the chronosequence may reflect the effect of silvicultural practices; *i.e.* both mono-culture of a soil-acidifying tree species on acidic soil and the tillage used to assist natural regeneration. Those practices may have dramatic impacts on burrowing earthworms. Furthermore, they reported low species number of litter-dwelling earthworms (5 species). The soil pH might be too much acid even in the youngest stands (pH_{water} 3.97) to permit high anecic earthworms abundance. Finally, according to Hedde *et al.* (2007) results, the observed changes in litter decay rates may not be related to earthworm's community structure and activity.

Other explanation lies on the high lignin content in beech litter (Karroum et al. 2005, Sariyildiz and Anderson 2005, Hobbie et al. 2006). *Fagus sylvatica* is a woody species which produces low quality leaf litter with high level of lignin and low level in nitrogen (Sariyildiz and Anderson 2003a, Hobbie et al. 2006) compared to early successional species such as birch (*Betula pendula*) or hornbeam (*Carpinus betulus*). Karroum *et al.* (2004; 2005) showed that lignin degradation occurring in the organic layers consists in an increase in the vanillic acid/aldhehyde ratio. The production of phenolic monomers during lignin degradation probably leads to strong acidification in both organic and organo mineral horizons during forest development. Consequently, microflora activity may decrease and affect litter decay rates. Nevertheless, this hypothesis did not explain why litter decay was higher in SP130 than in SP95. The return of higher litter decay in the oldest stands may suppose that beech litter quality change at the end of the chronosequence. An investigation of litter quality along the chronosequence may provide insights in our understanding in moder development with forest ageing. It would be also interesting to investigate the soil microbial community structure and function along the pure beech chronosequence in both the organic and organo mineral layers to validate this hypothesis.

4.2. Humus forms and litter dynamics co-variation patterns

Even if litter production changes were not significant at $P < 0.05$, we showed that they were positively correlated with the maximal thickness of the OF layer suggesting a possible effect of litterfall increase on moder development. It was also highly correlated with the percentage of bleach leaves in the OLv layer and negatively correlated with the number of earthworms casts in the OLn layer. It is probable that higher beech leaf litter production favored the fungal community due to high recalcitrant compounds (lignified carbohydrates) content in litter, which may accumulate within the forest floor along the chronosequence. This hypothesis is supported by Karroum *et al.* (2005) investigation of morphological evolution of beech litter and biopolymer transformation in humus forms from Fougères forest in France. They showed that fungal attack occurred mainly within

the OL and OF layers while bacteria activity seemed efficient within the OF and especially the OH layers where they were responsible for structural polysaccharide degradation. The gradually higher amount in rich-lignin leaf litter falling each year with stands ageing may promote the fungal community within the OL layer, spatial segregation of soil decomposers and consequently the thicken of the fragmentation layer (Lebret et al. 2001, Salmon et al. 2006). Our results also showed that litter production was significantly correlated with the abundance of live roots in the OLv layer. We indeed observed higher root colonization in the OF and OH layers along the chronosequence in this present study. This statement corroborate with Ehrenfled *et al.* (1992) works who showed that as forest floor materials accumulate along forest development, the percentage of fine roots increase within the organic horizons of a variety of coniferous forests. Since beech is a strongly ectomycorrhizal species (Taylor et al. 2000), the presence of roots in the OF and OH layers may promote the ectomycorhizal fungal community in the organic litter layers and in turn limit microbial community activity (Ehrenfeld et al. 1997).

We showed that litter decay rates were highly correlated with the OH maximal thickness suggesting that the development of moder may result from the decrease in litter decay rates in SP65 and SP95. Litter decay was also negatively correlated with the OF maximal thickness and the percentage of skeletonized and brown leaves in the OLv, suggesting that changes in mesofauna and enchytraeids community may take place along the chronosequence and affect greatly litter decay rates (Zaitsev et al. 2002, Ponge 2003, Chauvat 2004, Chauvat et al. 2007).

4.3. Soil nutrient availability as a controlling factor of humus forms development?

We did not observed significant changes in nutrient availability within the organo mineral horizon along the chronosequence. This is probably due to the stands selection which required the same climatic, geologic and historical management conditions to reconstitute the chronosequence. However, even insignificant, we observed a tendency in decreasing soil quality (pH and nutrient availability), *i.e.* pH_{water} was maximal in SP15 and minimal in SP95 (3.97 *versus* 3.80, respectively), pH_{KCl} showed the same pattern, with 3.33 in SP15 and 3.09 in SP95, Ca content in the organo mineral horizon was maximal in SP15 (1.43 cmol+ kg^{-1}) and minimal in SP95 (0.71 cmol+ kg^{-1}) while Al contents showed the inverse pattern. This tendency agrees with Aubert *et al.* (2004) results. They observed an acidification of the organo mineral horizon along 170-yr-old chronosequence of pure beech stands from the same site (Eawy forest, France). In the study of Aubert *et al.*, (2004) the sampling occurred in July while in this present work, we sampled the organo mineral horizon in May. It is possible that nutrients uptake by trees was higher in July than in May, resulting in stronger differences between silvicultural phases.

The decrease in soil nutrient availability along forest maturation was often related to higher nutrient uptake by trees during the intense growth phase of trees in response to nutrients requirements for wood building (Brais et al. 1995, Ponge 2003). Since soil alteration (a stable process in mineral element production along forest maturation) may not supply enough nutrients for beech trees requirements (N, K, P, base cations), soil quality may decrease in soil along the pure beech chronosequence (Brais et al. 1995, Aubert et al. 2004, Trap et al. 2009). This may affect litter decay, probably through modification in biotic environment. This hypothesis is support by Sariyildiz and Anderson (2003b) results who showed that after 12 months of laboratory incubation, both oak (*Quercus robur* L.) and beech litter decomposition rates varied

greatly according to soil quality. It wonder whether these changes in soil nutrient availability were enough significant to be responsible for organic materials accumulation along the pure beech chronosequence. We cannot reject the third hypothesis formulated in the introduction, *i.e.* the soil quality (nutrient availability) should decrease along the pure beech chronosequence and limit soil biological activity (direct effect) and consequently litter decomposition rates. However, we did not found significant linear correlations between soil parameters and litter dynamics, suggesting that soil nutrient availability effects on litter decay rates was limited. On the other hand, according to Sariyildiz and Anderson (2003b) works, soil nutrient availability may impact humus forms indirectly via litter quality. The slight decrease in soil nutrients availability may impact the amount of mineral elements in green leaves during the phase of intense growth of trees, affecting hence the decomposability of senescent leaves. Lower nutrients content in leaf may decrease litter decay along the chronosequence and favor organic materials accumulation. The characterization of litter quality and total mineral budget within both organic and deep mineral horizons is required to validate these hypotheses.

5. Conclusion

We can conclude that the shift mull-moder occurring at the onset of the pure beech chronosequence result in both higher litter production and lower litter decay rates (moder forming processes). However, changes in litter decay along the chronosequence were highly significant compared to litter production. Furthermore, only litter decay was significantly correlated with OH maximal thickness suggesting that the appearance of the humification layer was mainly due to the decrease in litter decay rates. The intense tree growth phase was the silvicultural phase implicated in the shift in both litter production and litter decay rates. Since nutrient uptake by trees was higher during their intense growth phase, soil nutrient availability may responsible for the decrease in litter decay rates. However, changes in the main properties of the organo mineral horizon appeared not enough significant to be responsible for litter dynamics shifts along the chronosequence. Soil nutrients availability changes along the chronosequence may affect indirectly soil functioning by promoting lower leaf litter quality. We can hence suppose that during the intense growth phase of trees, the chemical composition of beech leaves change and impact greatly soil biological activity. Complementary studies focusing on the quality of beech leaves along stand ageing should provide insights about the cause of humus forms and soil functioning changes at the rotation scale.

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III. Synthèse du chapitre 4

L'objectif de cette étude était d'identifier, parmi la production et la vitesse de décomposition de la litière de hêtre, le facteur responsable de l'apparition des moders. Nous avons montré que la production de litière ne variait pas significativement le long de la chronoséquence alors que la vitesse de décomposition diminuait durant la phase de croissance des peuplements (SP65 et SP95). Aussi, la vitesse de décomposition est significativement corrélée avec l'épaisseur de l'horizon OH. L'apparition de l'horizon d'humification semblerait donc être principalement le résultat de cette chute de la vitesse de décomposition de la litière. L'augmentation de la production de la litière joue un rôle secondaire mais semble contribuer au changement hemimoder-dysmoder.

Cette diminution de la vitesse de décomposition témoigne donc d'un changement de l'efficacité biologique à décomposer le matériel foliaire. Cette efficacité est directement dépendante de (i) la structure des communautés des organismes du sol et (ii) de la qualité de la litière. D'après l'étude menée par Hedde *et al.* en 2007, la composition spécifique et la biomasse de la macrofaune du sol présente peu de variations le long de la maturation des peuplements purs de hêtres de Haute Normandie. Nous allons donc nous intéresser dans un premier temps aux changements structurels et fonctionnels du compartiment microbien au sein des différents horizons de l'épisolum humifère le long de la chronoséquence (chapitre 5). Dans un second temps, nous allons étudier la variabilité de la qualité de la litière de hêtre le long de la chronoséquence (chapitre 6).

CHAPITRE 5
Les communautés microbiennes

I. Présentation du chapitre 5

Au cours du chapitre précédent, nous avons observé une diminution importante de la vitesse de décomposition de la litière durant la phase de croissance intense du peuplement (65-95 ans) qui semble être responsable de l'apparition de l'horizon d'humification OH. La vitesse de décomposition de la litière est sous le contrôle de nombreux facteurs, tels que le climat, le sol, la qualité des ressources et surtout l'activité biologique du sol. D'après le modèle hiérarchique des facteurs contrôlant la décomposition de la matière organique au sein des écosystèmes terrestres, proposé par Lavelle *et al.* (1993), dans des conditions climatiques et édaphiques similaires, les facteurs susceptibles de contrôler les processus de décomposition de la matière organique sont la végétation et les organismes du sol (Fig. 5.1).

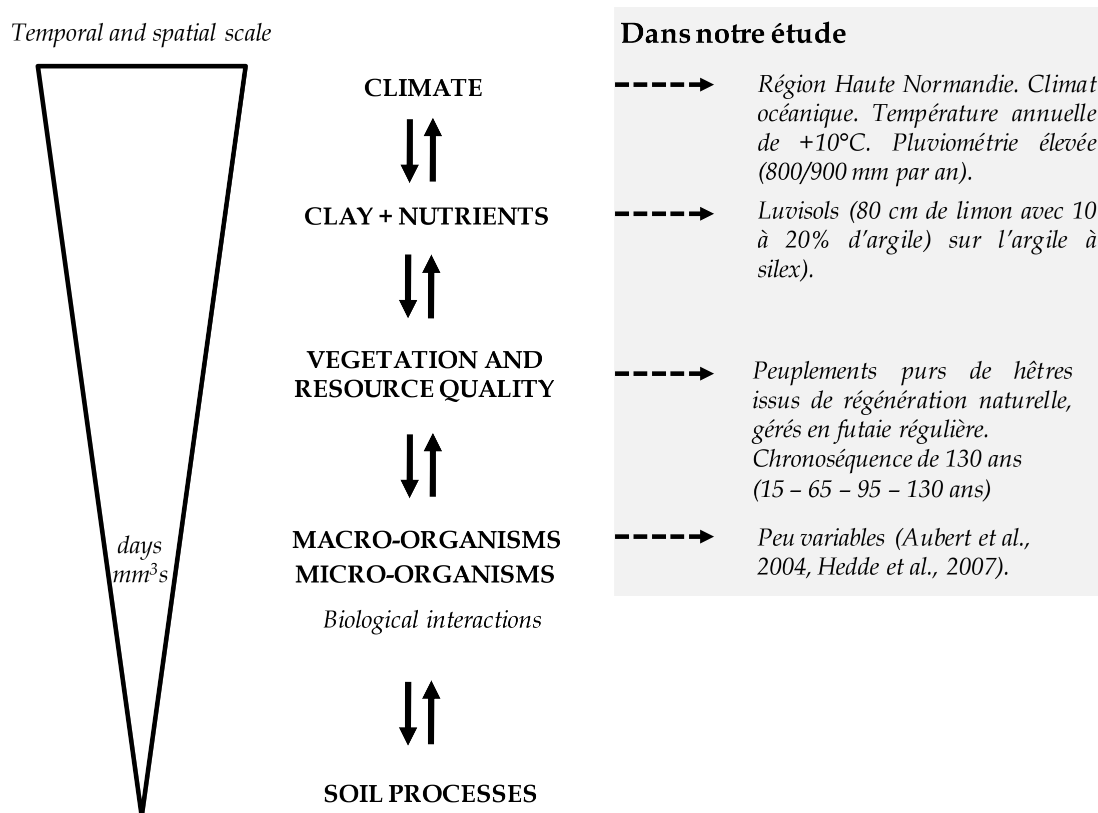

Figure 5.1. *Modèle hiérarchique des facteurs contrôlant les processus de décomposition de la matière organique au sein des écosystèmes terrestres appliqué à notre étude (d'après Lavelle et al. 1993).*

D'après Ponge (2003), la diminution de la disponibilité des éléments dans le sol et de l'abondance des communautés de lombricidés le long de la maturation des peuplements forestiers, sont les principaux facteurs responsables de la diminution de la vitesse de décomposition de la litière et donc de l'apparition de l'horizon d'humification des moders.

"During the phase of intense growth of trees, more nutrients are used for the build-up of wood than are released through litter decomposition and mineral weathering. During maturity of the forest stand the internal recycling of nutrients and the slower growth of trees yield more nutrients to the decomposer system. Earthworms are particularly sensitive to environmental changes occurring during tree stand development, especially in coniferous stands but also in deciduous stands. There is a global trend of decreasing abundance of earthworms during the phase of intense growth of trees (except a short spell following thinning operations), followed by progressive recovering as trees reach maturity then senesce. This process, which is probably controlled by tree physiology, can be considered as the driving force for the observed changes in humus forms."

Ponge, 2003:939

Or, Hedde *et al.* (2007) ont montré peu de changement de la composition spécifique, de la densité et de la biomasse des communautés de macro détritivores au sein de l'épisolum humifère le long d'une chronoséquence de hêtraie pure de 200 ans sur limons de plateau de Haute Normandie. Ainsi, l'absence de patron de variation de la macrofaune le long de la maturation des peuplements purs de hêtre suggère que seules les communautés microbiennes du sol et/ou la qualité de la litière peuvent être responsables de l'apparition des moders. L'étude présentée au sein de ce chapitre a pour objectif de caractériser simultanément les patrons de variations de la morphologie et des communautés microbiennes de l'épisolum humifère le long d'une chronoséquence de hêtraie pure de 130 ans.

II. Article 4

Soil microbial community and humus forms changes along a 130-yr-old chronosequence of pure beech (*Fagus sylvatica* L.) stands on loamy soils

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Abstract

Within forest ecosystems, organic matter recycling that involves soil microbial community is mainly achieved in the upper organic and organo-mineral horizons. Nevertheless, our understanding of microbial community structure and functioning within these horizons is patchy, especially about temperate deciduous forest. Moreover, the accumulation of organic materials during forest development, resulting in soil morphological shift from mull to moder humus forms, supposes great changes in microbial community. The aim of the present study was thus to characterize structural and functional profiling of microbial community within the unmodified litter layer (OL layer), the fragmented and humified layer (FH layer) and the organo-mineral horizon (A) along a 130-yr-old chronosequence of pure beech (*Fagus sylvatica* L.). We investigated the relationships between humus forms and soil microbial community in order to identify morphological variables that can potentially act as indicator for microbial structure and function. We measured the microbial biomass N (N_{mic}), the N_{mic} to total N ratio (N_{mic}-to-N_t), the ergosterol pools and the bacterial and fungal DNA biomass using quantitative (Q)-PCR within each soil layers. We also measured the potential metabolic profiling (BIOLOG method). We found great changes in both microbial structure and function between soil layers and stand age. The N_{mic} was higher within the organic layers compared to the A layer but did not change significantly between silvicultural phases. In contrast, the N_{mic}-to-N_t decreased along stands ageing in the OL layer. Fungal community dominated in the OL layer while bacteria community biomass was higher in the A layer whatever the stand age. The fungal biomass decreased in the OL layer at the onset of the chronosequence but increased in the FH and the A layer. The fungal/bacterial biomass ratio increased along stands ageing in the FH layer. The potential functional profiling of heterotrophic bacteria was higher in the organic layers. The functional diversity was higher in the oldest stands within the organic layers while the A layer showed higher functional richness in the youngest stands. We found significant correlations between morphological and microbial variables. We showed that beech forest maturation favored fungal community in the fragmentation and humification layers. Our results suggest the potential use of some morphological variables as indicators of the structural and functional profiling of soil microbial community along stand ageing.

Keywords

Soil microbial biomass N, fungal DNA biomass, bacterial DNA biomass, potential metabolic profiling, humus forms, temperate forest chronosequence, *Fagus sylvatica*.

1. Introduction

Soil microflora plays a key role in terrestrial ecosystems by driving many belowground processes critical to ecosystem functioning such as biogeochemical cycles (Nannipieri *et al*. 2003; van der Heijden *et al*. 2008). Changes in microbial community structure and functioning have important consequences, especially in forest ecosystems that, unlike agricultural ones, do not depend on external inputs but rather internal microbial-mediated processes to sustain plant growth (Priha *et al*. 2001; Lejon *et al*. 2005; van der Heijden *et al*. 2008).

Unfortunately, despite the impressive research effort carried out during the last decade, the amount of information about soil microbial community in forest ecosystems remains limited compared to agricultural ones (Henckel *et al*. 2000; Graystone and Prescott 2005). This is particularly true for works focusing on microbial community structure and activity within temperate deciduous forest. Concerning these ecosystems, changes in microbial communities within the organic layers at different litter decay stages (*i.e.* unmodified, fragmentation litter or humus layer) appear non-documented with regards to the amounts of literatures about boreal coniferous forests (Pennanen *et al*. 1999; Pennanen 2001; Priha *et al*. 2001; Kanerva and Smolander 2007). This is a serious shortcoming since the key soil processes such as carbon and nitrogen mineralization carried out by soil bacteria and fungi mostly occurred within the organic layers (Jussy *et al*. 2000; Trap *et al*. 2009). For instance, Persson *et al.* (2000) have investigated C mineralization in different soil layers from European forest sites (transect from North Sweden to Central Italy, NIPHYS/CANIF project) and have shown that it was ten fold higher than at 10 cm depth in the mineral soil.

Furthermore, the amount and the structure of the organic materials within the forest floor change considerably during forest development. Indeed, successional patterns of humus forms have often been described along both temperate managed (Aubert *et al*. 2004; Chauvat *et al*. 2007) and semi-natural forest maturation (Bernier and Ponge 1994; Ponge and Delhaye 1995). Early developmental stages have been associated with mull humus form (thin organic layers and deep organo-mineral horizon with clay-humus complexes) while moder (deep fragmented and humus layers with fine organic matter from fauna faeces, thin organo-mineral horizon) have been frequently observed under older stands on acidic forest soils (Ponge 2003; Salmon *et al*. 2006).

The shift mull-moder occurring along stands ageing is often paired with great changes in nutrients turnover and soil fertility, *e.g.* soil acidification and nutrients impoverishment (Brais *et al*. 1995; Aubert *et al*. 2004; Zeller *et al*. 2007; Trap *et al*. 2009). For instance, a decrease in nitrification along stand development was observed by several authors (Idol *et al*. 2003; Trap *et al*. 2009). It can be thus hypothesize that the shift from mull to moder occurring along forest maturation resulting in the appearance of deep fragmented and humus layers, strongly affects microbial structure and activity in the different soil layers leading to large shifts in nutrient turnover. We intended to test this hypothesis in this present study.

Our aim was thus to (i) characterize soil morphology and the structural and functional profiling of microbial community (bacteria and fungi) within the different upper soil layers along a 130-yr-old chronosequence of pure beech (*Fagus sylvatica* L.) and to (ii) search for co-variation patterns between morphological and microbial variables in order to identify morphological variables that potentially act as indicator for microbial structure and function. More specifically, the addressed questions were (1) How are microbial structure and function within the upper forest soil layers? (2) What

are the patterns of variation of this vertical spatial distribution along forest maturation? (3) Do theses patterns of changes correlate with humus forms changes along forest maturation?

For that purpose, we reconstituted an empirical 130-yr-old pure beech chronosequencel using space-for-time substitution. We investigated soil microbial community structure and function using biomass indices (microbial N, ergosterol pools and quantitative (Q)-PCR) and potential metabolic profiling (BIOLOG method). Macro-morphological description of humus was done in the field on the basis of variation visible to the naked eye

2. Materials and methods

2.1. Study site

The study site was located in the Eawy state forest (France, Upper Normandy, 01°18' E; 49°44' N; 7200 ha). The climate is temperate oceanic with a mean annual temperature of +10°C and a mean annual precipitation of 800 mm. A space-for-time substitution procedure (Pickett 1989) was used to empirically reconstitute an even-aged forest chronosequence: sixteen pure beech (*Fagus sylvatica* L.) stands were selected to represent four silvicultural phases of different ages: 13-18 years (SP15), 65-66 years (SP65), 91-103 years (SP95) and 121-135 years (SP130) (Table 2.1, page 45). Each phase was comprised of four replicated stands. All stands were managed as even-aged forest by the French Forestry Service (ONF). All of them were set in a flat (plateau) topographic situation (205 m a.s.l.). The soil was an endogleyic dystric Luvisol (FAO 2006) developed on more than 80 cm of loess (lamellated silt) lying on clay with flints. The understory vegetation was defined as a characteristic Endymio-Fagetum according to phytosociological classification (Durin *et al*. 1967).

2.2. Soil morphology

In each stand center, a 16 m^2 plot was materialized away from vehicle tracks and as far as possible from tree trunks to avoid any acidification due to organic matter accumulation (Beniamino *et al*. 1991). Macro-morphological descriptions of organic and organo-mineral layers were done within frames (25 cm x 25 cm) at three corners of the central plot according to the French nomenclature (Jabiol *et al*. 2007) in May 2007. 36 macro-morphological variables were described in the field on the basis of variation visible to the naked eye (see Table 2.2, page 47). We distinguished mull (mainly dysmull) and moder (hemimoder + eumoder + dysmoder) humus forms on the base of morphological characters (Table 2.2, page 47). A total of 48 humus profiles were described in the field (3 descriptions per stand × 16 stands). We differentiated:

(1) **the OL layer**. This horizon consists of almost unmodified leaf and woody fragments. Most of the original plant structures are easily discernible. Leaves may be recently fallen and unmodified (OLn) or bleached and slightly fragmented (OLv);

(2) **the OF layer** consisting of a mixture of coarse plant debris with fine organic matter (humus). This horizon is characterized by an accumulation of partly decomposed organic matter derived mainly from leaves and woody materials, which are fragmented and mixed with invertebrate faeces. The proportion of organic fine substances (mainly fecal pellets with some millimetric plant fragments) ranges from 10 to 70 % by volume;

(3) **the OH layer**. This horizon is an organic horizon characterized by an accumulation of humified organic matter. The original plant structures and materials are not discernible. Organic fine substance (mainly invertebrate faeces) accounts for more than 70 % by volume;

(4) **the A layer** or organo-mineral layer below the organic layers which differ in depth and structure among humus forms.

2.3. Soil sampling

Soil layers were sampled in May 2008 within frames (25 cm x 25 cm) located in three corners of the square plots. We sampled OL, OF, OH and A layers. Because the OH layer was sometimes discontinuous and not enough abundant, we decided to mix OF and OH layers in a single pool called FH layers. Samples were stored at 4°C during the transport (Forster 1995a). In the laboratory, the three subsamples of each soil layers were pooled to form one sample per stand. Leaves were roughly cut, brushwood, stones and large roots were removed and the A layer were sieved (2 mm). Microbial analyses were immediately performed on fresh samples except for fungal biomass for which soils were stored at -18°C before analyses. An aliquot each sample was dried at 105°C for 24 hours for the A layer and 65°C for 48 hours for organic material to obtain the gravimetric water content.

2.4. Microbial structural profiling

2.4.1. Microbial biomass N

Microbial biomass N (N_{mic}) was determined using the chloroform fumigation-extraction method (Brookes *et al*. 1985). After 24 h of fumigation with $CHCl_3$, followed by a persulfate digestion, mineral N in the OL, FH and A materials was extracted with K_2SO_4 solution (0.2 M). An aliquot of 5g (organic material) or 10g (A layer) of each sample was placed in beakers with K_2SO_4 0.2 M (200 or 100ml for organic and A materials, respectively) and shacked for 1 hour at 100 rev min^{-1} (Alef 1995; Forster 1995b). The obtained extractions were filtered through Schleicher & Schuell 0790 ½ filter papers and frozen for analyzed mineral N pools. Filters were pre-leached with 0.2 M K_2SO_4 in order to avoid any ammonium and/or nitrate contamination. Concentrations of NH_4^+-N and NO_3^--N were determined colorimetrically (AA3, BRAN+LUEBBE, Norderstedt, Germany). Also, aliquots of each soil layer were air-dried and total N (N_t) was measured by gas chromatography with a CHN pyrolysis micro-analyzer (Flash 2000 Series, CHNS/O Analysers Thermo Scientific, France). The microbial N quotient (N_{mic}-to-N_t) was calculated for each soil layers according to silvicultural phases.

2.4.1. Fungal biomass by ergosterol quantification

Fungal biomass (FB) was determined through ergosterol contents thanks to methanol extraction after 1 hour mechanic shaking at 320 rev min^{-1} according to Gong method (Gong and Witter 2001). The ergosterol was measured by High-Performance Liquid Chromatography at 282 nm (SPD-10A SHIMADZU LC-6A) and expressed as µg-ergosterol per g of dry matter (Rössner 1996).

2.4.2. Fungal and bacterial biomass by real-time PCR quantification

Total DNA was extracted from 0.5 g of moist sieved soils or 0.25 g of fresh organic materials using the BIO101 Fast DNA spin Kit for Soil. DNA was re-suspended in 50 µl sterile deionized water and quantified by spectrofluorimetry (Hoechst 33258-BIORAD). DNA extracts were stored at -18°C.

Fungal DNA quantification was realized using 18S rDNA real-time qPCR. 18S rDNA amplifications were carried out in a total volume of 50 µl. The qPCR mix was prepared as follows: 2 ng of soil microbial DNA, 0.25 µl of each primer (FU18S1 5'-GGAAACTCACCAGGTCCAGA-3' derived from Nu-SSU-1196 and Nu-SSU-1536 5'-ATTGCAATGCYCTATCCCCA-3' (Borneman and Hartin 2000), 25 µl of qPCR Mastermix for SYBR®Green I no ROX and 2.5 mg ml^{-1} BSA. A dilution series containing known amounts of *Fusarium graminearum* genomic DNA was used as the standard for quantification of sample DNA. After an initial denaturation and enzyme activation step of 10 min at 95°C, 40 cycles of PCR were performed in the iCycler iQ Real-Time PCR Detection System(BIORAD) as follows: 20 s at 95°C, 30 s at 62°C and 30 s at 72°C. A final 5-min extension step completed the protocol. Upon completing PCR, melting curve analysis was used to determine whether there was a detectable primer-dimer contribution to the SYBR®Green fluorescence measurement. Results were expressed as µg of fungal DNA per g of dry matter.

Bacterial DNA quantification was realized using 16S rDNA real-time qPCR. 16S rDNA amplifications were carried out in the same conditions as 18S rDNA PCR except for primers (63f 5'-CAGGCCTAACACATGCAAGTC-3', and BU16S4 5'-CTGCTGCCTCCCGTAGG-3' (Marchesi *et al*. 1998) derived from 341F (Muyzer *et al*. 1993), and amplification protocol (40 s at 95°C, 45 s at 62°C and 30 s at 72°C). A dilution series of *Pseudomonas aeruginosa* DNA was used as the standard and results are expressed as µg of bacterial DNA per g of dry matter. The ratio between total fungal biomass and total bacterial biomass (F/B ratio), as estimated by their respective DNA quantification, was also calculated and analyzed within each soil layers according to the silvicultural phases.

2.5. Microbial functional profiling

2.5.1. Microbial community metabolic profiles

Carbon source utilization profile of soil bacterial community was determined by the BIOLOG method (Garland and Mills 1991). The BIOLOG® EcoplatesTM (Biolog Inc., USA) used contained 96 wells preloaded with a buffered nutrient medium and a tetrazolium violet redox dye. The Ecoplates contained 3 repetitions of 31 carbon sources and a remaining well with water (control). The 31 different carbon sources were distributed among six substrate groups: carbohydrates (n=7), carboxylic acids (n=9), amides and amines (n=2), amino acids (n=6), polymers (n=4) and miscellaneous (n=3).

Bacteria extraction was performed by diluting an aliquot of fresh material from each of 48 samples in sterile NaCl solution (0.85%, ratio 1/10). All dilutions were shaking for 3 minutes on a vortex mixer at maximum speed following by centrifugation during 10 minutes at 1000 rpm (Eppendorf centrifuge 5810 R). After bacterial extraction, the bacteria suspensions were diluted (from 10^{-2} to 10^{-5} for OL and FH samples and from 10^{-2} to 10^{-4} for A samples) and inoculated in duplicate on R2A agar medium. All plates were incubated at 20°C and enumerated after 48 h in the dark. Inoculations of Biolog microplates were performed from bacteria extraction samples. The dilutions were adjusted to obtain a mean inoculum density about 1500 bacteria per

well. 150 µl of cell suspension was added to each well of the Biolog plates. The Ecoplates were then incubated for 48 h at 20°C in the dark. Three optical density measurements at 590 nm were performed, *i.e.* at the beginning of the incubation (t_0), 24 (t_{24}) and 48 (t_{48}) hours after the beginning with an Emax precision microplate reader (Microplate E-Max, Molecular Devices, Sunnyval, CA). The $O.D._{590}$ of the control well was subtracted from the $O.D._{590}$ of each wells containing a substrate (corrected $O.D._{590}$).

The potential Substrate Richness (SR) and the Average Well Color Development (AWCD) were calculated from Biolog data for each stand horizon (n=4 for each horizon). The SR index corresponds to the number of positive well. A well was considered to be positive when it corrected $O.D._{590}$ was superior at 0.25 (Calbrix *et al.* 2005; Plassart *et al.* 2008). Metabolic profiling by carbon sources were established from SR index for each soil layer. No transformation was performed.

2.6. Statistics

All tests were computed with the R freeware (R Development Core Team 2008) and statistical significance was set at $P < 0.05$. Means and standard errors were calculated by silvicultural phases for all microbial variables (4 true replications). Comparisons of means between silvicultural phases were performed using non-parametric tests (Kruskal-Wallis rank sum test and Multiple Comparaison test after non-parametric test). Three-way ANOVA was used to test the effects of the factors "silvicultural phase", "soil layer" and "substrate group" on the Average Well Color Development. Beforehand, data normality and variances homogeneity were checked using the Wilk-Shapiro and the Bartlett test, respectively. We performed a Principal Component Analysis (PCA1) on morphological variables (16 rows corresponding to stands and 36 columns corresponding to morphological variables). The PCA1 illustrated which morphological variables discriminated silvicultural phases along the chronosequence. Codes of morphological variables are listed in the table 2.2 (page 45). We performed a Principal Component Analysis (PCA2) on microbial variables to distinguish variables which discriminated the silvicultural phases (16 rows corresponding to stands and 26 columns corresponding to microbial variables). Codes of each microbial variable are listed in the table 5.1. We used Pearson linear correlations to describe co-variation patterns between macro-morphological and microbial variables along the forest chronosequence. The Truncated Product Method was used to adjust the *P* values (Zaykin *et al.* 2002).

3. Results

3.1. Microbial structural profiling

The microbial biomass N (N_{mic}) estimated by the fumigation-extraction method did not change significantly between silvicultural phases in both organic and organo-mineral layers (Table 5.2). However, within the OL layer, N_{mic} tended to be lower in young stands with around 241 µg-N g dry matter and higher in matures one with about 380 µg-N g dry matter (57% increase). We observed the same pattern in the FH layers with an increase of about 28%. In contrast, within the A layer, N_{mic} tended to be lower in SP95 and higher in SP15. N_{mic} was about 30-fold higher in the organic layers that within the A layer (Table 5.2).

Table 5.1. *Codes of each microbial variables used in the Principal Component Analysis.*

Soil layers	Microbial variables	Codes
OL	Microbial biomass N (N_{mic})	Mic-OL
	Microbial N quotient (N_{mic}-to-N_t)	MNQ-OL
	Fungal biomass	Fg-OL
	Bacterial biomass DNA	BaDNA-OL
	Fungal biomass DNA	FgDNA-OL
	Ratio fungal/bacterial biomass DNA (Ratio F/B)	F/B-OL
	Average Well Color Development (AWCD)	AWCD-OL
	Substrate Richness (SR)	SR-OL
FH	Microbial biomass N (N_{mic})	Mic-FH
	Microbial N quotient (N_{mic}-to-N_t)	MNQ-FH
	Fungal biomass	Fg-FH
	Bacterial DNA	Ba-FH
	Fungal DNA	FgDNA-FH
	Ratio fungal/bacterial biomass DNA (Ratio F/B)	F/B-FH
	Average Well Color Development (AWCD)	AWCD-FH
	Substrate Richness (SR)	SR-FH
A	Microbial biomass N (N_{mic})	Mic-A
	Microbial N quotient (N_{mic}-to-N_t)	MNQ-A
	Fungal biomass	Fg-A
	Bacterial DNA	Ba-A
	Fungal DNA	FgDNA-A
	Ratio fungal/bacterial biomass DNA (Ratio F/B)	F/B-A
	Average Well Color Development (AWCD)	AWCD-A
	Substrate Richness (SR)	SR-A

Within the OL layer, the N_{mic}-to-N_t ratio was significantly higher in SP130 (2.17%) and lower in SP15 (1.27%) and SP95 (1.19%). SP65 showed intermediate values. We observed the similar pattern in the FH layers, *i.e.* SP130 exhibited the higher value (1.76%) while SP95 showed the lowest one (1.15%). The N_{mic}-to-N_t within the A layer did not showed significant changes between silvicultural phases and corresponded to only 0.03% of total N, on average. It was about 50-times higher in the organic layers than in the A layer. Fungal biomass determined through ergosterol pools did not change significantly between silvicultural phases in the OL layer at $P < 0.05$ (Table 5.2). Nevertheless, it tended to be the highest in SP15 and the lowest in SP130. Within the FH layers, the fungal biomass was significantly higher in SP130 (75.3 µg-ergosterol g^{-1} dry matter) and lower in SP15 (57.9 µg-ergosterol g^{-1} dry matter). In the A layer, the fungal biomass was higher in SP130 and lower in SP65 while SP15 and SP95 exhibited intermediate values. The fungal biomass was 3-times higher in the OL layers compared to the FH layers and 80-times higher in the OL layers than in the A layer (Table 5.2).

Whatever the soil layer, the bacterial biomass obtained from DNA quantification (bacterial biomass DNA) did not change significantly between silvicultural phases but tended to be higher in SP15 within organic layers (Table 5.3). Bacterial biomass DNA was 4.3-fold higher in the OL layer compared to the A layer and it was 2.2-fold higher in the FH layer compared to the A layer. The fungal biomass obtained by DNA quantification (fungal biomass DNA) was significantly higher in OL layer from SP15 (Table 5.3). In contrast, the fungal biomass DNA within the FH layers was higher in SP130 and lower in SP15.

Table 5.2. *Microbial biomass N expressed (N_{mic}) as µg-N g^{-1} dry matter, the N_{mic}-to-N ratio and the fungal biomass expressed as µg-ergosterol g^{-1} dry within soil layers according to silvicultural phases.*

DNA biomass	**Soil layers**											
Silvicultural phases	**OL**				**FH**				**A**			
Bacterial DNA (µg g^{-1} of dry soil)												
SP15	3.47	(0.57)	a	**x**	1.25	(0.37)	a	**y**	0.52	(0.192)	a	**z**
SP65	2.05	(0.98)	a	**x**	1.22	(0.46)	a	**x**	0.62	(0.40)	a	**y**
SP95	2.97	(1.72)	a	**x**	1.33	(0.26)	a	**x**	0.77	(0.55)	a	**y**
SP130	2.12	(0.81)	a	**x**	1.66	(0.41)	a	**x**	0.55	(0.14)	a	**y**
Fungal DNA (µg g^{-1} of dry soil)												
SP15	19.71	(3.47)	**a**	**x**	1.02	(0.57)	**b**	**y**	0.11	(0.08)	a	**z**
SP65	9.56	(2.36)	**b**	**x**	1.45	(0.47)	**b**	**y**	0.08	(0.02)	a	**z**
SP95	11.08	(1.28)	**b**	**x**	1.37	(0.31)	**b**	**y**	0.12	(0.01)	a	**z**
SP130	9.61	(1.74)	**b**	**x**	3.41	(0.65)	**a**	**y**	0.08	(0.01)	a	**z**
F/B ratio												
SP15	5.91	(1.95)	a	**x**	0.84	(0.34)	**b**	**y**	0.18	(0.11)	a	**z**
SP65	5.54	(2.45)	a	**x**	1.06	(0.47)	**ab**	**y**	0.18	(0.11)	a	**z**
SP95	4.52	(2.05)	a	**x**	1.03	(0.10)	**ab**	**y**	0.21	(0.17)	a	**z**
SP130	5.60	(1.30)	a	**x**	2.05	(0.24)	**a**	**y**	0.16	(0.04)	a	**z**

Data are means (SD). Letters refer to significant differences between silvicultural phases (a and b) or soil layers (x, y and z) according to Kruskal-Wallis test ($P < 0.05$ level, n=4).

We did not observed significant changes in fungal biomass DNA between silvicultural phases in the A layer. The ratio between fungal biomass DNA and bacterial biomass DNA (F/B ratio) did not change significantly between silvicultural phases in the OL layer. In the FH layer, the F/B ratio was significantly the highest in SP135 and the lowest in SP15.

Table 5.3. *Bacterial and fungal DNA biomasses (expressed as µg g^{-1} dry matter) and fungal/bacterial DNA ratio within each soil layer according to silvicultural phases.*

Microbial biomass indices	**Soil layers**											
Silvicultural phases	**OL**				**FH**				**A**			
Microbial biomass N (µg-N g^{-1})												
SP15	240.57	(94.91)	a	**x**	223.50	(59.80)	a	**x**	10.75	(4.82)	a	**y**
SP65	305.43	(80.17)	a	**x**	221.73	(79.08)	a	**x**	9.27	(6.18)	a	**y**
SP95	298.42	(78.23)	a	**x**	219.99	(58.80)	a	**x**	5.72	(0.36)	a	**y**
SP130	379.65	(19.55)	a	**x**	286.38	(76.72)	a	**x**	9.84	(2.75)	a	**y**
Microbial N quotient (N_{mic}-to-N_t)												
SP15	1.27	(0.43)	**b**	**x**	1.36	(0.59)	a	**x**	0.03	(0.01)	a	**y**
SP65	1.53	(0.24)	**ab**	**x**	1.43	(0.68)	a	**x**	0.03	(0.02)	a	**y**
SP95	1.19	(0.37)	**b**	**x**	1.15	(0.41)	a	**x**	0.02	(0.00)	a	**y**
SP130	2.17	(0.15)	**a**	**x**	1.76	(0.75)	a	**x**	0.03	(0.01)	a	**y**
Fungal biomass (µg-ergosterol g^{-1})												
SP15	221.37	(34.69)	a	**x**	57.90	(10.14)	**b**	**y**	2.23	(0.77)	**ab**	**z**
SP65	203.90	(25.44)	a	**x**	76.88	(5.77)	**ab**	**y**	2.09	(0.14)	**b**	**z**
SP95	192.64	(27.15)	a	**x**	75.37	(10.08)	**ab**	**y**	2.75	(0.60)	**ab**	**z**
SP130	184.70	(28.18)	a	**x**	78.98	(7.33)	**a**	**y**	3.10	(0.24)	**a**	**z**

Data are means (SD). Letters indicate significant differences between silvicultural phases (a and b) or soil layers (x, y and z) according to Kruskal-Wallis test ($P < 0.05$, n=4)

Fungal biomass DNA was about 5-fold higher than bacterial one in the OL layer. Fungal biomass DNA was around 1.2–fold higher than bacterial one in mean. In the A layer, the F/B ratio did not change significantly between silvicultural phases. In contrast to the organic layers, bacterial biomass DNA was about 5-fold higher than fungal one in the A layer.

3.2. Microbial functional profiling

The BIOLOG® ECO plates accounted for the variation of the potential metabolic activity of the soil microbial communities extracted from the three soil layers. The potential Substrate Richness (SR) index within the OL layer was higher in SP130 (mean 10.33 positive substrates equivalent to 33.3% of metabolized C sources) and lower in SP15 (mean 4.50 positive substrates equivalent to 14.5%) (Table 5.4). SP65 and SP95 exhibited intermediate values. We observed the same trend within the FH layer (*i.e.* from 6.66 corresponding to 21.4% to 11.75 corresponding to 37.9%). However, the SR index did not change significantly between silvicultural phases in the FH layers. Within the A layer, we did not measure significant changes in the SR index but a sharp decrease in the SR occurred after 15 years old (from 4.00 to 1.25). In SP65 and SP130, the SR index was lower in the A layer compared to the FH layers. Within all soil layers, the Average Well Color Development (AWCD) did not change significantly between silvicultural phases (Table 5.4) even if it tended to be higher in mature stands. The FH layers showed the higher values in AWCD while the A layer exhibited the lowest ones.

Table 5.4. *The Substrate Richness (SR) and the Average Well Color Development (AWCD) indexes within each soil layer according to silvicultural phases.*

Indices	**Soil layers**		
Silvicultural phases	**OL**	**FH**	**A**
SR			
SP15	4.50 (3.20) **b** x	6.66 (2.49) a x	4.00 (5.24) a x
SP65	7.50 (3.62) **ab xy**	9.75 (3.26) a **x**	1.25 (2.12) a **y**
SP95	6.75 (1.64) **ab** x	8.75 (3.16) a x	2.25 (2.90) a x
SP130	10.33 (0.47) **a x**	11.75 (2.48) a **x**	3.00 (4.64) a **y**
AWCD			
SP15	1.41 (0.45) a **xy**	1.88 (0.64) a **x**	0.39 (0.41) a **y**
SP65	1.30 (0.44) a **x**	1.69 (0.52) a **x**	0.09 (0.04) a **y**
SP95	1.43 (0.67) a x	1.69 (0.73) a x	0.34 (0.52) a x
SP130	1.67 (0.44) a x	1.89 (0.62) a x	0.61 (0.75) a x

Data are means (SD). Letters refer to significant differences between silvicultural phases (a and b) or soil layers (x and y) according to Kruskal-Wallis test ($P < 0.05$ level, n=4).

The potential metabolic profiling showed strong changes in the nature of C sources used by heterotrophic bacteria between silvicultural phases and soil layers (Fig. 5.2). Within the OL layer, the stand ageing went with the appearance of several positive C sources such as "Glycyl–L-Glutamic Acid", "Methyl-D-Glucoside", "N-Acetyl-Glucosamine" and "Tween 80". We also observed more intense degradation (high Optical Density) of some substrates such as "D-Xylose" and "α-Cyclodextrin". Amines and carboxylic acids were poorly metabolized in the OL layer.

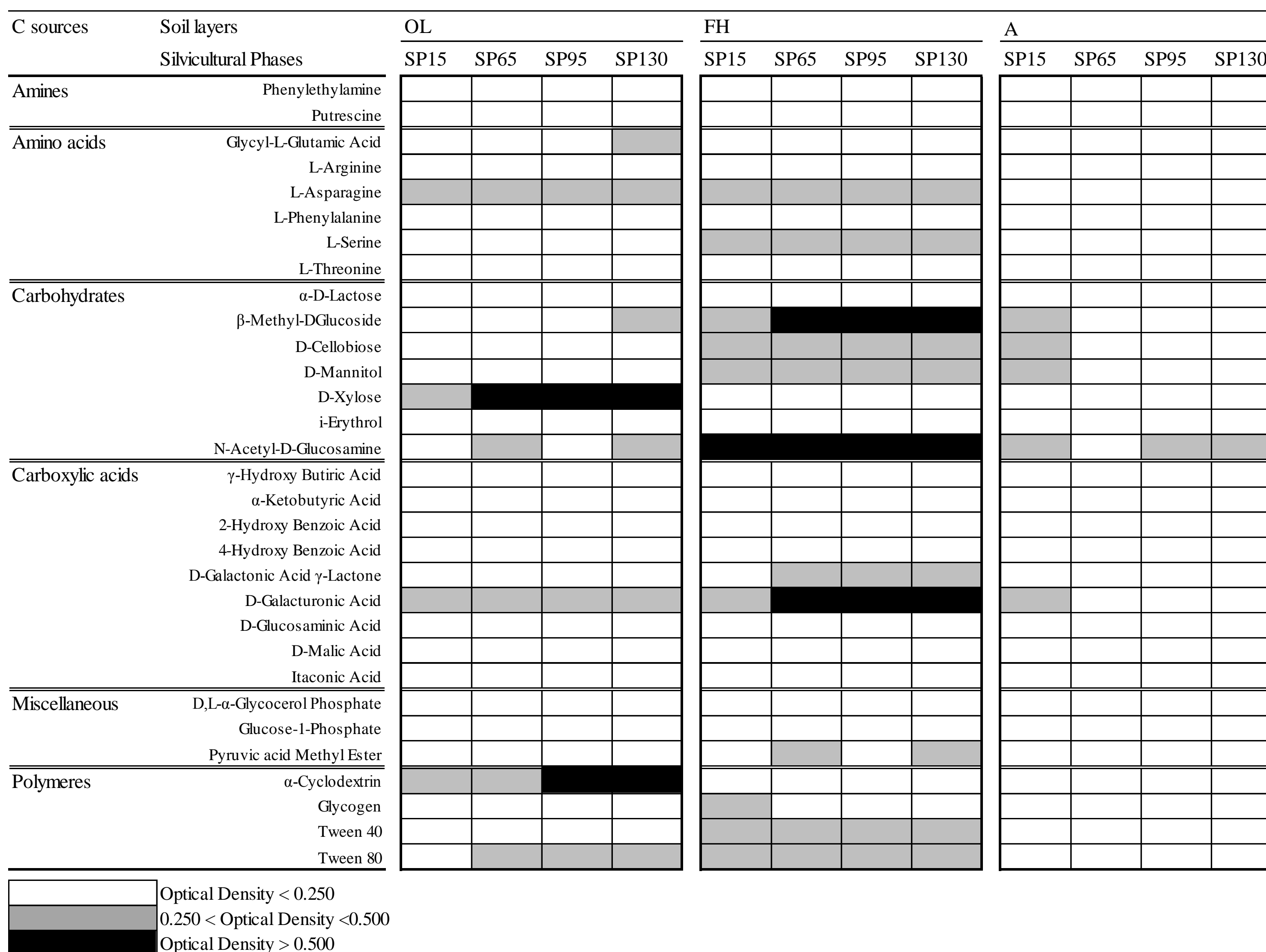

Figure 5.2. *Potential metabolic profiling of soil heterotrophic bacteria within each soil layer according to silvicultural phases.*

Within the FH layer, the maturation of stands was characterized by the degradation of "D-Galactonic Acid γ–Lactone" and "Pyruvic Acid Methyl Esther". Only 5 substrates were shared by both the OL and the FH layer while 8 substrates were specific to the OL or the FH layers. Carbohydrates, amino acids as well as polymers were the main C sources metabolized in the FH layer.

Table 5.5. *Results of the three-way ANOVA on the effects of the factors "silvicultural phase", "soil layers" and "substrate group" on the Average Well Color Development.*

Factors	df	MS	F	*P* level
Silvicultural phase (SP)	3	19.6	2.89	*
Soil layer (SL)	2	584.9	129.32	***
Substrate group (SG)	5	179.5	15.87	***
Interactions				
SP*SL	6	4.0	0.29	N.S.
SP*SG	15	15.7	0.46	N.S.
SL*SG	10	68.9	3.02	***
SP*SL*SG	30	17.5	0.25	N.S.

df. degrees of freedom.

MS. mean squares.

P level. N.S. > 0.05, * < 0.05, **< 0.01, ***<0.001.

Within the A layer, the maturation of stands was characterized by the disappearance of 4 substrates: "Methyl-D-Glucoside", "D-Cellobiose", "D-Mannitol" and "D-Galacturonic Acid". "N-Acetyl-Glucosamine" was the only C source considered as positive in the mature stands (SP95 and SP130). In this layer, the carbohydrates metabolized were the same as in the FH layer, *i.e.* "D-Cellobiose", "D-Mannitol", "Methyl-D-Glucoside" and "N-Acetyl-Glucosamine". A three-way ANOVA was performed on AWCD values to test the effect of the factors "silvicultural phase" (SP), "soil layer" (SL) and "substrate group" (SG) on the functional structure of bacteria community (Table 5.5). The most significant factors were SL ($P < 0.001$) and SG ($P < 0.001$) followed by SP ($P < 0.05$) (Table 5.5). We did not observe significant interactions between SL and SP and between SG and SP. In contrast, SL and SG interaction was highly significant ($P < 0.001$).

3.3. Microbial community and humus forms changes

3.3.1. Morphological versus microbial shifts along the chronosequence

A Principal Component Analysis (PCA1) was performed on morphological data set. The first two axes of the PCA1 explained 43 % of the total inertia (Fig. 5.2). Two shifts in soil morphology occurred along the chronosequence according to the PCA1. The first axis of the PCA1 (relative inertia = 29.8%) opposed the SP15 with negative coordinates to the three others phases (Fig. 5.2.a). This axis opposed OLn and macrofauna-based variables (negative scores) such as the number of earthworm casts in organic layers or the structure of the A horizon, to OLv, OF and OH variables (positive scores) (Fig. 5.2.b). This axis was interpreted as the shift mull-moder. The second axis

(relative inertia = 12.7%) opposed OLv-based variables (OLvcov, OLvmt, OLvBl, OLvCom) against OH-based variables (OHcov, OHMt, OHmt, OHffog, OHvfog and OHro). It segregated SP65 (negative scores) from SP95 and SP130 (positive scores). This axis was interpreted as the changes in moder humus forms, *i.e.* from hemimoder to dysmoder. The first two axes of the Principal Component Analysis (PCA2) performed on the microbial data set explained more than 33 % of the total inertia (Fig. 3.a'). In contrast with humus forms, only one shift in soil microbial community occurred along the chronosequence according to the PCA2. The first axis of the PCA2 (relative inertia = 24.4%) opposed the SP15 with negative coordinates to the SP130 with positive coordinates (Fig. 3.a').

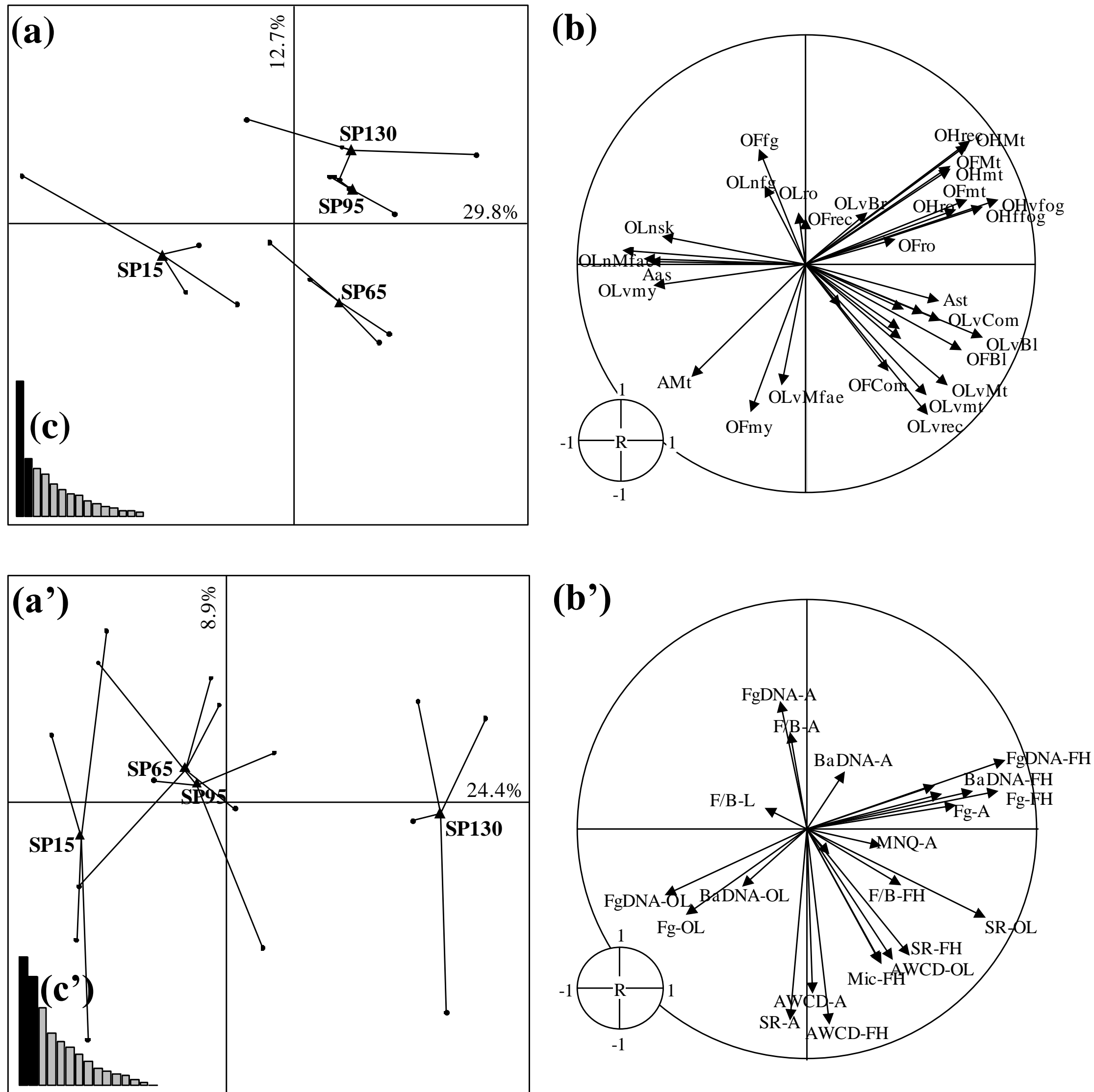

Figure 5.3. *Principal Component Analyses performed on morphological (PCA1, a, b and c) and microbial (PCA2, a', b' and c') data set. Silvicultural phases ordination represented by stars and barycentres (black triangle) on the first two axes of the PCA1 plan (a) or PCA2 plan (a'); each black point corresponds to a stand. Correlations circle for PCA1 (b) and PCA2 (b'). Eigenvalue diagrams of PCA1 (c) and PCA2 (c'). Only labels of the more relevant variables are indicated. Morphological and microbial variables codes are given in the table 2.2. (page 43) and 5.1 (page 99), respectively.*

This axis opposed fungal-based variables in the OL layer (negative scores) such as the fungal biomass DNA and ergosterol content in the OL layers, to (i) fungal-based variables in the FH layers such as fungal biomass DNA, the F/B ratio, the ergosterol content in the FH and (ii) bacterial-based variables such the SR and the bacterial biomass DNA in the OL layer (Fig. 3.b'). It was interpreted as an increase in fungal-based energy channel in both the FH and the A layers, an increase in microbial C and N mineralization and a decrease in microbial biomass in the OL layer along the chronosequence. The second axis of the PCA2 (relative inertia = 24.4%) opposed fungal-based variables (FgDNA-T, F/B-T) *versus* bacterial-based variables (AWCD-T, AWCD-FH, SR-T) in the A layer.

3.3.2. Linear correlations analysis

Seven microbial variables were significantly correlated with eight morphological variables (Table 6). For instance, the microbial biomass in the OL layer was negatively correlated with the percentage of skeletonized leaves in the OLv layer (R^2 = 0.72, P < 0.01) and the number of earthworms casts in the OLn layer (R^2 = 0.66, P < 0.05). The fungal biomass DNA in OL was negatively correlated with the percentage of OH covering (R^2 = 0.67, P < 0.05). In the FH layer, the F/B ratio was negatively correlated with the structure of the organo-mineral horizon (R^2 = 0.70, P < 0.01). The percentage of leaves fragments in the OF layer was positively correlated with the bacterial biomass DNA in this same layer (R^2 = 0.66, P < 0.05) and with microbial biomass in the A layer (R^2 = 0.79, P < 0.001). The fungal biomass DNA in the A layer was positively correlated with both the percentage of skeletonized leaves (R^2 = 68, P < 0.05) and leaves fragments (R^2 = 0.82, P < 0.001) in the OLv layer. However, the F/B ratio in the A layer was positively correlated with the OH maximal thickness (R^2 = 0.72, P < 0.01).

Table 6. *Linear correlations between microbial and morphological variables.*

Soil layers	Microbial variables	Morphological variables[a]			R² values	Slope
		Names	R² rank PCA1			
			Axis 1[b]	Axis 2[c]		
OL layer	Microbial biomass	Skeletonized leaves in OLv	21	25	**0.72****	(-)
	Microbial biomass	Earthworms casts in OLn	5	35	**0.66***	(-)
	Fungal DNA	OH cover	6	4	**0.67***	(-)
FH layer	Ratio F/B	A structure	18	29	**0.70****	(-)
	Bacterial DNA	Leaves fragment in OF	30	8	**0.66***	(+)
Topsoil	Microbial biomass	Compacted leaves in OLv	17	21	**0.65***	(-)
	Microbial biomass	Leaves fragment in OF	30	8	**0.79*****	(+)
	Fungal DNA	Skeletonized leaves in OLv	21	25	**0.68***	(+)
	Fungal DNA	Leaves fragment in OLv	32	28	**0.82*****	(+)
	Ratio F/B	OH maximal thickness	7	7	**0.72****	(+)
Total	***7***	***8*** ***Mean***	***19***	***19***		

[a] All the morphological variables are listed in the appendix 1

[b] The rank of the morphological variable according to its R² value on the first axis of the PCA1

[c] The rank of the morphological variable according to its R² value on the second axis of the PCA1

* P < 0.05
** P < 0.01
*** P < 0.001

Each morphological variable was ranked from 1 to 36 according to its R^2 correlation coefficient value with the two first axes of the PCA (36 ranks per PCA axis). On each axis, the first rank corresponded to the highest R^2 value, *i.e.* "living roots in OH" for the first axis 1 and "OLv cover" for the second one of the PCA. Conversely, the 36th rank corresponded to the lower R^2 value, *i.e.* "OF cover" for the first axis and "A aggregate size" for the second one of the PCA. This ranking thus reflected the relative "power" of each morphological variable in discriminating the silvicultural phases on the PCA axes since the first two axes opposed clearly the different silvicultral phases (Fig. 5.2.a). The morphological variables significantly correlated with microbial variables were positioned on average at the ranks 19 on both the first and the second PCA axes, respectively. The morphological variables with the highest ranks did not showed significant correlations with microbial variables, except for OH-based variables (OH maximal thickness and cover) which were highly correlated with the first two PCA axes and the number of earthworms casts in the OLn which was highly correlated with the first PCA axis (Table 6).

4. Discussion

4.1. Changes in microbial community between soil layers

Our study has shown that microbial biomass N was higher in the OL layer, intermediate in the FH layers and lower in the A layer, irrespective of stand age. This pattern was also found by other authors (Kanerva and Smolander 2007) and was associated to higher organic matter and oxygen availability. Kiikkilä *et al.* (2006), investigating dissolved organic carbon (DOC) and nitrogen (DON) from the OL, OF and OH layers of *Betula pendula*, *Picea abies* and *Pinus sylvestris*, showed that the proportions of the most degradable chemical fractions of DOC and DON were higher within the OL layers. Fresh litter contains considerable easily degradable material, while within the deeper layers fresh input is obtained only via leaching from the OL layer or from degradation of dead roots or root exudates. The high amount of microflora in the OL layer compared other layers, may be linked to the wide array of free organic substrates at all stages of decomposition and the high proportion of easily degradable chemical fractions. Moreover, the amount of organic matter in the A layer was very low (mean total C = 4.2% in mean, C/N = 15) compared to the OL (mean total C = 44%, C/N = 25) and FH layers (mean total C = 39%, C/N = 23). The availability of C resources may be limited in the A layer and strong competition for N between autotrophic bacteria, heterotrophic bacteria, saprotrophic and mycorhizal fungi may occur in this layer which may results in lower microbial biomass.

According to genetic fingerprints, the high amount of microflora within the OL layer was dominated by the fungal community, where the fungal biomass was about 5-times higher than bacterial one. The dominance of the fungal community in the OL layer whatever the stand age is probably related to the quality of beech litter. Contrary to early successional species such as *Betula pendula* or *Carpinus betulus*, the litter produced by late successional species such as *Fagus sylvatica* contains high level of lignin and low level in nitrogen (Hobbie *et al*. 2006). Wardle (2005) and Warlde *et al.* (2004) suggested that low litter quality (high C/N ratio and lignin content and low N content) may promote fungal-based energy channel while higher litter quality (low C/N and lignin content) may favor bacterial-based energy channel in soil. Fungi degrade the most refractory compounds such as lignin while heterotrophic bacteria do not degrade lignin as efficiently as fungi (Gobat *et al*. 2004). For instance, Möller *et al.* (1999) showed that fungi were more efficient at accessing the beech leaf C than bacteria. Suberkropp and

Weyers (1996) found 11 to 26 times higher fungal C production rates compared to bacteria in Yellow poplar decomposing leaves litter. We can thus hypothesize that the low quality of beech litter promoted the fungal community and limit bacteria community growth.

In contrast, in the FH layer, the biomass of the fungal community was only 1.2-times higher than bacterial one. These results suggest that bacterial activity in the FH layers may be important. The fauna activity in the FH layer and the supply of fine organic matter from fauna dejections in the FH layers (Brêthes *et al*. 1995; Jabiol *et al*. 2007) may provide new substrates for bacteria compared to the OL layer. It is also plausible that more simple molecules make available by fungal activity percolate from the OL layer and favored bacteria growth in the FH and the A layers. Bacterial community dominated (about 5-times higher) fungi within the A layer according to DNA fingerprints. The structure of the A layer was massive and no biological or chemical structure (soil aggregate) was observed, except in SP15 (mean bulk density = 1.20 g cm^3). Soil compaction due to harvesting may limit fungal mycelium colonization in this layer. However, the bacterial biomass was substantially lower in this horizon compared to the organic layers. The size of the microbial compartment is a function of the available C content and the organic matter content in the A layer was 10-times lower than in the OL and FH layers suggesting that microflora growth in the A layer was strongly limited by the supply of resources.

Besides microbial biomass, the functional structure of the bacterial community changed greatly between soil layers. The FH layers showed the higher SR and AWCD values while the bacterial biomass was maximal in the OL layer. This suggests that high microbial biomass does not necessary result in high functional diversity. The high amount of resource within the OL layer may favor the fungal biomass and decrease the specific richness of the bacterial community leading to lower SR values.

A high amount of substrates may percolates from the OL layers and originate from fauna activity leading to higher diversity of resources in the FH layer compared to the OL layer. This may lead to higher richness of microbial extracellular enzymes explaining hence the increase of substrate richness found with the metabolic profiling. Furthermore, in contrast to earthworms, microarthorpods which are abundant in the FH layer did not create favorable habitats for microorganisms (Lavelle and Spain 2001) which may explain the low microbial biomass found in this layer compared to the OL layer. It is also possible that greater predation by soil protists, nematodes and micro arthropods within the FH layers regulate bacteria community composition and favor the bacterial functional diversity.

In our study, 43% of the total AWCD correspond to carbohydrates and carboxylic acids degradation by bacteria while the microbial use of amino acids corresponded to only 12% and amides and amines corresponded to only 11%. Our results agree with Kjoller *et al.* (2000) investigations initiated from European coniferous forests (*Picea abies*, *Pinus sylvestris* and *Betula pubescens*) who measured within the organic layer a high activity of functional groups metabolizing carboxylic acids and carbohydrates while the microbial use of amides and amines seemed to be slown down. Furthermore, the interaction between the two factors “substrate group” and “soil layer” was highly significant ($P < 0.001$) while the interaction between “silvicultural phase” and “soil layer” was not significant. These results suggest that the potential activity pattern of functional groups were specific to soil layers. This confirms that the Biolog technique is a useful approach for measuring soil layer-specific differences in the functional structure of microbial communities.

4.2. Changes in microbial community along the chronosequence

The diverse tools used within our study provided some evidences that forest ageing goes with great changes in the structural and functional profiling of the microbial community within the different soil layers, especially fungi within the organic layers. The ageing of stands was paired with the increase in fungal community biomass while bacteria community tended to decrease within the FH layer. In our study, the A pH tended to decrease along the chronosequence and may be an important factor controlling soil microbial communities. Indeed, fungal/bacterial ratio in forest soil is known to vary in response to soil quality (*i.e.* water and nutrients availability), with the relative abundance of bacteria increases in response to increasing nutrients availability (Pennanen *et al*. 1999) and that increasing N availability (Leckie *et al*. 2004; Hogberg *et al*. 2007). Fungi may be more active in soil with lower nutrient concentrations compared to bacteria (Gobat *et al*. 2004; Wallenstein *et al*. 2006). A possible cause of fungal community promotion is soil acidification (Gobat *et al*. 2004) resulting from ammonium uptake by plants, nitrification of ammonium in soils, and nitrate leaching. In a study of forested sites representing a pH gradient, Baath and Anderson (2003) found that pH was positively correlated to substrate-induced respiration and negatively correlated to the fungal/bacterial ratio.

Beside microbial biomass, the functional structure of the bacterial community was also greatly different between the silvicultural phases. Higher functional diversity in the oldest phase was observed in the organic layers while the A layer showed lower SR index in mature stands. According to three-way ANOVA, the effect of the factor "silvicultural phase" on mean metabolic activity (AWCD) was significant but less important than "soil layers". Biolog method is hence a useful tool to measure forest development impacts on the functional structure of soil microbial communities. However, the interactions between the two factors "silvicultural phase" and "soil layer" was not significant, suggesting that intra-silvicultural phase differences ("soil layer" effects) in bacterial substrate used were more pronounced than inter-silvicultural phases differences.

As forest floor materials accumulate during forest development (Bernier and Ponge 1994; Ponge and Delhaye 1995), they are heavily invaded by roots. Indeed, Ehrenfled *et al.* (1992) showed that 30–60% of fine roots are in the organic horizons of a variety of coniferous forests. This in turn, affects microbial community structure and activity (Ehrenfeld *et al*. 1997). In our study, the amount of live roots increased gradually within the FH layer along the chronosequence but it decreased within the A layer. Since beech is a strongly ectomycorhizal species (Taylor *et al*. 2000), the increase in fungal biomass with the appearance of the fragmented and humified litter layers may be due to a fine-scale spatial distribution of ectomycorhizal roots. Whatever the soil layers, we showed that the N_{mic}-to-N_t tended to be lower in SP95. According to Bauhus *et al.* (1998) and Bauhus and Barthel (1995), a decrease in N_{mic}-to-N_t may indicate a decline in substrate quality. Thus, those results presume that soil organic matter quality may decline with stands age and/or beech trees may produce different litter quality according to stands age. This hypothesis is supported by Andersson *et al.* (2004) study who observed a decrease in C_{mic} within the humus layer (OH layer) of a spruce forest with stand age, suggesting a decline in soil organic matter quality with age. This hypothesis is also supported by others studies who showed substantial within-species variability in litter quality (Sanger *et al*. 1996; Sanger *et al*. 1998; Inagaki *et al*. 2004) often related to differences in site quality (soil water and nutrient availability and

climate conditions). For instance, Cordell *et al.* (1998) showed that differences in foliar N content among *Metrosideros polymorpha* populations was due in large part to water and nutrient availability among sites. Hence, our understanding of successional changes in microbial structure and function along forest development might be improved by monitoring litter quality along the chronosequence.

4.3. Humus forms: potential indicators of soil microbial community structure and function?

The PCA1 performed on morphological data set showed that the morphological variables discriminated clearly the four silvicultural phases and appeared robust as indicators of temporal changes in of forest functioning. The use of humus forms as indicator of forest ecosystem functioning has been proposed by several authors (Toutain 1987; Ponge 2003; Karroum *et al*. 2005; Jabiol *et al*. 2007). Mull humus is usually associated with high soil fertility and rapid nutrients turnover (Jabiol *et al.*, 2009). In a parallel study, we measured the potential N mineralization pathway within the different soil layers from the same chronosequence (Trap *et al.*, chapter 2). We observed higher potential net N mineralization within the organic layers in moder (oldest stands) compared to mull (youngest stands). We retrieve this trend for both microbial biomass and potential metabolic profile of bacteria. Finally, moder may not be synonymous to low microbial activity and biomass.

Among the morphological variables, the percentage of skeletonized leaves in OLv appears as an important indicator of microbial biomass and especially fungal biomass in the humic epipedon. The development of the OLv layer is mainly due to high fungal activity by leaves bleaching (Lavelle and Spain 2001; Jabiol *et al*. 2007) but the percentage of skeletonized leaves may directly due to the activity of macrofauna or mesofauna (Ponge 1999). Soil macroinvertebrates are for instance known to provide favorable conditions for bacteria growth (Lavelle and Spain 2001). For instance, earthworms grazing affect fungal population and favor N release and casting may provide mineral N by leaching promoting bacteria growth. The presence of macrofauna in mull may lead to higher bacteria biomass in the organic layer but probably limit fungal activity leading to lower microbial biomass N in the OL layer. This is supported by the negative correlation found between the microbial biomass N and the number of earthworms casts the OL layer.

The fungal biomass DNA in the OL layer was negatively correlated with the percentage of OH cover while the F/B ratio in the A layer was positively correlated with the OH maximal thickness. This suggeststhat the OH-based variables may constitute important indicators of fungal community biomass. Furthermore, the F/B ratio in the FH layer was negatively correlated with the structure of the A horizon (R^2 = 0.70, P < 0.01). Since the development of the OH layer is paired with lower clay-humic complexes abundance within the A horizon, the shift mull-moder clearly promote fungal community especially in the OH layer.

Finally, the morphological variables can be sort in three groups. The first group includes the most powerful morphological variables to discriminate silvicultural phases on the PCA and which explained the shift from mull towards moder humus forms (higher R^2 rank). It includes morphological variables from the organo-mineral horizon such as aggregation level and aggregate size. The second group gathers morphological variables significantly correlated with soil microbial community along the chronosequence. It includes the percentage of skeletonized leaves in OLv as well as the thickness of this horizon, the percentage of leaves fragment in OF and OLv and the

structure of the A horizon. The third one corresponds to morphological variables potentially indicators of both forest dynamics and soil microbial community. It includes the OH-based variables such as the thickness and the percentage cover of the OH layer as well as the number of earthworm casts in the OL layer. Hence, morphological variables identified as the best indicators of microbial community may not constitute necessarily the best morphological criteria to discriminate the main kinds of humus forms. They also suggest the potential use of selected macro-morphological variables as indicators of the structural and functional profiling of soil microbial community along stand ageing. OH-based and macrofauna-based variables were the best morphological variables indicators of both forest dynamics and soil microbial community. It appeared necessary to explore this question and provide datasets from contrasted pedoclimatic situations to calibrate humus-based ecological indices of soil microbial community.

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III. Synthèse du chapitre 5

Les résultats obtenus montrent une grande variabilité du profil structurel et fonctionnel des communautés microbiennes de l'épisolum humifère le long de la chronoséquence.

Changements du profil structurel

La quantité d'azote de la biomasse microbienne est plus élevée au sein des horizons organiques qu'au sein de l'horizon organo-minéral mais ne varie pas significativement le long de la chronoséquence. Néanmoins le ratio N_{mic}-to-N_t au sein de l'horizon OL diminue significativement le long de la maturation des peuplements. La communauté fongique domine dans l'OL alors que les bactéries dominent au sein du A quel que soit l'âge du peuplement. Le ratio biomasse fongique/biomasse bactérienne au sein de l'horizon FH augmente le long de la chronoséquence.

Changements du profil fonctionnel

La diversité fonctionnelle de la communauté bactérienne dans les horizons organiques est plus élevée dans les peuplements âgés. Au contraire, la diversité fonctionnelle est plus élevée dans le stade le plus jeune dans l'horizon A. Les substrats métabolisés diffèrent entre les horizons.

Relations microbes-formes d'humus

Nous avons observé de nombreuses corrélations significatives entre les variables morphologiques et microbiennes, ce qui confère aux formes d'humus un potentiel indicateur du profil structurel et fonctionnel des communautés microbiennes du sol. Par exemple, la biomasse microbienne au sein de l'OL est négativement corrélée avec le pourcentage de feuilles squelettisées dans l'OLv. Les résultats montrent que les variables morphologiques significativement corrélées aux variables microbiennes ne sont pas nécessairement celles dont la variance participe considérablement au changement mull-moder le long de la chronoséquence.

Bilan. La maturation des peuplements forestiers semblent favoriser la communauté fongique au sein des horizons de fragmentation et d'humification. Ces résultats supposent que la chute de la vitesse de décomposition de la litière durant la phase de croissance intense des peuplements, pourrait provenir de ces changements microbiens. Néanmoins, d'autres facteurs pourraient être impliqués, telle que la qualité du matériel organique apporté au sol, et expliquer les changements microbiens observés.

CHAPITRE 6
La qualité des litières

I. Présentation du chapitre 6

Au cours du chapitre 4, nous avons observé une diminution de la vitesse de décomposition de la litière de hêtres pendant la période de croissance intense des peuplements de hêtres (65-95 ans). Nous avons également mesuré des changements importants des profils structurel et fonctionnel des communautés microbiennes au sein de l'épisolum humifère le long de la chronoséquence. D'après le modèle hiérarchique de Lavelle (1993), dans des conditions climatiques et édaphiques similaires, la végétation est le facteur susceptible de contrôler le compartiment biologique du sol et les processus de décomposition de la matière organique. Dans l'étude qui va suivre, nous avons émis l'hypothèse que la qualité de la litière de hêtre varie au cours du vieillissement des peuplements et que cette variabilité est responsable des patrons de variations des formes d'humus et du cycle de l'azote. L'objectif de l'article 5 est de tester cette hypothèse.

II. Article 5

Litter quality variability as an adaptive trait? A multivariate study crossing litter quality, humus forms, soil properties and N dynamics

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Abstract

Forest dynamics leads to substantial shifts in belowground subsystem properties such as humus forms and N cycle. In this study, we put in test the hypothesis that the litter quality of a slow-growing species (*Fagus sylvatica* L.) change along a ageing gradient of pure beech (*Fagus sylvatica* L.) forest and significantly impacts humus forms, soil properties and soil N dynamics. We investigated the effects of litter quality changes on the belowground component properties by analyzing the data through two complementary statistical approaches: (i) a multiple co-inertia analysis (MCOA) to investigate the co-variation patterns between litter quality, soil N functioning, soil morphology and soil quality, and (ii) a stepwise multiple regression analysis to assess which litter quality attributes were the more significantly related to soil N dynamics and morphology. We observed two main shifts in litter element and fibers contents along the chronosequence. The first one occurred after 15 years of forest ageing and consisted in (1) a decrease in Mg, hemicellulose, cellulose, hemicellulose N and lignin N contents and (2) an increase in Mn, C/N and lignin/N ratios. The second shift occurred after 95 years of forest ageing and resulted in (1) a drop in lignin, lignin N, total cation (especially K and Mg) and (2) an increase in cellulose, hemicellulose N, relative N amounts in hemicellulose, cellulose and soluble. The MCOA suggested that A horizon chemical properties were weakly correlated with litter quality. However, the MCOA and the stepwise multiple regression analysis showed that three interrelated shifts occurred along the chronosequence (1) a morphological shift from dysmull towards moder, (2) a shift high quality *versus* low quality of beech leaf litter, and (3) a functional shift from nitrification-based functioning *versus* ammonification-based functioning. Those results suggest that litter quality variability is a key factor of belowground component functioning at the rotation scale but is poorly related to nutrient availability in the A horizon. The results permit us to hypothesize that within-species variability in litter quality along stands ageing, by promoting fungal-based energy channels, may allow a better N nutrition of the beech trees through bypassing microbial competition. Further investigations are required to test this hypothesis.

Keywords

Litter quality, mineral element, fiber, lignin/N ratio, humus forms, soil N cycle, soil nutrients availability, multiple co-inertia analysis, stepwise multiple regression analysis, *Fagus sylvatica*, 130-yr-old chronosequence.

1. Introduction

In terrestrial ecosystems, it is admitted that interactions between aboveground and belowground subsystems critically contribute to the regulation of ecosystem-level processes and properties (Wardle *et al.* 2004). For instance, plant species identity is often linked to fundamental properties of soil food webs. Fast-growing plant species (early-successional species) adapted to fertile soils produce high quality leaf litter favoring bacterial-based energy channels, while slow-growing species (late-successional species) adapted to infertile soils produce lower quality leaf litter promoting the fungal-based energy channel (Wardle *et al.* 2004; Wardle 2005). Furthermore, the dominance of bacterial-based energy channels is known to be link with high active humus forms (*i.e.* mull) with fast nutrients cycling, whereas fungal predominance may lead to less active humus forms (*i.e.* moder) with low nutrients cycling (Ponge 2003; Wardle 2005).

Humus formation processes (*i.e.* litter decomposition, humification and humus accumulation) are driven by a variety of factors, among which litter quality is probably one of the more important at a local scale (Olson 1963; Lavelle *et al.* 1993; Ponge 2003; Jabiol *et al.* 2007). For instance, litter decomposition rates among plant species are usually negatively correlated to initial lignin concentrations and positively related to initial nitrogen (N) concentrations (Melillo *et al.* 1982; Aerts 1997b). Differences in litter quality also exert an important feedback on soil N dynamics (Hobbie 2000; Hattenschwiler *et al.* 2003; Satti *et al.* 2003; Wurzburger and Hendrick 2009). Litter with high C-to-N and lignin-to-N ratios usually correlate with slow rates of soil net N mineralization and net nitrification and high N immobilization (Vitousek 1982; Scott and Binkley 1997; Satti *et al.* 2003). Litter quality is thus considered as a key plant trait to understand the way aboveground and belowground subsystems interact with each other's (Melillo *et al.* 1982; Aerts 1997b; Hobbie *et al.* 2006) and its variability among and within-species along soil or climate gradients has been the focus of a great number of recent studies (Hobbie 1996; Preston and Trofymow 2000; Madritch and Hunter 2002; Eviner 2004; Schweitzer *et al.* 2004; Wardle *et al.* 2009).

Forest dynamics leads to substantial shifts in belowground subsystem properties. Successional patterns of humus forms have often been described along forests ageing of both temperate semi-natural mixed (Bernier and Ponge 1994; Ponge and Delhaye 1995) and managed mono-specific stands (Aubert *et al.* 2004; Chauvat *et al.* 2007; Hedde *et al.* 2007). Early successional stages are usually associated with mull humus form (thin organic layers and deep organo-mineral horizon with clay-humus complexes) while moder (deep fragmented and humified organic layers with fine organic matter) have been frequently observed in older stages (Ponge 2003; Salmon *et al.* 2006). Humus forms succession along forest ageing is often followed by a modification of soil N functioning and a decrease in soil quality leading to soil acidification and growing lower nutrient availability (Brais *et al.* 1995; Aubert *et al.* 2004; Zeller *et al.* 2007; Trap *et al.* 2009). For instance, a decrease in nitrification along stand development was reported by several authors (Brais *et al.* 1995; Bauhus *et al.* 1997; Trap *et al.* 2009). Likewise, Aubert *et al.* (2004) observed an acidification of the organo-mineral horizon along a 170 years old pure beech chronosequence in France.

Within-species variability in woody litter quality has been already reported (Sanger *et al.* 1996; Sanger *et al.* 1998; Inagaki *et al.* 2004) and has been often related to local environmental conditions (soil water and nutrient availability and climate). For instance, Cordell *et al.* (1998) showed using a common garden experiment that differences in foliar N content among *Metrosideros polymorpha* Gaud. populations are

due in large part to water and nutrient availability among sites. It can thus be hypothesize in line with Wardle (2004) principles, that forest species may influence the belowground subsystem shifts occurring along both semi-natural and managed forest maturation by producing different leaf litter quality depending on the age of individual trees.

In this study, we thus put in test the hypothesis that the litter quality of a slow-growing species (*Fagus sylvatica* L.) change along a ageing gradient of pure beech (*Fagus sylvatica* L.) forest and significantly impacts humus forms and soil N dynamics. We investigated the effects of litter quality changes on the belowground component properties by analyzing the data through two complementary statistical approaches: (i) a multiple co-inertia analysis to investigate the co-variation patterns between litter quality, soil N functioning, soil morphology and soil quality, and (ii) a stepwise multiple regression analysis to assess which litter quality attributes were the more significantly related to soil N dynamics and morphology.

2. Materials and methods

2.1. Study site

The study sites were located in the Eawy state forest (France, Upper Normandy, 01°18' E; 49°44' N; 7200 ha). The climate is temperate oceanic with a mean annual temperature of +10°C and a mean annual precipitation of 800 mm. Within-species variability in leaf litter quality and its effects on soil functioning and morphology cannot be investigated in a short-term experiment since litter quality changes may impact soil functioning year after year over a long time period. Consequently, we designed a space-for-time substitution procedure (Pickett 1989) to empirically reconstitute a 130 years old beech forest chronosequence. *F. sylvatica* is an ideal candidate species for such a study, because is the more abundant and dominant late successional tree species in European temperate forest (Rameau 1997; Gessler *et al.* 2007) and because it occupies a broad habitat range (Sariyildiz and Anderson 2003b; Sariyildiz and Anderson 2005). Moreover, *F. sylvatica* is often chosen as a model in plant trait studies, thus allowing potentially useful literature comparisons (Dyckmans *et al.* 2002; Sariyildiz and Anderson 2003b; Sariyildiz and Anderson 2003a; Sariyildiz and Anderson 2005; Novak and Slodicak 2008; Kooijman and Martinez-Hernandez 2009).

Sixteen pure beech stands were thus selected to represent four silvicultural phases of different ages: 13-18 years (SP15), 65-66 years (SP65), 91-103 years (SP95) and 121-135 years (SP130) (Table 2.1, page 45). Each phase was comprised of four replicated stands. All stands were managed as even-aged forest by the French Forestry Service (ONF). All of them were in a flat topographic situation (205 m a.s.l.). The soil was an endogleyic dystric Luvisol (FAO 2006) developed on more than 80 cm of loess (lamellated silt) lying on clay with flints (Lautridou 1985; Laignel *et al.* 1998). The understory vegetation was defined as a characteristic *Endymio-Fagetum* according to phytosociological classification (Durin *et al.* 1967). At the centre of each stand, a 16 m² square plot was delimited. These square plots were located away from vehicle tracks and tree trunks to avoid any acidification due to local organic matter accumulation (Beniamino *et al.* 1991).

2.2. Litter quality

Beech leaves were collected within each stand between September and November 2007. Litter material consisted of freshly fallen leaves gathered in litterfall collectors (1m^2, 30cm deep and 1m height). Three collectors were placed in each stand along a 40m transect at 0, 20 and 40m to take into account intra-stand variability . Litter was air-dried at room temperature, oven-dried at 65°C for 48h and stored at room temperature (Verhoef 1995). Subsamples of leaf litter were ground in a laboratory mill (Culatti) to a mesh fraction smaller than 1 mm and analyzed for (1) mineral elements, (2) fibers (hemicellulose, cellulose and lignin) and (3) N content in fibers. Mineral elements (calcium, magnesium, sodium, manganese, potassium) were analyzed by atomic absorption spectrophotometer (AAS, ICE 3000 SERIES, Thermo Scientific, China) after acid digestion (Mircowave System MARSx). Fibers were determined on an Fiber analyzer according to the Van Soest (1994) method (French Norm XPU44-162). We distinguished (i) soluble, (ii) hemicellulose and bound protein, (iii) cellulose and (iv) lignin plus other recalcitrants, determined on an ash-free dry mass basis. Total C and N were measured in both leaf litter and fibers by gas chromatography with a CHN pyrolysis micro-analyser (Flash 2000 Series, CHNS/O Analysers Thermo Scientific, France).

2.3. Humus forms

Macro-morphological descriptions of organic and organo-mineral layers were previously done within frames (25 cm x 25 cm) at three corners of the central plot according to the French nomenclature (Jabiol *et al.* 2007) in May 2007. A total of 36 macro-morphological variables were described in the field on the basis of variation visible to the naked eye (Table 2.2, page 47). We distinguished mull (mainly dysmull) and moder (hemimoder + eumoder + dysmoder) humus forms on the base of morphological characters (Jabiol *et al.* 2007). A total of 48 humus profiles were described (3 descriptions per stand × 16 stands). We differentiated:

(1) **the OL horizon** consisting of almost unmodified leaf and woody fragments. Most of the original plant structures are easily discernible. Leaves may be recently fallen and unmodified (OLn) or bleached and slightly fragmented (OLv);

(2) **the OF horizon** consisting of a mixture of coarse plant debris with fine organic matter (humus). This horizon is characterized by an accumulation of partly decomposed organic matter derived mainly from leaves and woody materials, which are fragmented and mixed with invertebrate faeces. The proportion of organic fine substances ranges from 10 to 70 % by volume;

(3) **the OH horizon**. This horizon is an organic horizon characterized by an accumulation of decomposed organic matter. The original plant structures and materials are not discernible. Organic fine substance (invertebrate faeces) accounts for more than 70 % by volume. The OH horizons differs from the OF horizon by more advanced humification due to the action of soil organisms;

(4) **the A horizon** or **organo-mineral horizon** different in depth and structure among humus forms.

2.5. Soil N cycle

Field decomposition experiment-We used a litter bag protocol to investigate leaf litter N dynamics during leaf litter decomposition (Bocock and Gilbert 1957; Verhoef 1995). Litter bags (15 x 20cm) were made of nylon net with 0.175mm mesh size (Aubert *et al.* 2010) and filled with 10g of dried leaves collected in the same stand where the bags were further placed. In each stand, 6 of these bags were placed between the OLn and the underlying horizon. A total of 96 litter bags (6 litterbags x 16 stands) were thus placed in the field on December 2007. In each silvicultural phase, 4 replicate bags (one per stand) were removed 1, 3, 5, 7, 10 and 12 months after the beginning of the experiment, packed in plastic bag and transported to the laboratory. The material was then oven dried at 60°C until constant weight and total C and N were determined by gas chromatography with a CHN pyrolysis micro-analyser (Flash 2000 Series, CHNS/O Analysers Thermo Scientific, France). The final remaining mass of litter after 12 months of field incubation was determined (see chapter 5). We calculated the maximum amount of N immobilized into decomposing leaf litter (N_{max}) by identifying the sampling date at which the proportion of initial N was at its maximum. We used the following equation.

$$\mathbf{N_{max} = ((P_{max} \times N_i) - N_i)/L_i}$$

Where P_{max} = the maximum proportion of N during the field experiment, N_i = the initial litter N content and L_i = initial litter mass (Hobbie *et al.* 2006).

Laboratory incubation experiment- Soil net N mineralization and nitrification were determined in a separate incubation experiment. We sampled the OL, the OF, the OH and A layers sampled in may 2007 within frames (25 cm x 25 cm) in the same three corners of the 16m^2 square plot. Because the OH layer was sometimes discontinuous and not enough abundant, we decided to mix OF and OH layers in a single pool called "FH layers". Samples were stored at 4°C for the transport (Forster 1995b). In the laboratory, leaves were roughly cut, brushwood, stones and large roots were removed and the A horizon was sieved at 2 mm. Aliquots of each sample were dried at 105°C for 24 hours (A horizon) or at 65°C for 48 hours (organic layers) to obtain gravimetric water content. The A material moisture was adjusted at 85% of its maximum water holding capacity. The organic material was adjusted to 75% of fresh weight before the incubation (Pansu and Gautheyrou 2003).

Aerobic incubations were conducted by introducing separately 15 g of OL, 15 g of FH and 35 g of A in tight glass beakers during 28 days, in darkness conditions and at 28°C (Hart *et al.* 1994). To avoid anoxic conditions, the beakers were weekly open and aerate. Acetylene 2% (v/v) was used to distinguish between autotrophic *versus* heterotrophic nitrification (De Boer and Kowalchuk 2001). Acetylene concentration was checked at the beginning and at the end of the incubation using a Gas Chromatograph equipped with a flame ionization detector (Girdel 30 GC, Spherosil XOB 75 column, France). At the end of incubation, for each sample, an aliquot of 5g (organic material) or 10g (A horizon) was placed in beakers with K_2SO_4 (0.2 M) solution (200 or 100ml for organic and A material, respectively) and shacked for 1 hour at 100 rev min^{-1} (Alef 1995; Forster 1995a). The obtained extractions were filtered through Schleicher & Schuell 0790 ½ filter papers (pre-leached with 0.2 M K_2SO_4) and stored at -18°C before analysis. Concentrations of NH_4^+-N and NO_3^--N were determined colorimetrically (AA3, BRAN+LUEBBE, Norderstedt, Germany). Aliquots of each horizon were air-dried and total carbon and nitrogen were measured by gas chromatography with a CHN pyrolysis micro-analyzer (Flash 2000 Series, CHNS/O Analysers Thermo Scientific, France).

We determined the potential net N mineralization and nitrification in each soil layers by subtracting initial concentrations of NH_4^+ and NO_3^- from post-incubation concentrations. Potential net N mineralization and nitrification were expressed as µg-N g^{-1} C d^{-1}. A negative value indicates microbial net N immobilization (Hart *et al.* 1994; Persson *et al.* 2000). We also determined N mineralization rates by subtracting the potential net N mineralization by total N content at the beginning of the incubation. N mineralization rate was expressed as ‰ of total N mineralized after 28 days of incubation.

2.4. Chemical properties in the A horizon (A quality)

The A horizon was sample in may 2007 within frames (25 cm x 25 cm) in three corners of each $16m^2$ sampling square. A total of 17 chemical variables were measured and grouped in a single data set referred as to "A quality" (Table 6.1.). Total organic matter, C and N contents were measured by gas chromatography with a CHN pyrolysis microanalyser. The C/N ratio, pH_{water} and pH_{KCl}, ΔpH (Baize 2000), available phosphorous contents (Duchaufour and Bonneau 1959) and cation exchangeable capacity were also determined in this horizon (Ciesielski and Sterckeman 1997) as well as total elements (Ca, Mg, K, Mn, Na, Al, Fe, H) by the cobaltihexamine exchangeable method.

2.6. Statistical analyses

Statistics were done using the R software (ADE4 and Statistics packages; R Development Core Team, 2008). Statistical significance was set at $P < 0.05$. Comparisons of means between silvicultural phases were done using Kruskal-Wallis rank sum test. We calculated a coefficient of variation along the chronosequence (standard deviation/mean × 100) for each litter quality attributes. We investigated the relationships between litter quality, chemical properties of the A horizon (A quality), N functioning and soil morphology by using two complementary statistical approaches.

(1) We performed a Multiple CO-inertia Analysis (MCOA) among the four data sets: litter quality (table L), humus forms (table H), soil N cycle (table N) and A quality (table A). MCOA is an ordination method designed for the simultaneous analysis of *K* tables (Chessel and Hanafi 1996; Bady *et al.* 2004). MCOA allows an optimization of the variance within each individual table and of the correlation between the scores of each individual table (individual ordination). It provides synthetic scores that correspond to a reference ordination also called compromise. MCOA analyzes whether each table deviates from this reference and shows variables driving the common structure or responsible for the deformation of the common structure. For that purpose, Principal Component Analyses (PCA) were previously performed individually on each table. The four tables contained 16 rows (*i.e.* the number of stands) and, respectively, 26, 36, 26 and 16 columns for L, H, N and A tables, respectively. The value of the squared covariance from the MCOA between the scores of individual tables and the synthetic variable of the same axis (1st or 2nd) corresponds to the contribution of a table in the construction of the synthetic axes. Hence, we classified the tables according to their squared covariance from 1 to 4 for each axis. The first rank corresponded to the highest squared covariance value. This ranking thus reflected the relative contribution of each table in the construction of the synthetic axes. The significances of the vector correlation coefficients (RV coefficient) obtained from both the compromise and each table were tested with a Monte Carlo permutation test (10000 permutations).

(2) We used stepwise multiple regressions with backwards elimination to identify which litter quality attributes were correlated with soil N functioning and morphology. Multiple regressions were performed after screening of potential independent variables for significant autocorrelation. We considered that variables were auto correlated when $R^2 > 0.90$. Additionally, we used linear regressions to assess more specifically the relationships between some litter quality attributes and some measures of soil functioning.

3. Results

3.1. Variability in beech litter quality along the chronosequence

The litter concentration in total cation was higher in SP15 and lower in SP130 (Table 6.1). Both Mg and K had their highest values in SP15 (1.33 and 4.37 mg g^{-1}, respectively). Mn was the only nutrient with the highest value in SP95 (4.16 mg g^{-1}). Ca and Na did not show significant changes between silvicultural phases (Table 6.1). Litter hemicellulose and cellulose exhibited lower values in SP65 and SP95 and higher ones in SP15 and SP130. In contrast, lignin concentration in litter was significantly higher in SP65 and SP95 (34.87% and 35.06%, respectively) compared to SP15 and SP130 (28.51% and 29.12%, respectively). Total N showed the lowest values in mature stands (SP130) with only 0.88%.

Table 6.1. *Litter quality attributes according to silvicultural phases.*

Leaf litter quality attributes	Silvicultural phases											
	SP15			SP65			SP95			SP130		
Nutrients (mg element/g litter)												
Ca	5.56	(1.39)	a	5.67	(0.98)	a	5.77	(0.55)	a	5.70	(0.75)	a
Mg	1.33	(0.22)	**a**	0.84	(0.09)	**b**	0.83	(0.14)	**b**	0.73	(0.08)	**b**
K	4.37	(1.22)	**a**	3.50	(0.52)	**a**	3.11	(0.65)	**ab**	2.42	(0.43)	**b**
Na	0.32	(0.10)	a	0.32	(0.05)	a	0.38	(0.07)	a	0.35	(0.05)	a
Mn	3.46	(0.15)	**b**	3.58	(0.31)	**ab**	4.20	(0.49)	**a**	3.91	(0.33)	**b**
Total cation	14.63	(1.44)	a	13.91	(1.33)	a	14.24	(0.92)	a	13.11	(0.91)	a
C fractions (% ash-free dry mass)												
Soluble	36.09	(1.02)	a	37.12	(1.77)	a	37.47	(0.92)	a	38.70	(2.06)	a
Hemicellulose	13.83	(1.47)	**a**	10.24	(1.64)	**b**	10.20	(1.54)	**b**	11.88	(2.25)	**ab**
Cellulose	21.58	(4.53)	**ab**	17.77	(2.66)	**b**	19.44	(2.70)	**b**	23.03	(2.09)	**a**
Lignin	28.51	(2.89)	**b**	34.87	(2.18)	**a**	35.06	(2.74)	**a**	29.12	(2.07)	**b**
N within fractions (%)												
Soluble	0.85	(0.35)	a	0.66	(0.14)	a	0.64	(0.09)	a	0.72	(0.09)	a
Hemicellulose	0.19	(0.05)	**a**	0.03	(0.04)	**b**	0.01	(0.01)	**b**	0.16	(0.08)	**a**
Cellulose	0.44	(0.15)	a	0.28	(0.08)	a	0.31	(0.07)	a	0.46	(0.08)	a
Lignin	1.88	(0.17)	**a**	1.46	(0.14)	**b**	1.35	(0.06)	**b**	1.04	(0.17)	**c**
Total N	1.20	(0.17)	**a**	0.96	(0.05)	**ab**	0.93	(0.07)	**ab**	0.88	(0.05)	**b**
N within fractions to total N ratio (%)												
Soluble	43.96	(10.19)	a	43.04	(8.37)	a	42.93	(3.58)	a	51.51	(4.75)	a
Hemicellulose	2.08	(0.91)	**a**	0.33	(0.47)	**b**	0.09	(0.11)	**b**	2.42	(1.41)	**a**
Cellulose	6.00	(1.41)	**b**	5.35	(1.38)	**b**	6.16	(3.07)	**b**	11.98	(2.21)	**a**
Lignin	45.29	(8.14)	**ab**	51.28	(9.32)	**a**	50.82	(3.24)	**a**	34.09	(6.39)	**b**
Ratio												
C/N	40.16	(5.51)	**b**	49.07	(2.70)	**a**	50.67	(4.82)	**a**	53.55	(3.12)	**a**
Lignin/N	24.09	(3.68)	**b**	36.50	(4.23)	**a**	37.87	(2.86)	**a**	33.25	(5.05)	**a**

Means (SD). Letters (a, b and c) refer to significant differences between silvicultral phases according to Kruskal-Wallis rank sum test ($P < 0.05$, n=4).

The percentage of N in hemicellulose was significantly higher in litter from SP15 (0.19%) and SP130 (0.16%) and lower in litter from SP65 (0.03%) and SP95 (0.01%). The percentage of N in cellulose did not change between silvicultural phases. N content in lignin was higher in SP15 (1.88%) and lower in SP130 (1.04%). The hemicellulose N to total N ratio was lower in SP65 and SP95 while the cellulose N to total N ratio was maximal in SP130 with 11.9%. The lignin N to total N ratio was significantly maximal in leaf litter from SP95 (51.2%) and minimal in SP130 (34.0%). No significant change along the chronosequence was observed for the percentage of total N in soluble (Table 6.1). Litter C/N ratio was significantly lower in SP15 (40.1) compared to the three others silvicultural phases. SP130 showed the highest C/N ratio with 53.5. The lignin/N ratio was significantly lower in litter from SP15 (24.0) compared to the three others silvicultural phases and was higher in SP95 (37.8).

The N content in hemicellulose is the variable with the highest variation (more than 80%) while Ca and soluble contents are the attribute with the lowest variation (less than 2%) (Fig. 6.1). Mg and K contents as well as cellulose N and lignin N showed more than 20% of variation along the chronosequence.

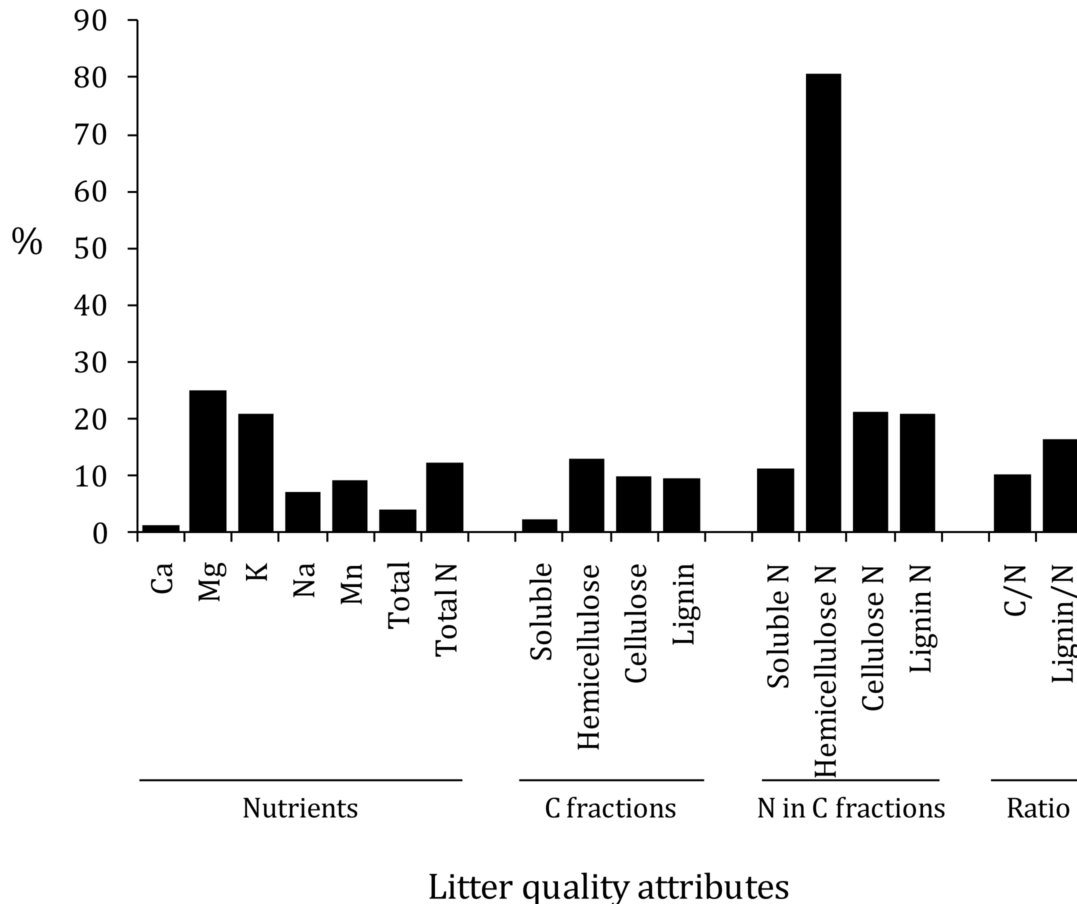

Figure 6.1. *Coefficient of variation (standard deviation/mean × 100) in F. sylvatica leaf litter quality attributes along stands ageing.*

3.2. Relationships between litter quality, soil N cycle, humus forms and A quality

3.2.1. Multiple CoInertia Analysis

Two axes were kept to interpret the Multiple CO-inertia Analysis (MCOA) according to the Eigenvalue diagram (Fig. 6.2.c). The first axis of the compromise opposed the younger stands (SP15, negatives scores) to the mature ones (SP95 and SP130, positives scores) (Fig. 6.2.a). The projection of individual PCA axes onto compromise plan showed that the first axis of each table, except A quality, was correlated with the first axis (Fig. 6.2.b). Based on the values of the squared covariance (between each individual table and the compromise), the contribution of each table in the construction of this first synthetic axis was ranked as follow: litter quality > soil morphology > soil N cycle > A quality.

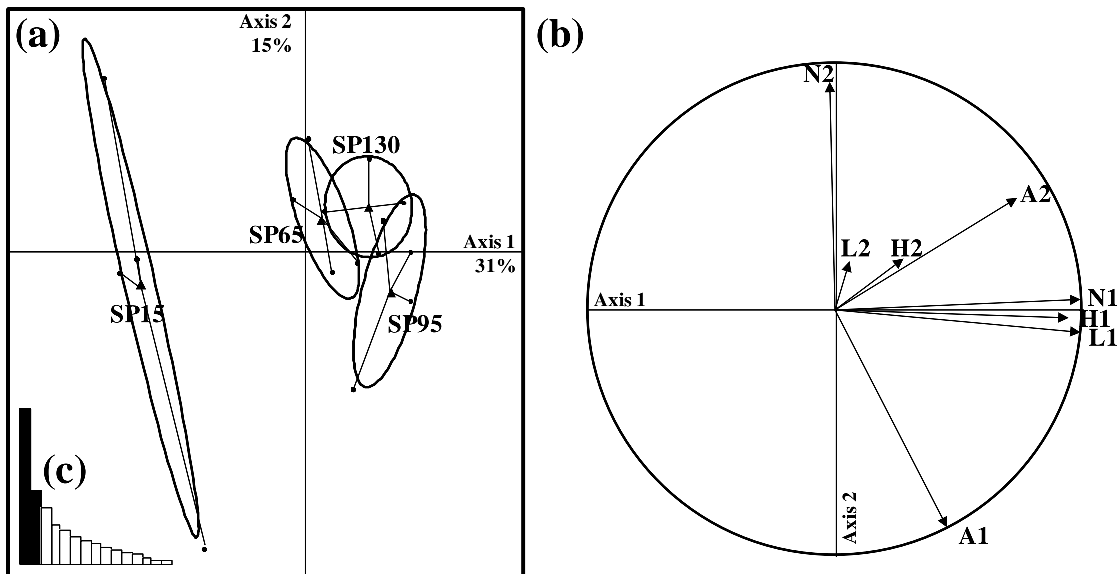

Figure 6.2. *Multiple coinertia analysis performed on litter quality (Table L), soil N cycle (Table N), humus forms (Table H) and A quality (Table A) data sets. Two axes were saved. (a) Silvicultural phases ordination represented by ellipse of dispersion and barycentres (black triangle) on the first two axes of the compromise. (b) Projection of the first two axes of each table onto the first two synthetic axes of the common structure. (c) Eigenvalue diagram. Coding for each variable is given in the annexes 6.1 (Table L), 6.2 (Table H), 6.3 (Table N) and 6.4 (Table A).*

The second axis of the common structure did not segregate the silvicultural phases (Fig. 6.1.a). However, in contrast to the first axis, we observed high intra-SP variability on the second axis (*i.e.* high deformation of ellipse of dispersion) for all silvicultural phases, especially for the SP15 (Fig. 6.2.a). Also, the first axis of the PCA performed from the table A was correlated with this second axis of the compromise (Fig. 6.1.b). The second synthetic axis opposed the pH_{water}, pH_{KCl}, and ΔpH variables (positives scores) against OM, C/N, CEC, H^+, Al and, Fe variables (negatives scores) (Fig. 6.3.d). The contribution level of each table to this second axis was ranked as follow: A quality > humus forms > soil N cycle > litter quality.

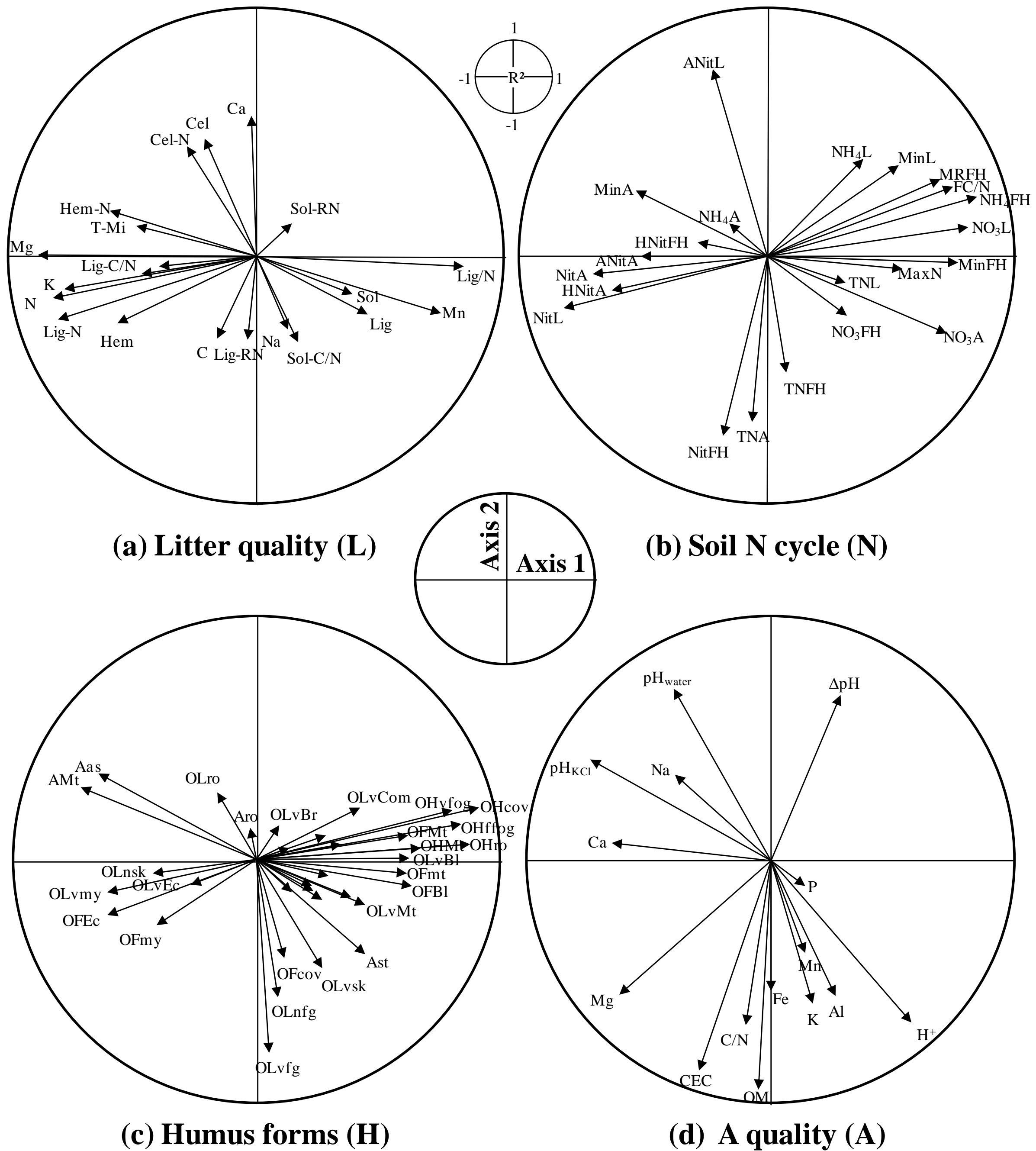

Figure 6.3. *Circles of correlations between variables from table L (a), N (b), H (c) and A (d) and the first two axes of the common structure of the MCOA. For clarity, labels of middle variables were removed.*

All tables had significant RV coefficients with the compromise ordination (Table 6.2), the lower value being observed for the A quality table (RV coefficient = 0.65, P value = 0.0002, Monte-Carlo test with 10000 permutations). Litter quality, soil N cycle and morphology tables were highly correlated together (Table 6.2), whereas no significant RV coefficient was observed with the table A. Soil morphology and litter quality tables exhibited the highest RV value (RV coefficient = 0 .65, P value = 0.0002, Monte-Carlo test with 10000 permutations).

Table 6.2. *Matrix of RV coefficients between each table including the compromise from the MCOA.*

Data sets	Litter quality (L)	Soil N cycle (N)	Humus forms (H)	A quality (A)	Compromise
Litter quality (L)	1				**0.80*****
Soil N cycle (N)	**0.58****	1			**0.71*****
Humus forms (H)	**0.65*****	**0.59****	1		**0.83*****
A quality (A)	0.45	0.40	0.42	1	**0.64*****

* *P* < 0.05.
** *P* < 0.01.
*** *P* < 0.001.

3.2.2. Multiple regression analyses: litter quality - humus forms

The number of earthworm casts in the OLn layer was positively related to soluble, lignin N and total C, and negatively related to K, lignin and lignin/N ratio ($P < 0.01$, $R^2 = 0.86$) (Table 6.3). In contrast, the number of earthworms cast in the OF layer was positively related to Mn, N content in hemicellulose and total C and negatively related to Mg, N content in cellulose, lignin and lignin/N ratio ($P < 0.001$, $R^2 = 0.91$). The percentage of skeletonized leaves in the OLv layer was positively related to N content in lignin, cellulose and lignin/N ratio and negatively related to Ca, soluble and total N ($P < 0.01$, $R^2 = 0.88$). Na, total N and lignin/N ratio were the most significant attributes related to the percentage of bleached leaves in the OF layer ($P < 0.001$, $R^2 = 0.96$). Fine organic matter (FOM) from both fauna faeces and vegetal debris was negatively related to total N while lignin content was positively related to vegetable FOM. The maximal thickness of the OH layer was positively related to Na and negatively related to K contents ($P < 0.001$, $R^2 = 0.68$). Finally, among litter quality attributes, N, lignin, lignin/N as well as Mn, Ca, K and Mg emerged as the best predictors of soil morphology changes (Table 6.3).

3.2.2. Multiple regression analyses: litter quality - soil N cycle

The *in situ* maximum N immobilization during litter decomposition was positively related to K, Mn, total N and lignin/N ratio, and negatively related to total C and lignin content ($P < 0.001$, $R^2 = 0.92$) (Table 6.4). Potential net N mineralization rates in the OL layer was significantly positively related to Ca, Na and Mn and negatively related to Mg and K. In contrast, in the FH layers, Mg and Na were the only explanatory attributes significantly related to N mineralization rates ($P < 0.01$, $R^2 = 0.79$). Mg, hemicellulose, soluble, N content in cellulose and total C were negatively related and while K and Mn were positively related to *in situ* ammonium pools in the FH layer ($P < 0.01$, $R^2 = 0.92$). Mg was the most significant litter quality trait related to net nitrification in the A horizon. Finally, mineral elements, especially Mn, Mg and K, as well as lignin, total C and lignin/N were the best predictors of soil N changes (Table 6.4).

Table 6.3. *Stepwise multiple regressions with backwards elimination (P < 0.01) of humus forms variables with litter quality attributes.*

Litter quality attributes	**Morphological variables (Table H)**						
	Earthworms casts		Skeletonized leaves	Bleach leaves	FOM[a] from fauna faeces	Vegetable FOM	Maximal thickness
Soil layers	*OLn*	*OF*	*OLv*	*OF*	*OH*	*OH*	*OH*
Ca			X(-)***			X(-)	
Mg		X(-)*		X(-)			
K	X(-)*						X(-)***
Na				X(-)**		X(-)	X(+)*
Mn		X(+)*		X(+)			
Soluble	X(+)		X(-)**				
Cellulose			X(+)**	X(-)*			
Hemicellulose		X(-)		X(-)*	X(-)*	X(-)	
Lignin	X(-)**	X(-)**	X(+)**	X(+)**		X(+)**	
Hemicellulose N		X(+)*				X(+)	
Cellulose N		X(-)			X(-)		
Lignin N	X(+)		X(+)				
Total C	X(+)	X(+)	X(+)**				
Total N			X(-)*	X(-)***	X(-)**	X(-)***	
Lignin/N	X(-)**	X(-)***	X(+)**	X(+)***			
P value	**	**	**	***	**	***	***
R^2	**0.86**	**0.91**	**0.88**	**0.96**	**0.70**	**0.91**	**0.68**

An ''X'' indicates that the variable was included in the model. Blank cells indicate nonsignificance (P >0. 05). The sign of the relationship between significant litter quality and soil morphological variables is indicates between brackets.

[a]FOM: fine organic matter

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Table 6.4. *Stepwise multiple regressions with backwards elimination ($P < 0.01$) of N variables with litter quality attributes.*

Litter quality attributes	**Field N variables**		**Laboratory N variables**		
	Maximum N immobilization	*in situ* NH_4^+ pools	N mineralization rates		Net nitrification
Soil layers		*FH*	*OL*	*FH*	*A*
Ca			X(+)		
Mg		X(-)*	X(-)	X(-)***	X(+)**
K	X(+)***	X(+)	X(-)**		
Na			X(+)	X(+)**	
Mn	X(+)***	X(+)	X(+)**		
Soluble		X(-)			
Hemicellulose		X(-)			
Lignin	X(-)**				
Hemicellulose N		X(+)			
Cellulose N		X(-)			
Total C	X(-)*	X(-)*			X(+)
Total N	X(+)				
Lignin/N	X(+)*				
P value	***	**	**	***	**
R^2	**0.92**	**0.92**	**0.79**	**0.79**	**0.63**

An ''X'' indicates that the variable was included in the model. Blank cells indicate nonsignificance (P >0. 05). The sign of the relationship between significant litter quality and soil N variables is indicates between brackets.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

4. Discussion

4.1. Variability in beech litter quality along ageing forests

Our study clearly demonstrates that *F. sylvatica* produce a leaf litter with contrasted quality attributes along stand ageing with homogeneous edaphic and climatic conditions. We observed two successive shifts along the chronosequence (Fig. 6.4). The first one occurred between SP15 and SP65 and consisted in (1) a decrease in Mg, hemicellulose, cellulose, hemicellulose N and lignin N contents and (2) an increase in Mn, C/N and lignin/N ratios. The second shift occurred after 95 years of forest ageing and resulted in (1) a drop in lignin, lignin N, total cation (especially K and Mg) and (2) an increase in cellulose, hemicellulose N, relative N amounts in hemicellulose, cellulose and soluble. The variability of *F. sylvatica* leaf litter quality is known to be important towards soil water and nutrient availability (Sariyildiz *et al.* 2005), light availability (sun *versus* shade leaves) (Sariyildiz and Anderson 2003a) or leaf age (green *versus* senescent leaf) (Sariyildiz and Anderson 2005; Tyler 2005) (see annex 6.5). We discussed how these factors may explain the observed trends in litter quality along the gradient of forest maturation.

A first explanation may be related to the fact that trees usually produce leaves with contrasted properties depending on their light exposure (Sariyilidz and Anderson, (2003a). For instance, it is know that light availability induce 7% and 17% of variation in N and lignin content in beech litter (calculated from Sariyilidz and Anderson, 2003a). Sariyilidz and Anderson (2003a) found that shade leaves of *F. sylvatica* contain less lignin and more cellulose than sun leaves. Single tree may thus produce sun and shade leaves with different chemical properties. The changes in litter quality observed along the chronosequence may thus be due to a modification in the ratio between shade and sun leaves in litter. We did not found any study focusing on the proportion between shade and sun leaves across forest ageing gradients. Addressing it would be interesting to characterize the chemistry of both sun and shade leaves and the shade/sun leaves ratio in litter along stand ageing.

Besides light availability, a second explanation may be found in the capacity of trees to modify energy allocation depending on the competitive context. Intraspecific competition is for example known to influence the allocation of photosynthates in individual beech trees (Ryan *et al.* 1997). In the SP65 stands, trees are expected to experience an intense growth phase (aggradation phase) and a strong competitive stress, and they may therefore allocate larger photoassimilate fractions to wood production (Kaufmann and Ryan 1986; Nilsson and Albrektson 1993; Ryan *et al.* 1997). A similar conclusion was proposed by Vanninen and Makela (2000), who found that suppressed trees of *Pinus sylvestris* L. allocate more energy to stem wood than dominant trees, and have a higher stem growth per unit of foliage mass. This would explain the decrease in litter N content during the aggradation phase. Competition may also promote higher lignin synthesis to sustain wood production. In the SP130 stands, photosynthate allocation to wood production may decline in favor to reproductive organs. This would explain the second shift observed in our results for lower litter lignin content. Litter quality may thus constitute a response trait to physiological behavior of tree during the aggradation phase.

Another physiological behavior is the ability of tree species to re-absorbe nutrients from its own leaves before abscission, which may result in a substantial decrease in nutrients contents between green and senescent leaves (Aerts 1996; Aerts 1997a). This important feedback mechanism may concern more than half of the nutrient

content in leaf (Eckstein *et al.* 1999). We can thus hypothesize that during the aggradation phase, both litter decomposition and soil alteration may not provide enough nutrients for wood building. This may lead to higher NRE after 15 years of tree ageing and have a consequence on leaf litter quality. This hypothesis is supported by the observation that conspecific mature trees often show higher leaf N resorption efficiency compare to younger ones (Nordell and Karlsson 1995; Eckstein *et al.* 1999)

A last explanation lies on the variability in genetic diversity of *F.sylvatica* stands along the chronosequence. As observed for most forest tree species with widespread temperate distributions, *F. sylvatica* has a high within-population genetic diversity resulting in high variability in many traits such as litter quality (Leonardi and Menozzi 1996; Sander *et al.* 2000; Schweitzer *et al.* 2004). We can suppose that the genetic structure of beech stands may change over time owing to both human and natural tree selection occurring during stand maturation. These changes may induce a modification of many traits including litter quality. While our study was set up at the stand level, thus making difficult any conclusion at the tree level, it is reasonable to hypothesis that the population genetic structure may change along the chronosequence and affect litter quality.

4.2. Litter quality and organo-mineral horizon properties relationships

We showed that the quality of the organo-mineral horizon (nutrients availability, pH) strongly deviated from the compromise ordination, thus suggesting that A horizon chemical properties were weakly correlated with litter quality. The lack of significant patterns of A quality along the chronosequence paired with constant climate conditions eliminates the possibility that litter quality was a response trait driven by site or A quality. Our results are not agree with Cordell *et al.* (1998) who showed that differences in foliar N content among *Metrosideros polymorpha* Gaud. populations are mostly related to soil water and nutrient availability among sites. This statement was further supported by Austin and Vitousek (2000) who concluded that local environmental characteristics such as water and nutrient availability may influence dramatically litter properties of *M. polymorpha* along a rainfall gradient in Hawai. However, it is important to note that A properties were responsible for the intra-silvicultural phase variability (axis 2 of the MCOA), especially in the youngest stands (SP15). Indeed, in contrast to moders observed under mature stands, dysmulls in the youngest stands are characterized by a high level of bioturbation by soil invertebrates, which is known to generate soil heterogeneity at different spatial scales (Ponge 2003; Aubert *et al.* 2004). The shift mull-moder may probably lead to low intra-silvicultural phase variability in A properties. On the other hand, forest development is paired with forest floor materials accumulation forming the humification layer and we observed an increase in the number of living roots in the OH layer along the beech chronosequence. Additionally, ectomycorrhizal beech roots may permit direct uptake of nutrients, including complexe molecules, from the OH horizon by-passing competition with soil microbes for nutrients, especially N (Schimel and Bennett 2004). Finally, older trees may uptake water and nutrients directly in upper organic layers (OF and OH layers) and it is possible that this ability limit the role of the organo-mineral horizon in topsoil functioning shift along the chronosequence.

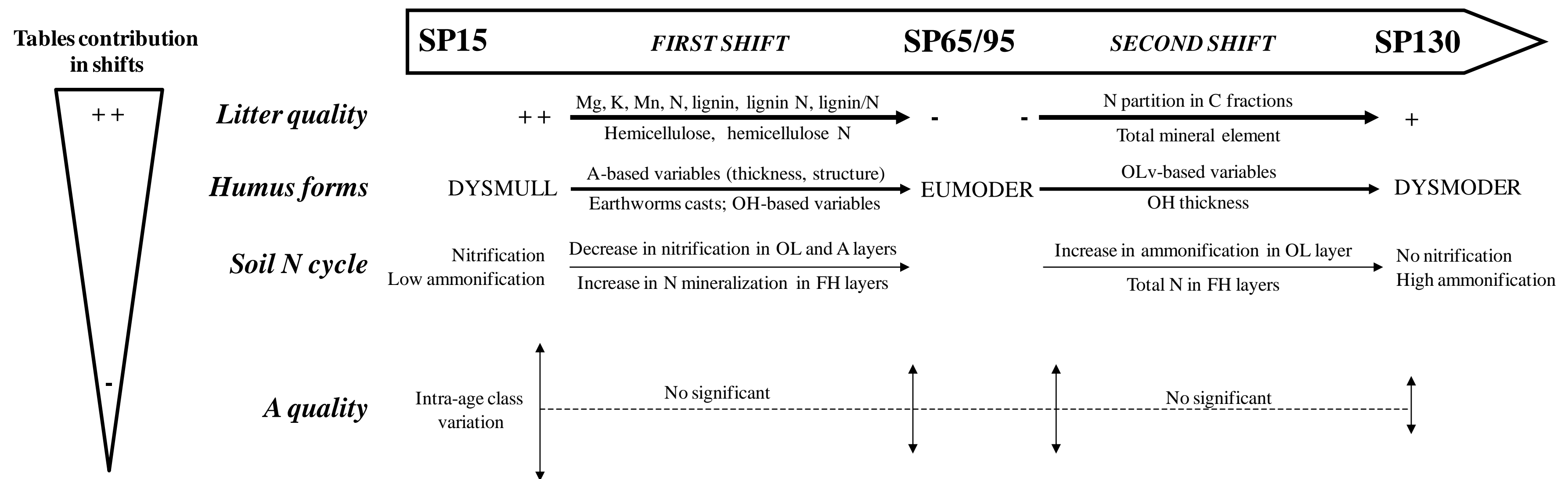

Figure 6.4. *Main shifts in litter quality, humus forms and soil N cycle along the pure beech chronosequence according to the Multiple CO-inertie Analysis.*

4.2. Litter quality as a driving factor of soil N cycle and morphology in ageing forests?

The contributions of the individual variables of each initial table to the compromise ordination (Fig. 6.3.a, b, c and d) suggest that this first axis correspond to (1) a morphological shift from dysmull (A-based variables such as Aas, Amt and earthworms casts) towards moder (OH-based variables such as OHro, OHffog, OHMt, OHcov), (2) a shift high quality (Mg, K, total N, N content in lignin and hemicellulose) *versus* low quality (lignin:N ratio, lignin and Mn) of leaf litter, and (3) a functional shift from nitrification-based functioning (NitL, NitA, HNitA, HNitFH and ANitA) *versus* ammonification-based functioning (MinFH, NH4FH, MRFH, MinL, TML).

Litter quality had the highest contribution to both axis 1 (first shift) and axis 3 (second shift) of the MCOA. We also found high significant relationships between a number of litter quality variables and both N and morphological variables. This suggests that litter quality may be an important driving factor of both soil N dynamics and morphological shifts along the chronosequence. For instance, low litter quality (high lignin content and high lignin/N ratio) was negatively correlated to the abundance of earthworm casts. Earthworms are able to digest relatively little of the cell wall constituents of the plant litter they ingest such as cellulose or simple phenolic materials, but probably no lignin (Curry and Schmidt 2006). The increase in lignin and the decrease in N during the intense tree growth phase may probably limit earthworm's activity leading hence to the development of moder (Beyer *et al.* 1991) but may favor enchytraeids (moder organisms) abundance. Indeed, the abundance of skeletonized leaves, a direct result of enchytraeids activity, was in fact positively correlated with litter cellulose, lignin and lignin/N and negatively related with litter Ca content. The antagonisms between earthworms and enchytraeids has been considered as an important factor to explain the shift from mull to moder observed along forest maturation gradients (Ponge 2003). Our results suggest that the ratio between both groups of invertebrates may be controlled by litter quality changes, *i.e.* earthworms being enhanced by higher Ca levels in litter and the consecutive increase in soil pH, while lower litter quality (high lignin and lignin/N ratio) may favor enchytraeid activity.

Besides enchytraeids, the first shift in litter quality occurring after 15 years ageing may also supports fungal community. For instance, we observed a strong relationship between the percentage of bleached leaves and lignin, total N and lignin/N ratio. Higher litter lignin content coupled with lower N content may in fact favor saprofagous fungal activity, and successively may result in a higher percentage of beached leaves. In a parallel study, we investigated soil fungal community biomass within the same stands used to reconstitute the chronosequence via ergosterol pools assessment. We found significant linear correlations between fungal biomass in the fragmentation and humification layers and lignin N ($R^2 = 0.62$, $P < 0.001$) or Mn contents in litter ($R^2 = 0.31$, $P < 0.05$). A possible explanation of this pattern lies on the importance of Mn as a cofactor of fungal extra cellular enzymes such as peroxidases that are efficient to degrade complex molecules such as lignin (Hatakka 1994; Erland and Taylor 2002; Baath and Anderson 2003; Gobat *et al.* 2004). Higher litter Mn content paired with lower N content in lignin may favor fungal enzymes activity and increase soil fungal biomass and richness along the chronosequence. For instance, Lu *et al.* (1999) examined sporocarp production in *Eucalyptus globules* Labill. plantations in Western Australia and found that the species richness was positively related to stand age (from 1 to 8 years old) and negatively correlated to soil pH. Twieg *et al.* (2007)

confirmed these results by studying the succession of ectomycorrhizal fungal communities along a 100 years old chronosequence of Douglas-fir (*Pseudotsuga menziesii*) and paper birch (*Betula papyrifera*) stands. Litter Mn content was also negatively related to the thickness of the organo-mineral horizon and positively related to maximal OH layer thickness and the number of livings roots in OH layer. We can conclude that Mn is probably a key litter attributes in the formation of moder.

The control of soil fungal-based channel by litter quality along the chronosequence is also supported by our results of soil N dynamics. For example, mineral elements, especially K and Mn, were strongly related with maximal N immobilization during litter decomposition and potential N mineralization rates in the FH layers. It is well know that field N immobilization during litter decomposition is almost exclusively due to uptake by fungal hyphae from the surroundings of the litter (Berg and Laskowski 2006). The initial concentration of N in litter definitely has an influence on whether there will be a net accumulation of N or not (Berg and Laskowski 2006). The decrease in total N in litter may thus favor fungal-based channel since N requirements by bacteria are higher due to high rates of cell division, and because bacteria use amino-acids as osmoregulator rather than carbohydrates. Also, the lower N requirement by C unit of fungi compare to bacteria may responsible for higher potential net N mineralization in soils where fungi dominate (Lavelle and Spain 2001; Kooijman *et al.* 2009). This may explain the significance of the correlations between Mn content and soil N transformations as highlighted by the multiple regression analyses.

5. Conclusion

Our study showed that beech species produce leaf litter with different quality according to the age of stands. It appears that the investigation on litter quality trait changes with forest ageing by broad comparison between two contrasted stand ages is by far too simplistic to highlight the whole complexity of mechanisms involved. Obviously, the method used does not permit us to conclude on the causal effect of litter quality on humus forms and soil functioning variations along the chronosequence. Experimental studies are clearly needed to assess the role of litter quality on soil functioning shifts along forest ageing. However, the variation in litter quality was highly correlated with soil morphology and N dynamics shifts but poorly related to the availability in nutrients within the A horizon. Those results suggest that litter quality variability is a key factor of belowground component functioning at the rotation scale but is poorly related to nutrient availability in the A horizon.

We wonder if positive N feedback emerges from these litter quality changes (Wurzburger and Hendrick 2009). Wardle (2005) and Aerts (2002) discussed on the assumption that the low quality of late successional species and the saprotrophy of mycorhizal symbionts can function together promoting positive N feedback. Indeed, several studies provided evidence of an intricate N feedback where plant litter chemistry would influence N cycle to maximize N acquisition by the host's mycorrhizal roots while hindering microbial N acquisition (Aerts 2002; Neff *et al.* 2003; Schimel and Bennett 2004; Wurzburger and Hendrick 2009). The development of mycorrhizae increases the ability of roots to absorb nutrients such as organic N from the soil. The degree to which this ability is enhanced depends on the extent of mycorhizal colonization. The litter-induced promotion of fungal-based channel and probably late successional ectomycorrhizal fungal species along forest maturation by higher Mn and lower N content in lignin may favor root colonization by mycorhizal species. *Fagus sylvatica* is a strong mycorhizal species (Simard *et al.* 2002; Smith and Read 2008) and it

is well know that ectomycorrhizal fungal species are especially efficient in taking up both organic and inorganic N (Gessler *et al.* 1998; Wallenda and Read 1999). We can thus hypothesize that within-species variability in litter quality along stands ageing, by promoting fungal-based energy channels, may allow a better N nutrition of the trees through bypassing microbial competition (Schimel and Bennett 2004; Wardle 2005). Such N feedback would thus determine a clear competitive advantage of beech trees over microbial species.

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III. Synthèse du chapitre 6

La qualité de la litière de hêtre présente une variabilité importante le long de la chronoséquence de 130 ans. Les résultats mettent en avant deux principaux changements. Le premier changement entre le stade de 15 ans et le stade 65 ans correspond à (1) une diminution des teneurs en Mg, en hémicellulose, en cellulose, en azote hémicellulosique et ligninique et (2) une augmentation des teneurs en Mn, du C/N et du ratio lignine/N. Le second changement après 95 ans de vieillissement correspond à (1) une baisse de la teneur en lignine, des cations (surtout K et Mg) et de l'azote ligninique et (2) une augmentation de la cellulose, de l'azote hémicellulosique, et du pourcentage d'azote total dans l'hémicellulose, la cellulose et les solubles. Les résultats issus des analyses univariées et multivariées témoignent du rôle majeur de la qualité de la litière dans les changements fonctionnels et morphologiques de l'épisolum humifère le long de la chronoséquence. Les résultats montrent également que la disponibilité des nutriments dans l'horizon A joue un rôle mineur dans les patrons de variations des formes d'humus et du cycle de l'azote à l'échelle de la rotation sylvicole. Une approche expérimentale permettrait de préciser les rapports de causalité entre la qualité de la litière et le cycle de l'azote.

PARTIE III

Approche expérimentale
Les facteurs de contrôle du cycle interne de l'azote

PEPINIERE DES ESSARTS - FORÊT D'EAWY - JUIN 2008

CHAPITRE 7
Approche expérimentale

I. Présentation du chapitre 7

Au cours des chapitres précédents, nous avons montré des changements importants du recyclage de l'azote (chapitres 2 et 3) et du profil structurel et fonctionnel des communautés microbiennes (chapitre 5) au sein de l'épisolum humifère le long de la chronoséquence de hêtre. Parallèlement, nous avons observé des changements importants de la qualité des litières (chapitre 6) qui pourraient être impliqués dans le contrôle du cycle de l'azote et des communautés microbiennes. Outre la qualité de la litière, le peuplement de hêtres modifie le fonctionnement de l'épisolum via les racines en association avec les partenaires fongiques. L'objectif de cette étude est donc de tester l'hypothèse selon laquelle une variation des paramètres foliaires et racinaires entraîne une modification des transformations de l'azote dans le sol et des communautés microbiennes du sol. Pour cela, une expérimentation en mésocosme de six mois a été menée lors de laquelle nous avons testé les effets de la litière (apport et qualité) et de la mycorrhization sur le cycle de l'azote et les communautés microbiennes du sol.

II. Article 6

Beech (*Fagus sylvatica* L.) leaf litter and ectomycorrhizal roots promote soil ammonium net production and fungal community

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Abstract

Forest dynamics is usually accompanied by substantial shifts in soil N pathways, *i.e.* the contribution of ammonification in total mineral N production increases along forest ageing in contrast to nitrification. Those N shifts are often paired with changes in soil microbial community, *i.e.* from bacterial-based energy channel towards fungal-based energy channels. Trees may actively control these belowground shifts occurring during forest maturation via two main pathways: "leaf litter effects" and "root effects". The aims of this study were thus to test the hypothesis that tree species do control concurrently soil N cycle and microbial community via both "litter effect" (qualitative and quantitative) and "root effects" (qualitative and quantitative, *i.e.* uptake and mycorrhizal status), and to shed light on the different effects of leaf litter and live roots on both soil N pathways and microbial community. For that purpose, we conducted a 6-months pot experimentation based on the presence/absence of (i) beech leaf litter with different initial quality with or without (ii) beech live roots strongly or not ectomycorrhized. At the end of the experiment, soil N pools and fluxes as well as microbial community were characterized in the laboratory. Potential N mineralization and nitrification were determined using anaerobic and aerobic incubations. We measured microbial biomass N (N_{mic}), the N_{mic} to total N ratio (N_{mic}-to-N_t), and ergosterol pools as fungal biomass index. Functional profiling of microbial community was determined by measuring the potential metabolic profiling (BIOLOG method). We also weighted the remaining litter initially placed on the top of the pot and calculated the litter mass loss. We observed substantial changes in soil N mineralization and microbial community in soil covered by litter layer and in the presence of live roots. We showed that litter input favored potential ammonium accumulation by inhibiting net potential nitrification, microbial N immobilization and fungal dominance but decreased N_{mic}-to-N_t ratio. Also litter input altered bacterial community structural and functional profiling probably by providing new C resources. Litter quality (lignin/N ratio, mineral elements content) did not affect significantly both soil N cycle and microbial community but lead to lower litter decay rates. Live roots favored ammonium accumulation in soil by enhancing soil potential ammonification and inhibiting net potential nitrification. Only ectomycorrhizal roots led to an inhibition of nitrification suggesting a possible role of fungal partner in the control of nitrate production. The presence of litter with ectomycorrhizal live roots led to higher soil mineral N content compare to soil with non-mycorrhizal root. This suggests that ectomycorrhizal roots take up N directly in the organic layer compare to non ectomycorrhizal roots. We also observed lower fungal biomass after litter input when ectomycorrhizal roots were present in soil probably due to high competition between saprophytic and mycorrhizal fungi. Others studies are required to identify the mechanisms (competition or allelopathy) implicated in the control of both soil N cycle and microbial community.

Keywords

Fagus sylvatica, leaf litter, litter quality, ectomycorrhizal roots, net ammonification, net autotrophic nitrification, soil microbial community, microbial N biomass, fungal biomass, metabolic profiling.

1. Introduction

Our perception of the functioning of soil nitrogen (N) cycling in forest ecosystem has undergone considerable changes during the last ten years (Schimel and Bennett 2004; Chapman *et al*. 2006; van der Heijden *et al*. 2008). The new paradigm recognizes that tree species may take up organic N (dissolved organic N such as amino acids) and not exclusively mineral N (Nasholm *et al*. 1998; Nasholm *et al*. 2009). In consequence, trees actively compete for N with microbes and are not a priori the least competitive organisms (Schimel and Bennett 2004). They thus could actively control N processes in soils (Paavolainen *et al*. 1998; Chapman *et al*. 2006; van der Heijden *et al*. 2008). This control can take place through diverse pathways (Jones *et al*. 1997; Schimel and Bennett 2004) but two seem to emerge as central: "leaf litter effects" and "roots effects".

"Leaf litter effects" consist in substantial inputs of organic carbon (C), N and nutrients directly available for soil biota (Saetre and Baath 2000; Subke *et al*. 2004; Sayer 2006). However, besides quantity, trees affect greatly soil N dynamics and microbial community by producing different leaf litter quality (Scott and Binkley 1997; Hobbie 2000; Hattenschwiler *et al*. 2003; Satti *et al*. 2003; Wurzburger and Hendrick 2009). Litters with high C/N and lignin/N ratios usually predict slow rates of soil net N mineralization and net nitrification and high bacterial N immobilization (Vitousek 1982; Scott and Binkley 1997; Satti *et al*. 2003). Considerable studies have therefore investigated litter quality variability among and within-species and its impact on soil N functioning (Hobbie 1996; Preston and Trofymow 2000; Madritch and Hunter 2002; Eviner 2004; Schweitzer *et al*. 2004; Wardle *et al*. 2009). Litter can also affect soil functioning by the formation of organic layers which modify abiotic soil conditions ("covering effects") and limit light and water penetration as well as soil transpiration (Arpin *et al*. 1995; Sayer 2006).

"Roots effects" consist in three main pathways. Live roots initially affect the soil N cycle via the uptake of water, organic and mineral N and nutrients, thus competing with microbial demand (Ehrenfeld *et al*. 1997; Schimel and Bennett 2004; Chapman *et al*. 2006; Dannenmann *et al*. 2009). A second way consists in root exudation (passive or active) which supports a large rhizosphere biota (Grayston and Campbell 1996; Raynaud *et al*. 2006). A part of the carbohydrates produced by photosynthesis is transported via the phloem and released into the soil. Depending on the quality of roots exudates, it can either enhance or inhibit microbial activity. Root exudates and root turnover (root litter) constitutes a major source of labile C for heterotrophic microbial processes (Colin-Belgrand *et al*. 2003; Hogberg and Read 2006). Those C-rich compounds may facilitate tree N uptake by activating soil decomposers and increasing mineralization of organic N (Raynaud *et al*. 2006). Also, most trees in forest ecosystems are in symbiosis with mycorrhizal fungi (Pöder 1996; Smith and Read 2008) which facilitate tree N acquisition where N mineralization is poor (Colin-Belgrand *et al*. 2003). Mycorrhizal roots may (i) compete with heterotrophic microorganisms (bacteria and fungi) and with autotrophic bacteria towards both organic and mineral N (Schimel and Bennett 2004; Nasholm *et al*. 2009; Wurzburger and Hendrick 2009) and (ii) provide organic substances by exudation with different impact on soil decomposers and by mycorrhizal hyphae turnover (Colin-Belgrand *et al*. 2003).

Forest development is usually accompanied by substantial shifts in soil N pathways, *i.e.* the contribution of ammonification in total mineral N production increases along forest ageing while nitrification tend to decrease (Chapter 1 and 2, Paré and Bergeron 1996; Jussy *et al*. 2000; Clinton *et al*. 2002; Fisk *et al*. 2002; White *et al*. 2004;

Welke and Hope 2005; Trap *et al*. 2009). Those N shifts along forest development are paired with changes in soil microbial community, *i.e.* from bacterial-based energy channel towards fungal-based energy channels (Bauhus *et al*. 1998; Ponge 2003; Wardle 2005). These changes can be ascribed to changes in soil fauna (Ponge 2003; Chauvat *et al*. 2007; Chauvat *et al*. 2009), soil quality (water and nutrients availability) and/or litter quality (Inagaki *et al*. 2004b).

In a previous work, Trap *et al*. (chapter 6) measured great variability in *Fagus sylvatica* leaf litter quality along a 130-yr-old chronosequence of pure beech stands in north-western France. Within-species variability in litter quality appeared to be responsible for soil N patterns and fungi dominance along the chronosequence. Since heterotrophic bacteria do not degrade lignin as efficiently as fungi do, higher lignin and lower N contents in litter produced by older stands may favor soil fungal-based channel along forest maturation. In contrast, soil quality (soil water and nutrient availability) as well as soil fauna (Aubert *et al*. 2004b; Hedde *et al*. 2007) did not exhibit significant patterns along the chronosequence suggesting that soil microflora and trees are the main driving factors of soil N cycle along pure beech stands ageing.

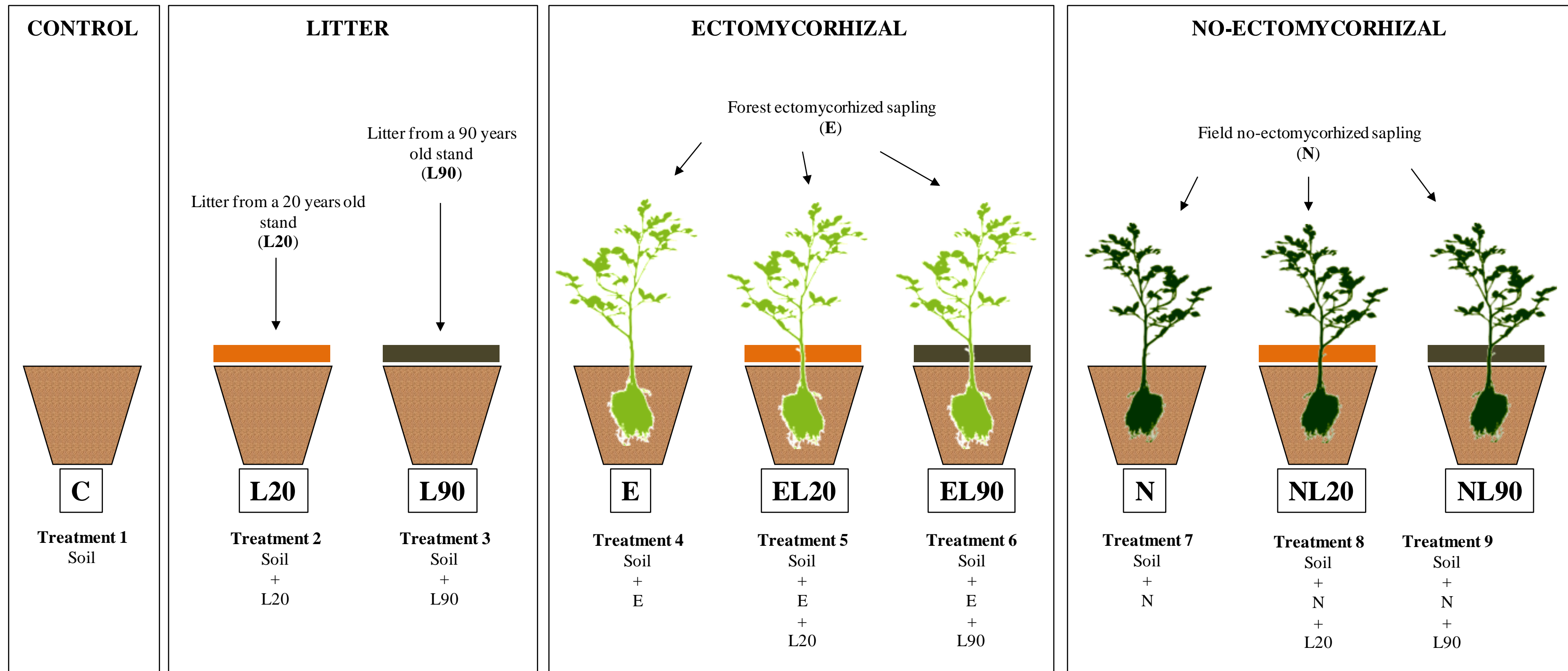
The aim of this study was thus to shed light on the differing effects of leaf litter and live roots on both soil N pathways and microbial community. For that purpose, we conducted a 6-months pot experiment based on the presence/absence of (i) beech leaf litter with different initial quality with or without (ii) beech live roots strongly or not ectomycorrhized. We hypothesized that beech promote concurrently soil ammonium pathways and fungal-based energy channel via both "litter effect" (inputs and quality) and "root effects" (inputs, uptake and mycorrhizal statue).

2. Materials and methods

2.1. Experimental design

The experiment was set up in microcosms consisting of PVC plant pots (inner diameter 22.5 cm, height 20.5 cm) which were sealed at the bottom with 270 µm plastic mesh. The microcosms were filled with 5 kg of sieved soil (5 mm). The soil (sand 46.5%, silt 27.2%, clay 12.6%, organic matter 7.2%, water content 21.3%, holding capacity 31.4%) was taken from grassland located on a plateau. Prior to use, the soil was sterilized by autoclaving 2 times for 1 hour at 101°C. Microbiota extractions from both forest and grassland growing on similar soils were performed in sterile water (1:20 soil:water) after 1 hour of agitation and filtration through a 270 µm plastic mesh. Two 500-ml portions of each filtrate were added after putting the soil into the microcosms. During the experiment, microcosm position within the greenhouse was randomized every 4 weeks. Also, the water regime was controlled by irrigating several times per week with 250 ml of water into all microcosms. After 6 months of experiment, saplings were removed from microcosms and the soil was sampled including both bulk and rhizospheral soil. The soil was 2 mm-sieved and stored at 4°C prior to chemical and microbial analyses.

Figure 7.1. *Experimental design. Nine treatments with five replicates per treatment (45 mesocosms).*



Nine treatments were realized with five replications (Table 7.1). Independent variables were (i) beech leaf litter effects (presence/absence), (ii) the quality of beech leaf litter according to the age of the stands from which the litter was collected (20-years-old *versus* 90-years-old), (iii) the inputs of live roots and their ectomycorrhizal status (null *versus* high) of beech saplings and (iv) interactions between litter and the ectomycorrhizal status of saplings (Fig. 7.1.).

Table 7.1. *Codes for each treatment.*

Treatments	Codes
Uncovered soil corresponding to control	C
Beech litter from a 20 years old stand placed on the top of the soil	L20
Beech litter from a 90 years old stand placed on the top of the soil	L90
Soil with a forest ectomycorhized beech sapling	E
Soil with a forest ectomycorhized beech sapling + beech leaf litter from a 20 years old stand	EL20
Soil with a forest ectomycorhized beech sapling + beech leaf litter from a 90 years old stand	EL90
Soil with a field no-ectomycorhized beech sapling	N
Soil with a field no-ectomycorhized beech sapling + beech leaf litter from a 20 years old stand	NL20
Soil with a field no-ectomycorhized beech sapling + beech leaf litter from a 90 years old stand	NL90

2.2. Leaf litter effects

Two stands were selected in the Eawy forest (France, région Haute Normandie), *i.e.* a 20-years-old (8.0 ha, dysmull, soil pH_{water} = 4.48, soil P = 0.13 g kg^{-1}, soil CEC = 6.19 cmol+ kg^{-1}, soil C/N = 16.6) and a 93-years-old pure beech stands (16.4 ha, eumoder, soil pH_{water} = 3.83, soil P = 0.22 g kg^{-1}, soil CEC = 5.56 cmol+ kg^{-1}, soil C/N = 15.4). Stands were managed as even-aged high forest by the French Forestry Service (ONF). The climate is a temperate oceanic with a mean annual rainfall and temperature of +10°C and 800 mm, respectively (Hedde 2006). Stands were located on LUVISOLS in the world reference base (FAO 2006) with more than 80 cm of loess as parent material.

Beech leaf litter was collected in both stands using a net tight one meter above the forest floor in order to avoid any colonization by soil organisms. The leaf litter collected from the 20-years-old stand was coded L20 while the litter from the 90-years-old stand was coded L90 (Table 7.1).

Table 7.2. *Initial litter quality according to the age of stands from which litter was collected.*

Litter quality	L20			L90		
Mg (g mg)	1.33	(0.22)	**a**	0.83	(0.14)	**b**
Mn (g mg)	3.24	(0.83)	**b**	4.16	(0.58)	**a**
Lignin (%)	28.51	(2.89)	**b**	35.06	(2.74)	**a**
Total N (%)	1.20	(0.17)	a	0.93	(0.07)	a
C/N	40.16	(5.51)	**b**	50.67	(4.82)	**a**
Lignin/N	24.09	(3.68)	**b**	37.87	(2.86)	**a**

Means (SD). Letters a and b refer to significant differences between L20 and L90 according to Kruskal-Wallis rank sum test (P < 0.05, n=4).

Fresh fallen leaves were air-dried during three months and cut into pieces about 3 cm length. 10.29 grams of dry leaf litter was placed on the top of the soil, for each treatment requiring litter occurrence *i.e.* L20, L90, EL20, EL90, NL20 and NL90. This amount of litter corresponded to the field annual litter production in both stands since we did not observe significant differences in annual litter production between 20 and 90 years old stands (chapter 4). To avoid any loss of litter material, a net was placed on the top of the microcosms. The quality of the two litter type was previously characterized (Table 7.2).

2.3. Ectomycorrhizal roots effects

Beech saplings were collected in March 2008 in a 130-years-old stand of "Eawy" forest (18.7 ha, eumoder, soil pH_{water} = 3.91, soil P = 0.15 g kg^{-1}, soil CEC = 5.75 cmol+ kg^{-1}, soil C/N = 14.8) and from nursery (Essarts nursery, France, Upper Normandy region, soil pH_{water} = 7.30, soil P = 0.61 g kg^{-1}). Stands were managed as pure beech even-aged high forest by the French Forestry Service (ONF). The stand was located on LUVISOLS in the world reference base (FAO 1998) with more than 80 cm of loess as parent material. The field beech saplings were 2-yrs-old. The height, the shoot and root biomasses as well as the shoot-to-root ratio of saplings was similar (one way ANOVA $P<0.05$, n=5) between forest and field sapling at the beginning of the experiment: mean height 58 cm, mean short-to-root ratio 1.69, mean shoot biomass 7.58 dry g, and mean root biomass 4.65 dry g. The mass percentage of ectomycorrhizal roots (mass of ectomycorrhizal fines roots divided by total roots) of each beech saplings type (forest *versus* field) were determined (Table 7.3).

Table 7.3. *Ectomycorrhizal root biomass of forest and field beech saplings expressed as µg-ergosterol per g of fine root or total root biomass, respectively.*

Modalities	Ectomycorhizal biomass of fine roots (*µg-ergosterol per g of dry fine root*)	Ectomycorhizal biomass of saplings (*mg-ergosterol per total root biomass*)	% of fine roots
Natural beech sapling	84.32 (45.23) **a**	2.46 (0.99) **a**	24.61 (11.46) **a**
Cultural beech sapling	7.00 (3.75) **b**	0.08 (0.04) **b**	3.03 (2.50) **b**

Means (SD). Letters (a and b) refer to significant differences between natural and cultural sapling according to one-side Student t test (P=0.05 level, n=5)

The mass percentage of ectomycorrhizal roots of forest beech saplings was 30-times higher (2.46 mg-ergosterol per total root biomass) compared to field ones (0.08 mg-ergosterol per total root biomass) according to one side Student test $P<0.05$ (n=8). After sampling, the roots were gently washed with water. Beech saplings were transplanted into microcosms. The forest ectomycorrhizal saplings were coded E while the field no-ectomycorrhizal saplings were coded N (Table 7.1). After six months of experiment, beech saplings were removed from microcosms. Final live root biomass was measured to check the living statue of roots (Table 7.4). Ectomycorrhization of beech saplings was checked using both macroscopic and microscopic observations.

2.4. Litter and soil sampling

After six months of experiment, both litter and soil were sampled. Litter was dried during 48h at 60°C and the final remaining mass was determined. Both bulk and rhizospheric soils were removed from the microcosms and mixed together without dead fine roots. After sampling, soil was stored at 4°C. Soil subsamples were stored at -20°C for fungal biomass quantification.

Table 7.4. *Final live root biomass (g dry) for each treatment.*

Treatments	Root biomass
Initial	
Ectomycorhized	4.3 (2.0) **c**
No-Ectomycorhized	5.0 (0.9) **c**
Final without litter	
Ectomycorhized	8.0 (2.9) **bc**
No-Ectomycorhized	8.4 (2.4) **bc**
Final with L20	
Ectomycorhized	14.6 (5.8) **ab**
No-Ectomycorhized	29.7 (9.2) **a**
Final with L90	
Ectomycorhized	12.8 (2.0) **ab**
No-Ectomycorhized	28.0 (7.0) **a**

Mean (SD). Letters (a, b and c) indicates differences according to one way ANOVA and Tukey HSD post hoc test ($P < 0.05$, n=5)

2.5. Soil N cycle

2.5.1. Soil N extraction and quantification

An aliquot of 20g of each soil was placed in beakers with 100ml K_2SO_4 (0.2 M) and shaken for 1 hour at 100 rev min^{-1} (Alef 1995; Forster 1995a). The obtained extractions were filtered through Schleicher & Schuell 0790 ½ filter papers and frozen for analyze. Filters were pre-leached with 0.2 M K_2SO_4 in order to avoid any ammonium and/or nitrate contamination. Concentrations of NH_4^+-N and NO_3^--N were determined colorimetrically with an Autoanalyser (AA3, BRAN+LUEBBE, Norderstedt, Germany). Aliquots of each soil were air-dried and total C and N were measured by gas chromatography with a CHN pyrolysis micro-analyzer (Flash 2000 Series, CHNS/O Analysers Thermo Scientific, France). Aliquots were dried at 105°C for 24 hours to obtain the gravimetric water content. Aliquots of each soil were air-dried and total C and N were measured by gas chromatography with a CHN pyrolysis micro-analyzer.

2.5.2. Incubations experiments

A 7 days anaerobic incubation was conducted in order to determine potential net ammonification. Anaerobic incubations were conducted in triplicate for each sample using 40 g of soil introduced in sealed glass beakers covered during 7 days in the dark at 40°C (Alef 1995). A 28 days aerobic incubation was conducted to determine potential net nitrification (Hart *et al*. 1994). Aerobic incubations were conducted in triplicate for each sample using 40 g of soil introduced in glass beakers covered during 28 days in the dark at 28°C. In aerobic incubation, the soil moisture was adjusted at 85% of its maximum water holding capacity (Pansu and Gautheyrou 2003). To avoid anoxic conditions, the beakers were frequently opened and aerated. In order to discriminate autotrophic from heterotrophic nitrification, acetylene was used to inhibit chemolitho-autotrophic nitrification(Hynes and Knowles 1978). In scintillation flasks, acetylene was generated by adding water to calcium carbide. Each week, scintillation flasks were

replaced in order to maintain a final acetylene concentration reaching 2% (v/v). Acetylene production was checked using GC equipped with a flame ionization detector (Girdel 30 GC, Spherosil XOB 75 column, France).

Immediately after soil samples and at the end of anaerobic and aerobic incubations, concentrations of NH_4^+-N and NO_3^--N in soil were determined as described previously (2.5.1.). Mineral N in water used in anaerobic incubation was also determined and added to mineral N pools. Potential net ammonification and nitrification expressed as µg N g^{-1} C d^{-1} were determined by subtracting initial concentrations of NH_4^+ and NO_3^- from post-incubation concentrations. Ammonification and nitrification rates were also calculated and expressed as ‰ of total N after 7 and 28 days of incubation, respectively (Hart *et al*. 1994; Robertson *et al*. 1999).

2.6. Soil microbial community

2.6.1. Potential functional profiling of heterotrophic bacteria

Carbon source utilization profiling of soil bacterial community was determined by the Biolog method (Garland and Mills 1991). The Biolog® Ecoplates™ (Biolog Inc., USA) used contained 96 wells, 3 repetitions of 31 carbon sources and a remaining well with water (control). Bacteria extraction was performed by diluting an aliquot of fresh soil from each of 45 microcosms in sterile NaCl solution (0.85%, ratio 1/10). All dilutions were shaken for 3 minutes on a vortex mixer at maximum speed followed by centrifugation during 10 minutes at 1000 rpm (Eppendorf centrifuge 5810 R). After bacterial extraction, the bacteria suspensions were diluted from 10^{-2} to 10^{-4} and inoculated in duplicate on R2A agar medium. All plates were incubated at 20°C and enumerated after 48 h in the dark. Inoculations of Biolog microplates were performed from bacteria extraction samples. The dilutions were adjusted to obtain a mean inoculum density of 1500 bacteria per well. 150 µl of cell suspension was added to each well of the Biolog plates. The Ecoplates were then incubated for 48 h at 20°C in the dark. Three optical density measurements at 590 nm were performed, *i.e.* at the beginning of the incubation (t_0), 24 (t_{24}) and 48 (t_{48}) hours after the beginning with an Emax precision microplate reader (Microplate E-Max, Molecular Devices, Sunnyval, CA). The $O.D._{590\ nm}$ of the control well was subtracted from the $O.D._{590\ nm}$ of each of the other wells. The potential *Substrate Richness* (SR) and the *Average Well Color Development* (AWCD) were calculated from Biolog data for each treatment. The SR index corresponds to the number of metabolized Biolog substrates. A substrate was considered to be metabolized if its mean $O.D._{590}$ measured after 48 h was higher than 0.250. Metabolic profiling were established with highly positive substrate ($O.D._{590}$ higher than 0.500).

2.6.2. Structural profiling

Microbial biomass N (N_{mic}) was determined using the chloroform fumigation-extraction method (Brookes *et al*. 1985) after a 24 h fumigation with $CHCl_3$, followed by a persulfate digestion. Soil materials were extracted with K_2SO_4 solution (0.2 M) and nitrates in the filtrate were determined with AA3 (Öhlinger 1996). The relative N amount in microbial biomass of total N (N_{mic}-to-N_t) was calculated and expressed as % of total N. Fungal biomass was determined through ergosterol contents thanks to methanol extraction after 1 hour mechanic shaking at 320 rev min^{-1} according to Gong method (Gong and Witter 2001). The ergosterol concentration was measured by HPLC at 282 nm (SPD-10A SHIMADZU LC-6A) and expressed as µg-ergosterol per g^{-1} dry matter (Rössner 1996).

2.7. Statistics

All tests were computed with the R freeware (2008) and statistical significance was set at $P < 0.05$. Means and standard errors were calculated by treatments for all variables (5 true replications). Comparisons of means between treatments were performed using one way ANOVA and Tukey HSD post hoc test. Beforehand, the normality and homogeneity of variances were checked using the Wilk–Shapiro test and the Bartlett test, respectively. When necessary, logarithmic transformations were applied. When normality or homoscedasticity condition was refuted, non-parametric tests (Kruskal-Wallis rank sum test and paired Kolmogorov-Smirnov, respectively) were preferred We used linear correlations to investigate the relationships between variables. The Truncated Product Method was used to adjusted the P values (Zaykin *et al*. 2002).

3. Results

3.1. Soil water content

Soil water content was higher in soil covered by litter (L20 and L90) but it was lower in presence of ectomycorrhizal roots (E) (Fig. 7.2). The presence of bare roots (N) did not change the water content compared to control. The presence of both L90 and ectomycorrhizal roots (EL90) led to lower water content. We retrieve this trend for NL20 (L20 plus no-ectomycorrhizal roots.)

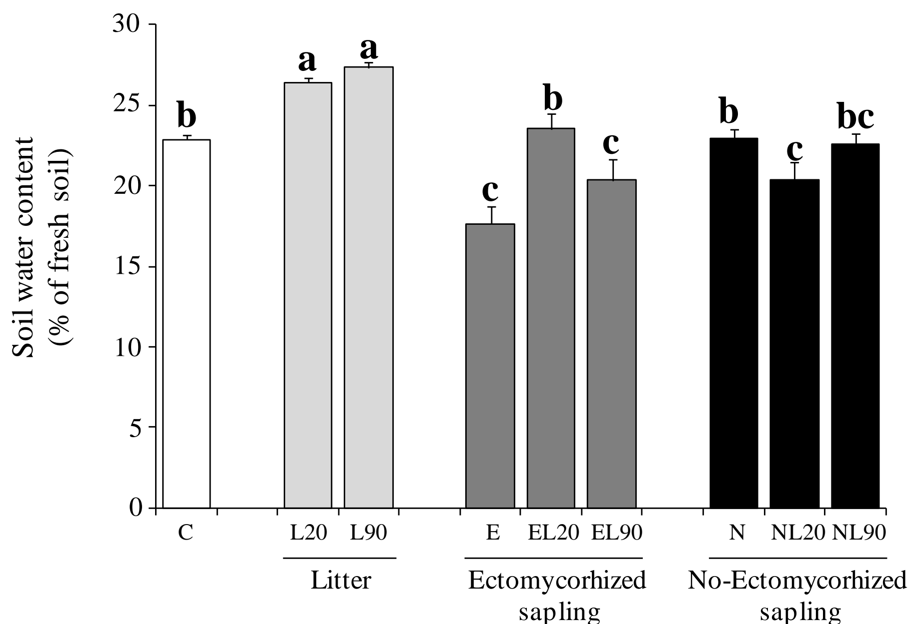


Figure 7.2. *Soil water content (% of fresh soil) at the end of the experiment. Vertical bars correspond to standard deviation. Letters (a, b and c) indicates differences according to Kruskal-Wallis (P < 0.05, n=5).*

3.2. Soil N cycle

Soil N pools - Total N in soil after 6 months of experiment was higher compared to control (C) for treatments with leaf litter (L20 and L90) (Fig. 7.3.A). This increase was higher with L90. The presence of live roots in soil, ectomycorrhizal (E) or not (N), did not change significantly total N. Soils covered by litter in the presence of ectomycorrhizal roots (EL20, EL90) showed higher values in total N compared to the

control (Fig. 7.3.A). In contrast, soils covered by litter in the presence of no-ectomycorrhizal roots (NL20 and NL90) did not exhibit high N contents (Fig. 7.3.A). The C/N ratio (Fig. 7.3.B) did not change significantly between treatments (mean 11.5) except for the control which showed higher value (mean 19.8). Ammonium content in soil was lower (near 0 µg N dry g) in presence of live roots (both E and N) compared to control (Fig. 7.3.C).

The presence of a litter layer (L20 and L90) did not lead to different ammonium contents. Despite the presence of ectomycorrhizal roots, ammonium content in soil covered by litter (EL20 and EL90) did not differ than control. However, ammonium content for NL20 and NL90 was lower than control (Fig. 7.3.C). Nitrate content was similar between L20, L90, NL20 and NL90 (Fig. 7.3.D). Nitrate content was lower in presence of live roots (both E and N) and for NL20 and NL90 compared to control (Fig. 7.3.D).

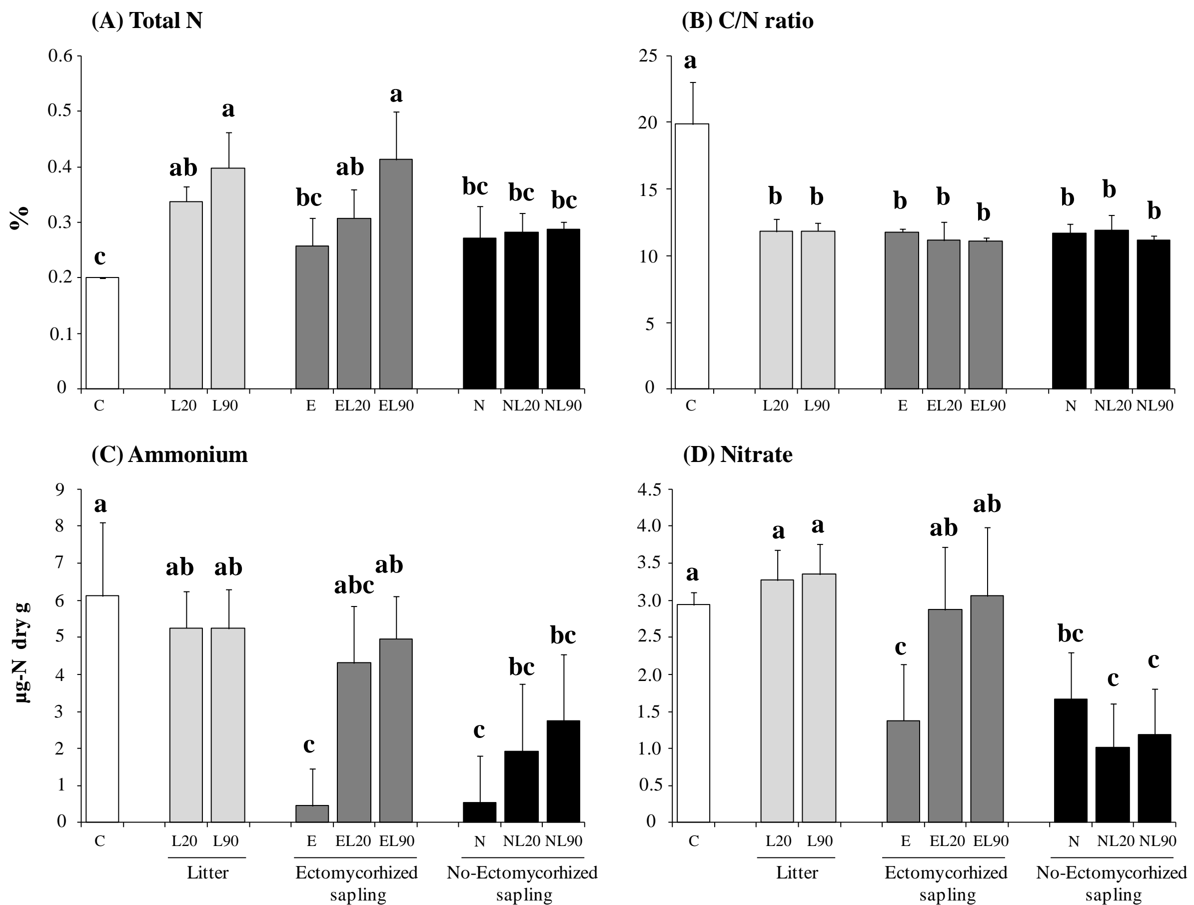


Figure 7.3. *Total N in % of dry soil (A), C/N ratio (B), ammonium (C) and nitrate (D) contents expressed as µg N dry g in soil for each treatment after 6 months of experiment. Vertical bars correspond to standard deviation. Letters (a, b and c) indicates differences according to one way ANOVA and Tukey HSD post hoc test (P < 0.05, n=5).*

Soil N fluxes – Potential net ammonification did not change in soil covered by litter (L20 and L90) (Fig. 7.4.A). However, the presence of roots (E and N) increased net ammonium production from 110 (control) to about 170 µg N g^{-1} C d^{-1} (Fig. 7.4.A). The increase of net ammonium production by roots occurrence was reduced by litter inputs, especially with L90. Ammonification rates (% of total N) decreased after litter inputs (L20 and L90) compared to control (1.5) (Fig. 7.4.B). The presence of roots did not change ammonification rates. However, ammonification rates tended to decrease when

both roots and litter were present, especially with L90. The presence of litter (L20 and L90) and ectomycorrhizal roots without (E) or with litter (EL20 and EL90) showed lower soil potential net nitrification and nitrification rates contrary to non ectomycorrhizal roots which did not affect those N fluxes (Fig. 7.4.C.D). The presence of litter with no-ectomycorrhizal roots (NL20 and NL90) tended to decrease both potential net nitrification and nitrification rates. The use of acetylene showed that the net nitrification process was exclusively autotrophic in all treatments.

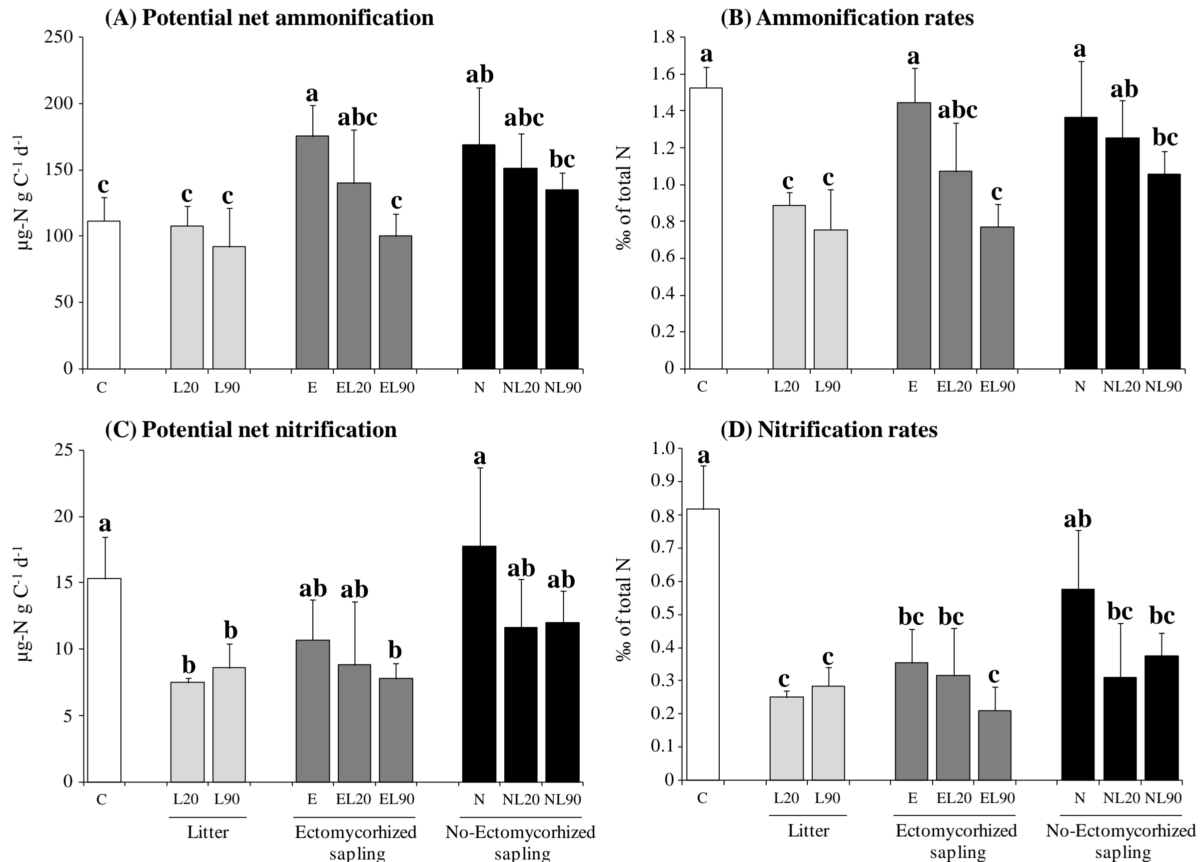


Figure 7.4. *Soil potential net ammonification (A), ammonification rates (B), potential net nitrification (C) and nitrification rates (D) in soils according to treatments. Potential net ammonification and nitrification were expressed as µg N g*$^{-1}$ *C d*$^{-1}$. *Ammonification and nitrification rates were expressed as ‰ of total N after 7 and 28 days of incubation, respectively. Vertical bars correspond to standard deviation. Letters (a, b and c) indicates differences according to one way ANOVA and Tukey HSD post hoc test (P < 0.05, n=5).*

3.3. Soil microbial community

The Average Well Color Development (AWCD) was higher for L20 and L90 compared to control (Fig. 7.5.A). The presence of live roots showed the same values of AWCD than control. Roots + litter treatments (EL20, EL90, NL20 and NL90) showed intermediate values. The treatment with ectomycorrhizal roots alone (E) showed the lower Substrate Richness (SR) values (Fig. 7.5.B). The SR was higher for the treatment L20 and L90. Roots + litter treatments showed intermediate values. The metabolic profiling presented the “positives” (O.D. > 0.5) and “negatives” (O.D. < 0.5) C sources for each treatment (Fig. 7.6). The presence of litter (L20 and L90) led to 5 more positive sources than control, *i.e.* “phenylethylamine”, “putrescine”, “L-arginine”, “4-hydroxy benzoic acid” and “pyruvic acid methyl esther”. In contrast, ectomycorrhizal roots

treatment (E) showed few positive substrates and 3 substrates became negative compared to control: "L-serine", "L-asparagine" and "D-cellobiose", while no-ectomycorrhizal roots treatment (N) showed the same profile than control. Roots + litter treatments (EL20, EL90, NL20 and NL90) showed intermediate profiling. Some C sources became negative such as "Hydroxy-Butiric acid" while others became positive such as "Putrescine", Pyruvic acid methyl esther" or "Tween 40" (Fig. 7.6).

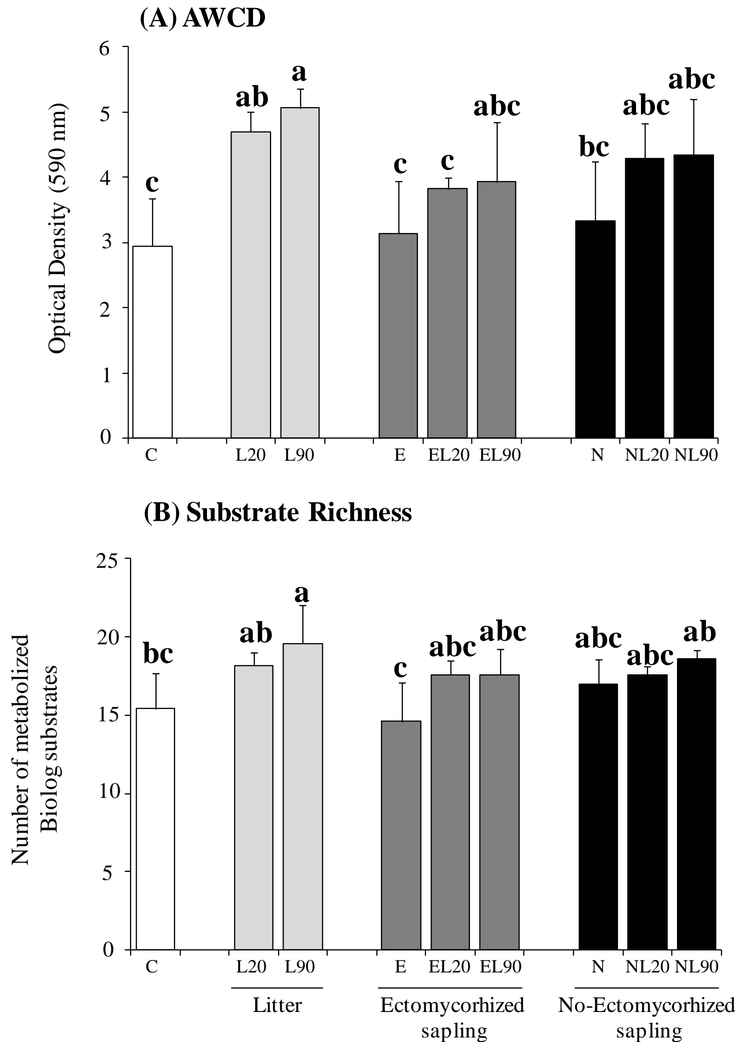


Figure 7.5. *Soil potential AWCD (A) and substrate richness or SR (B) according to treatments. Vertical bars correspond to standard deviation. Letters (a, b and c) indicates differences according to one way ANOVA and Tukey HSD post hoc test (P < 0.05, n=5).*

The microbial biomass N (N_{mic}) was significantly higher with litter (L20 and L90) compared to control (Fig. 7.7.A). The presence of roots did not affect N_{mic} but the presence of litter + ectomycorrhizal roots lead to higher values. We did not retrieve this pattern with no-ectomycorrhizal roots + litter. The relative amount of N in microbial biomass (N_{mic}-to-N_t) was lower with litter (0.9%) compared to control (1.4%) (Fig. 7.7.B). N_{mic}-to-N_t decreased also with roots input, especially with no-ectomycorrhizal roots (0.8%). EL20 and EL90 showed values similar to control while NL20 and NL90 exhibited intermediate values. Fungal biomass increased with litter inputs (L20 and L90) compared to control and ectomycorrhizal roots (E treatment) (Fig. 7.7.B).

Carbon sources	Treatments	Control	Litter		Ectomycorhized sapling			No-ectomycorhized sapling		
	Codes	C	L20	L90	E	EL20	EL90	N	NL20	NL90
Amines/amides	Phenylethylamine	□	■	■	□	□	□	□	□	□
	Putrescine	□	■	■	□	■	□	□	■	■
Amino acid	Glycyl-L-Glutamic acid	□	□	□	□	□	□	□	□	□
	L-Arginine	□	■	■	□	□	□	□	□	■
	L-Asparagine	■	■	■	□	■	■	■	■	■
	L-Phenylalanine	□	□	□	□	□	□	□	□	□
	L-Serine	■	■	■	□	■	■	■	■	■
	L-Threonine	□	□	□	□	□	□	□	□	□
Carbohydrates	α-D-Lactose	□	□	□	□	□	□	□	□	□
	β-Methyl-DGlucoside	■	■	■	■	■	■	■	■	■
	D-Cellobiose	■	■	■	□	■	■	■	■	■
	D-Mannitol	■	■	■	■	■	■	■	■	■
	D-Xylose	□	□	□	□	□	□	□	□	□
	i-Erythrol	□	□	□	□	□	□	□	□	□
	N-Acetyl-D-Glucosamine	■	■	■	■	■	■	■	■	■
Carboxylic acid	γ-Hydroxy Butiric acid	■	■	■	□	□	□	■	□	■
	α-Ketobutyric acid	□	□	□	□	□	□	□	□	□
	2-Hydroxy Benzoic acid	□	□	□	□	□	□	□	□	□
	4-Hydroxy Benzoic acid	□	■	■	□	□	□	□	□	■
	D-Galactonic acid γ-Lactone	■	■	■	■	■	■	■	■	■
	D-Galacturonic acid	■	■	■	■	■	■	■	■	■
	D-Glucosaminic acid	□	□	□	□	□	□	□	□	□
	D-Malic acid	□	□	□	□	□	□	□	□	□
	Itaconic acid	□	□	□	□	□	□	□	□	□
Miscellaneous	D,L-α-Glycocerol Phosphate	□	□	□	□	□	□	□	□	□
	Glucose-1-Phosphate	□	□	□	□	□	□	□	□	□
	Pyruvic acid Methyl Ester	□	■	■	■	□	■	□	■	■
Polymeres	α-Cyclodextrin	■	■	■	■	■	■	■	■	■
	Glycogen	■	■	■	■	■	■	■	■	■
	Tween 40	□	■	■	□	■	■	□	■	□
	Tween 80	□	□	□	□	□	□	□	□	□

□ Optical density < 0.500

■ Optical density > 0.500

Figure 7.6. *Metabolic profiling of soil bacterial for each treatment. A substrate was considered positive when its optical density was superior at 0.500.*

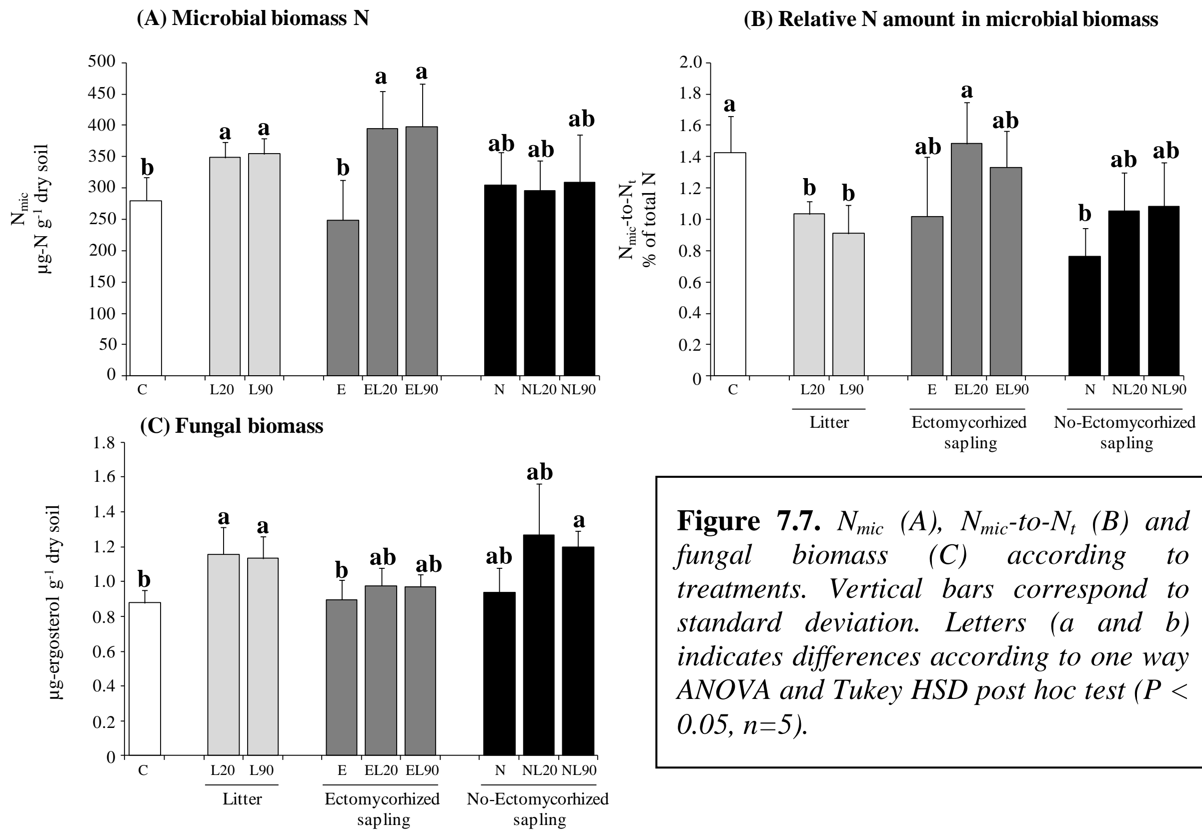


Figure 7.7. *N_{mic} (A), N_{mic}-to-N_t (B) and fungal biomass (C) according to treatments. Vertical bars correspond to standard deviation. Letters (a and b) indicates differences according to one way ANOVA and Tukey HSD post hoc test (P < 0.05, n=5).*

3.4. Litter decay

The litter mass loss (% of initial mass) after 6 months of experiment was higher for the litter from the 20 years old stand (L20) with about 18% compared to the litter from the 90 years old (L90) with about 8% (Fig. 7.8).

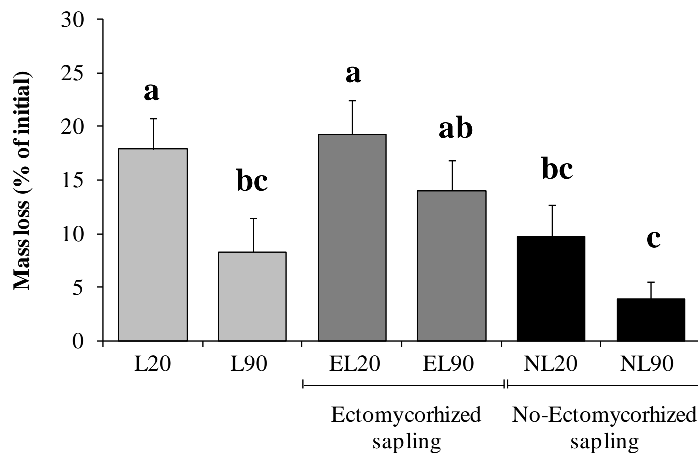


Figure 7.8. *Litter mass loss expressed in % of initial mass after 6 months of experiment in presence or absence of bare roots ectomycorrhizal roots. Vertical bars correspond to standard deviation. Letters (a, b and c) indicates differences according to Kruskal-Wallis and Tukey Kramer post hoc tests (P < 0.05, n=5).*

The presence of live ectomycorrhizal roots did not affect the decomposition rate of L20 but tended to increase the decomposition rate of L90. In contrast, the presence of live bare roots led to lower decay rates of both L20 and L90.

4. Discussion

4.1. Litter and roots provide organic C and N

The study has shown that the both the presence of litter and live roots, ectomycorrhizal or not, provide organic N into soil (+85% and +32% in mean, respectively, figure 7.3). Nevertheless, the input of organic N from roots was higher (+20% in mean) when roots were no ectomycorrhizal. It is possible that in the absence of ectomycorrhizal partner, beech saplings release more exudates stimulating hence the microbial activity in the root's vicinity (Subke *et al*. 2004). This may facilitate N release from soluble organic matter (SOM) through the increased decomposition rate and compensate the absence of fungal partner in the uptake efficiency of N forms. In contrast, the presence of live roots (whatever the mycorrhizal status) led to lower initial mineral N content in soil compared to uncovered and covered soil. Gessler *et al*. (1998) showed that beech trees take up preferentially ammonium rather than nitrate. We retrieve this pattern since soil ammonium contents were lower than nitrates at the end of the experiment suggesting that ammonium was the mineral N form preferentially used by saplings.

4.2. Litter input inhibited autotrophic nitrification

Litter input inhibited net autotrophic nitrification compared to uncovered soil. The quality of dissolved organic carbon (DOC) from litter was often proposed as the main factor inhibiting nitrification activity (Strauss and Lamberti 2002; Aubert *et al*. 2005; Zeller *et al*. 2007). Indeed, autotrophic nitrification may be inhibited by allelopathic compounds such as monoterpenes (White 1988; Ward *et al*. 1997; Paavolainen *et al*. 1998) or polyphenols (Northup *et al*. 1995; Hattenschwiler *et al*. 2003; Kraus *et al*. 2003) present in DOC. Lorenz *et al*. (2004) measured higher identifiable tannins in beech (56.0 mg g^{-1}) compared to oak (11.1 mg g^{-1}) leaf litter collected from stands located Mannheim-Käfertal forest in South-Western Germany. Beech leaf litter also showed high phenolic compounds content (31.2 mg g^{-1}) (Lorenz *et al*. 2004). It is probable that beech litter provided tannins and polyphenols which may inhibit the activity of ammonia oxidizing bacteria populations. Nitrification may also be inhibited by competition for ammonium between nitrifying bacteria and heterotrophic microorganisms (bacteria and fungi). It is possible that the supply of organic C and N by litter promoted the growth of heterotrophic organisms (heterotrophic bacteria and fungi). This may increase their demand for ammonium and reduce hence autotrophic nitrification. This hypothesis is supported by the investigation of both fungal biomass which increased with litter input and heterotrophic bacterial functional profiling. Indeed, heterotrophic bacteria community structure may be strongly affected by litter input since both SR and AWCD profiling increased significantly in soil covered by litter. Litter may provide new resources, especially amine and amino acids, to heterotrophic bacteria favoring hence species diversity. Zeller *et al*. (2007) discussed that the beech litter itself do not play a direct role in beech stands, but rather microbial degradation products. Complementary studies are required to identify the mechanisms implicated in the inhibition of nitrification.

4.3. Litter favor fungal community

As discussed previously, soil covered by litter showed higher fungal biomass than control, suggesting that beech leaf litter favored soil fungal-based energy channel. The promotion of fungi can be explained by the quality of beech leaf litter. High lignin (35% for L90) and Mn (4.16 mg g^{-1} for L90) contents may limit bacterial growth and favor fungal activity since fungi possess lignolytic enzymes such as the Mn-peroxidase (Gobat

et al. 2004). Furthermore, in a parallel study, we have shown that more than 50% of total leaf N was associated with lignin fraction (see chapter 6) suggesting that the large amount of N supply by litter during the experiment may consist in complex N-compounds hardly decomposable by bacteria. Fungal biomass promotion by litter may explain why we observed higher microbial biomass N and lower N_{mic}-to-N_t despite higher N content in soil covered by litter. Indeed, the N requirement per C unit of fungi is very low compared to bacteria (Lavelle and Spain 2001; Kooijman *et al*. 2009). The supply of labile N may be rapidly immobilized by fungi explaining why soil covered by litter did not lead to higher potential net ammonium production and higher mineral N pools in soil compared to uncovered soil. We thus assume that litter occurrence has increased gross fungal ammonium production but concomitant microbial N immobilization has lead to similar soil net ammonium production and mineral N pools in pots with and without litter.

4.4. Litter quality did not affect soil N cycle and microbial community

Surprisingly, despite clear differences in initial beech litter quality (see table 7.2), we did not observe significant differences in both soil N cycle and microbial community between L20 and L90 treatments even if some tendencies were observed, especially for soil N pathways. This statement is in contradiction with previous field soil N and microbial measures (see chapter 6). The absence of soil N and microbial response to litter quality is probably due to the time scale of the experiment, the age of beech saplings used, and/or the lack of soil fauna. The short-time experiment conducted may not be long enough to allow significant effects of litter quality. Also, we did not input earthworms within the microcosms to avoid confusing effects between leaf litter and earthworms. Consequently, leaves were not mixed with mineral material. On the field, litter quality effects on soil functioning is endorsed by fauna activity leading to positive or negative feedbacks (Lavelle and Spain 2001). It would be interesting to conduct a similar experiment including soil fauna or testing litter quality using entire humic epipedon core including OF, OH layers and the organo-mineral layer or litter solution extraction.

However, the litter quality may affect litter decay rates since the litter mass loss after 6 months of experimentation was higher for the litter from the 20 years old stand (L20) with about 18% compared to the litter from the 90 years old (L90) with about 8%. This is probably due to higher lignin contents, C/N and lignin/N ratios and lower N contents in L90 compared to L20. Those results showed that variability in litter quality of beech impact significantly litter decay dynamics and probably soil functioning since initial microbial community were similar between the two treatments but not N cycle during the 6 months of experiment.

4.5. Live roots favored soil ammonium pathways

The presence of live roots (whatever the mycorrhizal status), led to higher net ammonium production compared to control. It is recognize that the inputs of available C by roots exudates increases microbial and microfaunal activity in the rhizosphere (Grayston and Campbell 1996; Grayston *et al*. 1997; Raynaud *et al*. 2006). The rhizosphere activity may prime decomposition of SOM and promote soil N depolymerization and net ammonification. Tree girdling, by interrupting the transport of labile C compounds from the shoots to the roots as well as root exudation, has been reported to result in a decrease in heterotrophic microbial activity in soil (Subke *et al*.

2004; Hogberg and Read 2006; Zeller *et al*. 2008) suggesting hence that roots promote SOM decomposition. It is also possible that after a certain period, fine roots might die leading to an increase in N mineralization (Hogberg and Read 2006; Zeller *et al*. 2008).

Those results suggest that the production of SOM decomposition may occur via easily decomposable compounds by root exudations. Furthermore, we observed an inhibition of both soil net autotrophic nitrification in presence of ectomycorrhizal roots compared to control. Some authors showed that clear cutting rapidly initiated nitrification which suggests an inhibitory control by roots (Duggin *et al*. 1991; Smolander *et al*. 1998). In our works, the presence of ectomycorrhizal fungi is needed to observe an inhibition of nitrification. Similarly to litter effects on nitrification, we can formulate two hypotheses: an allelopathy and a competition hypothesis (Bremmer and McCarty 1993; Strauss and Lamberti 2002; Aubert *et al*. 2005). The allelopathy hypothesis suggests that autotrophic bacteria may be inhibited by the release of inhibiting compounds by tree roots. It was established that root exudates contain nitrification-inhibiting compounds (Subbarao *et al*. 2006; Subbarao *et al*. 2007). Smolander *et al*. (2005) showed low nitrification under spruce and detected in the humus layer of spruce high concentration of proanthocyanidins (condensed tannins) and some phenolic acids (ferulic acid, p-coumaric acid) which may act as nitrification inhibitors. It is possible that the presence of ectomycorrhizal fungi promotes or regulates the release of these compounds. The competition hypothesis suggests a competition for ammonium between nitrifying bacteria and tree roots (Kaye and Hart 1997; Hodge *et al*. 2000; Chapman *et al*. 2006). Ectomycorrhizal roots are efficient in the uptake of ammonium reducing thus substrate availability for autotrophic nitrification (Schimel and Bennett 2004; Dannenmann *et al*. 2009). This last hypothesis appears less robust since ammonium content was also lower in soil with no-ectomycorrhizal roots compared to control. Perhaps a complementary study focused on the identification of inhibitory compounds release by roots may highlight tree root mechanisms involved in the inhibition of nitrification.

However, strong competition between ectomycorrhizal fungi and heterotrophic bacteria probably took place in soil. Indeed, the presence of ectomycorrhizal roots may lead to substantial changes in heterotrophic bacteria community structure since the metabolic profiling was different to control (Fig. 7.6). In contrast, the presence of no-ectomycorrhizal roots led to the same metabolic profiling than control. This may reflect the competition between ectomycorrhizal fungi and heterotrophic bacteria towards resources leading to structural shift in the community of heterotrophic bacteria. High competition towards N may occur since “L-Asparagine” and “L-serine” were the amino acids which segregate the two metabolic profiling.

4.6. Litter altered roots effects on soil N cycle and microbial community

Our results have shown that the presence of an organic horizon not only significantly affected N dynamics and microbes in the soil, but also altered the occurrence of root effects on these belowground parameters. Mineral N contents in soil with ectomycorrhizal roots appeared low compared to control except if soil was covered by litter. In comparison, soil covered with litter without roots showed similar values as control, suggesting that litter provides mineral N which was directly immobilized by microbes. This trend was not retrieve with no-ectomycorrhizal roots. These results suggest that ectomycorrhizal roots uptake mineral N directly in the organic layer rather than within organo-mineral material. This may explain why litter decay rates increase with the presence of ectomycorrhizal roots but not with no-ectomycorrhizal roots. It

would be interesting to investigate the effects of live roots (ectomycorrhizal or not) on soil N cycle and microbial community within the organic layers collected from two contrasted stand age.

Litter-root interaction was also altered by the ectomycorrhizal status of root. For instance, we observed lower fungal biomass after litter input when ectomycorrhizal roots were present in soil. This observation can be explained by the method used to quantify soil fungal biomass. The method (Gong and Witter 2001) based on ergosterol extraction by methanol may reflect saprophytic fungal biomass rather than mycorrhizal one. Indeed, fungal biomass was not measured in the rhizospheric soil but in a subsample of soil including both rhizospheric soil and bulk soil where saprophytic fungi dominated. Intense competition between saprophytic (promoted by litter input) and mycorrhizal fungi may occur, and is probably responsible for the inhibition found. This hypothesis is supported since no-ectomycorrhizal roots did not affect fungal community promotion by litter.

Also, the presence of ectomycorrhizal roots favored the decomposition of the L90 but not the L20 while the presence of no-ectomycorrhizal roots inhibited the decomposition of both L20 and L90 (Fig. 7.8). It is possible that the mycelium of ectomycorrhizal fungi colonized the litter and took up nutrients directly in litter allowing better litter decomposition rates. Also, we showed that the release of organic N from roots was higher (+20%) when roots were no ectomycorrhized. Roots exudates may provide more decomposable compounds than those provided by leaf litter, leading to lower litter decay rates.

5. Conclusion

We can conclude that litter input favored potential ammonium accumulation by inhibiting net nitrification potential and promote fungal-based energy channel. Litter input altered heterotrophic bacterial community structural and functional profiling probably by providing new C resources. In contrast, litter quality did not affect significantly both soil N cycle and microbial community but lead to lower litter decay rates. Live roots favored potential ammonium accumulation by enhancing ammonification potentials and inhibiting potential net nitrification. Only ectomycorrhizal roots led to an inhibition of nitrification suggesting a possible role of fungal partner in the control of nitrate production. The presence of litter with live roots led to higher soil mineral N content compared to soil with live roots but only when roots were ectomycorrhized.

Several studies provided evidence of an intricate N feedback where plant litter chemistry would influence N cycle to maximize N acquisition by the host's mycorrhizal roots while hindering microbial N acquisition (Aerts 2002; Neff *et al*. 2003; Schimel and Bennett 2004; Wurzburger and Hendrick 2009). The development of mycorrhizae increases the ability of roots to absorb nutrients such as organic N from the soil. The degree to which this ability is enhanced depends on the extent of mycorrhizal colonization. Since *Fagus sylvatica* is a strong ectomycorrhizal species (Simard *et al*. 2002; Smith and Read 2008) and ectomycorrhizal fungal species are especially efficient in taking up both organic and inorganic N (Gessler *et al*. 1998; Wallenda and Read 1999), beech litter-induced promotion of fungal-based channel may allow high root colonization by ectomycorrhizal fungal species and favor trees N nutrition through bypassing microbial competition (Schimel and Bennett 2004; Wardle 2005). Furthermore, it was showed that beech uses preferentially ammonium as mineral N source (Gessler *et al*. 1998). We can thus formulate the hypothesis that the inhibition of

nitrification by both litter and ectomycorrhizal roots and the promotion of ammonification by live roots is likely part of an adaptation mechanisms to maximize N acquisition by beech trees (Schimel and Bennett 2004; Subbarao *et al*. 2006). Such N feedbacks would thus determine a clear competitive advantage of beech trees over microbial species.

Acknowledgements

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III. Synthèse du chapitre 7

(1) La litière de hêtre, indépendamment de sa qualité initiale, inhibe la nitrification autotrophe et favorise la communauté fongique. L'apport de litière affecte fortement la communauté bactérienne et pourrait, via l'apport de nouvelles sources de carbone, favoriser la richesse spécifique de la communauté bactérienne hétérotrophique.

(2) Toutes les racines favorisent l'ammonification potentielle et seules les racines ectomycorrhizées inhibent la nitrification autotrophe. Les racines pourraient contrôler le cycle de l'azote via (i) les prélèvements racinaires, (ii) les exsudats racinaires, (iii) la présence du partenaire symbiotique fongique et/ou (iv) le turnover racinaire et mycélien.

(3) La qualité de la litière n'affecte pas le cycle de l'azote et les communautés microbiennes du sol. Néanmoins, la perte de masse de la litière après six mois d'expérience a été plus importante pour celle issue du peuplement de 20 ans.

(4) La présence de litière affecte l'effet des racines et vice-versa (interaction litière/racine). Par exemple, la teneur en azote minéral dans le sol est faible en présence de racines vivantes ectomycorrhizées, excepté en présence de litière. Ce patron n'a pas été observé pour les racines nues. Les racines ectomycorrhizées ont donc certainement prélevé l'azote directement dans la litière. Aussi, contrairement aux racines nues, la présence de racines ectomycorrhizées diminue l'effet des litières sur la communauté fongique.

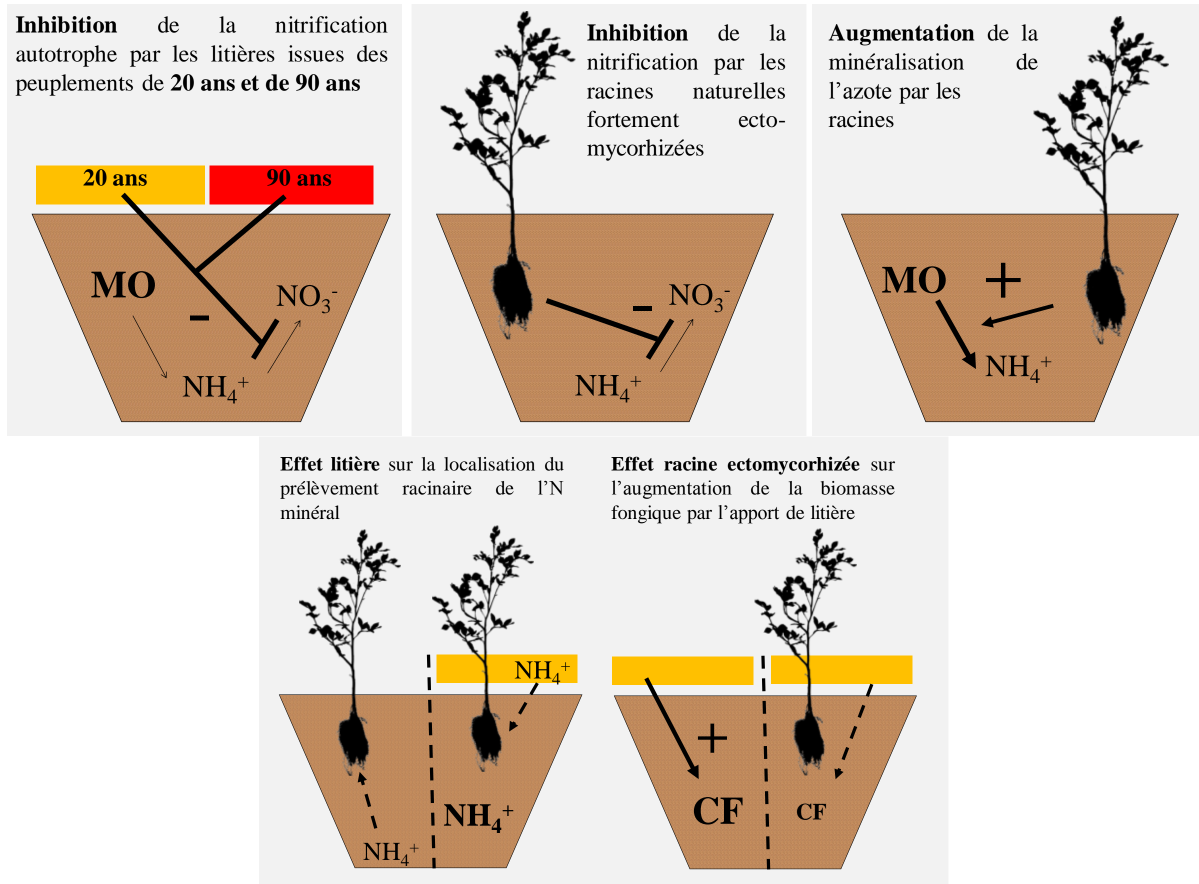

Figure 7.9. *Schéma bilan (M.O.=Matière organique; CF=Communauté fongique)*

Conclusion Générale et Perspectives

FORÊT D'EAWY – AVRIL 2008

1. Objectifs de la thèse

L'objectif de cette thèse était de contribuer à la compréhension :

(1) des relations morphologie/cycle de l'azote au sein de l'épisolum humifère le long de la maturation des peuplements,

(2) du cycle de l'azote au sein des différents horizons de l'épisolum humifère et de sa dynamique le long de la maturation des peuplements forestiers;

(3) des facteurs écologiques (*e.g.* les microorganismes, la qualité de la litière, la production de litière, les propriétés physico-chimiques du A, la vitesse de décomposition) responsables du développement des formes d'humus de type moder et régulant le cycle interne de l'azote dans le sol à l'échelle de la rotation sylvicole.

L'objectif de ce chapitre est de statuer sur les hypothèses formulées dans l'introduction et de synthétiser l'ensemble des résultats obtenus dans cette étude afin de présenter un modèle général du cycle interne de l'azote dans le sol le long de la maturation des peuplements purs de hêtres.

2. Partie I : Relations morphologie/cycle de l'azote au sein de l'épisolum humifère

L'objectif était de caractériser la dynamique du cycle de l'azote au sein de l'épisolum humifère le long d'une chronoséquence de 130 ans de hêtraie (*Fagus sylvatica* L.) pure et d'identifier les variables macro-morphologiques susceptibles d'être indicatrices des patrons de variations des transformations de l'azote.

Hypothèse H1. Le cycle interne de l'azote (*i.e.* ammonification et nitrification) au sein de l'épisolum humifère varie le long de la maturation des peuplements forestiers et cette variation est en adéquation avec les patrons de variations des formes d'humus.

Deux approches complémentaires ont été utilisées pour caractériser la variabilité du cycle de l'azote le long de la chronoséquence: une approche au laboratoire et une approche *in situ*. Nous avons observé une variabilité importante de la production nette potentielle et *in situ* d'azote minéral, aussi bien au sein des horizons organiques, qu'au sein de l'horizon organo-minéral. L'ammonification potentielle nette augmente avec l'âge des peuplements au sein des horizons OL et FH alors que la nitrification potentielle et *in situ* nette diminue au sein des horizons OL et A. Outre la variabilité temporelle, nous avons également observé une variabilité spatiale verticale importante. Par exemple, la nitrification potentielle nette est essentiellement localisée au sein des horizons OF et OH. L'ammonification (exprimée par gramme de carbone) est toujours plus élevée au sein des horizons organiques. De même, les transformations fongiques dominent nettement au sein de l'horizon OL alors que les processus bactériens semblent être principalement localisés dans l'horizon A. Les analyses statistiques ont mis en évidence des patrons de covariations très significatifs entre le cycle de l'azote et les formes d'humus le long de la chronoséquence. Les résultats issus de ces expériences nous permettent donc d'**accepter l'hypothèse H1**.

3. Partie II : Les facteurs de contrôle des formes d'humus et du cycle de l'azote le long de la chronoséquence

Cette étude avait pour second objectif d'identifier les facteurs écologiques impliqués dans les changements morphologiques de l'épisolum humifère le long de la chronoséquence de hêtraie pure. La première hypothèse formulée était la suivante.

Hypothèse H2. L'accumulation de matériels organiques transformés le long de la maturation des peuplements (apparition d'un horizon d'humification) résulte d'une augmentation de la production de litière de hêtres.

Nous n'avons pas observé de variabilité temporelle significative des retombées de litière le long de la chronoséquence. Cependant, nous avons observé une corrélation linéaire significative entre la production de litière et l'épaisseur de l'horizon OF. De plus, lorsque l'on compare la production de litière en fonction des différentes formes d'humus rencontrées dans notre étude, la quantité de litière produite est significativement plus importante sous eumoder et dysmoder (Fig. 8.1.a). La forme d'humus dysmull est celle dont la production de litière est la plus faible. Malgré l'absence d'augmentation significative de litière le long de la chronoséquence, nous pouvons donc **accepter l'hypothèse H2**.

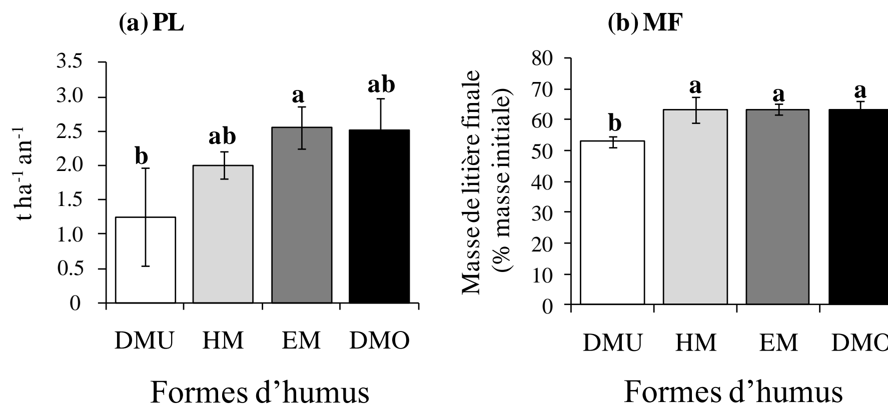

Figure 8.1. *Production de litière de hêtre (PL) et masse finale de litière (MF) après 12 mois d'incubation sur le terrain en fonction des formes d'humus (DMU = Dysmull (n=3), HM = Hemimoder (n=3), EM = Eumoder (n=8), DMO = Dysmoder (n=2)). Les barres verticales correspondent à l'écart-type et les lettres aux différences significatives d'après le test de Kruskal-Wallis test à P < 0.05.*

Hypothèse H3. L'accumulation de matériels organiques transformés le long de la maturation des peuplements (apparition d'un horizon OH) résulte d'une diminution de la vitesse de décomposition de la litière.

La méthode des « litterbags » nous a permis d'observer une forte variabilité temporelle de la vitesse de décomposition de la litière. Les peuplements de 65 et 95 ans présentent une vitesse de décomposition plus faible que les peuplements de 15 et 130 ans. De plus, la vitesse de décomposition de la litière est fortement corrélée à l'épaisseur des horizons OF et OH. De même, lorsque les résultats sont représentés par formes d'humus, la vitesse de décomposition de la litière augmente entre le dysmull et l'hemimoder (Fig. 1.8.b). Nous pouvons donc **valider l'hypothèse H3**. Ces résultats supposent donc que la chute de la vitesse de décomposition de la litière serait

responsable du passage mull-moder alors que la production de litière jouerait un rôle secondaire mais contribuerait au changement hemimoder-dysmoder.

Hypothèse H4. La disponibilité des nutriments au sein de l'horizon organo-minéral diminue au cours de la maturation des peuplements.

Les résultats du chapitre 4 montrent peu de variations des propriétés physico-chimiques de l'horizon organo-minéral. Nous n'avons pas observé de corrélations significatives entre les variables morphologiques et fonctionnels. De plus, l'analyse de multi co-inertie montre que la disponibilité des nutriments dans l'horizon A joue un rôle mineur dans les patrons de variations des formes d'humus et du cycle de l'azote à l'échelle de la rotation sylvicole. L'**hypothèse H4** est donc **rejetée**.

Hypothèse H5. L'apparition d'un horizon OH le long de la maturation des peuplements suppose des modifications importantes des communautés microbiennes de l'épisolum humifère.

La biomasse microbienne et le profil fonctionnel des communautés microbiennes du sol varient considérablement le long de la chronoséquence. Plus précisément, la quantité d'azote de la biomasse microbienne est plus élevée au sein des horizons organiques qu'au sein de l'horizon organo-minéral, mais ne varie pas significativement le long de la chronoséquence. Néanmoins le ratio N_{mic}-to-N_t (azote de la biomasse microbienne/azote total) au sein de l'horizon OL diminue significativement le long de la maturation des peuplements. La communauté fongique domine dans l'OL, alors que les bactéries dominent au sein du A quel que soit l'âge du peuplement. Le ratio biomasse fongique/biomasse bactérienne au sein de l'horizon FH augmente le long de la chronoséquence. La diversité fonctionnelle de la communauté bactérienne dans les horizons organiques est plus élevée dans les peuplements âgés. Au sein de l'horizon A, la diversité fonctionnelle est plus élevée dans le stade le plus jeune. De manière générale, la maturation des peuplements forestiers semble favoriser la communauté fongique au sein des horizons OF et OH et la diversité fonctionnelle des communautés bactériennes au sein des horizons organiques. Nous avons observé de nombreuses corrélations significatives entre les variables morphologiques et microbiennes, ce qui confère aux formes d'humus un potentiel indicateur du profil structurel et fonctionnel des communautés microbiennes du sol. Par exemple, la biomasse microbienne au sein de l'OL est négativement corrélée avec le pourcentage de feuilles squelettisées dans l'OLv. L'**hypothèse H5** est donc **acceptée**.

Hypothèse H6. L'apparition d'un horizon OH le long de la maturation des peuplements suppose des changements importants dans la qualité de la litière restituée au sol.

La qualité des feuilles sénescentes de hêtres présente une variabilité importante le long de la chronoséquence de 130 ans. Les résultats mettent en avant deux shifts majeurs. Le premier shift après 15 ans de vieillissement correspond à (1) une diminution des teneurs en Mg, en hémicellulose, en cellulose, en azote dans l'hémicellulose et dans la lignine et (2) une augmentation des teneurs en Mn, de la lignine, du C/N et du lignine/N. Le second shift après 95 ans de vieillissement correspond à (1) une baisse de la teneur en lignine, des cations (surtout K et Mg) et de l'azote dans la lignine et (2) une augmentation de la cellulose, de l'azote dans l'hémicellulose, et du pourcentage d'azote total dans l'hémicellulose, la cellulose et les solubles. L'utilisation de tests statistiques univariés (régression multiple) et multivariés

(MCOA) supposent des relations de causes à effets entre la qualité de la litière de hêtre et la morphologie de l'épisolum humifère. Ces résultats nous permettent donc **accepter l'hypothèse H6**.

4. Partie III : Approche expérimentale - Les facteurs de contrôle du cycle de l'azote

Le troisième objectif était d'identifier les facteurs écologiques impliqués dans le contrôle du recyclage de l'azote au sein de l'épisolum humifère. Nous nous sommes intéressés plus spécifiquement aux effets de la litière (apport et qualité) et des racines (nues ou ectomycorhizées) de hêtres sur le cycle interne de l'azote et les communautés microbiennes.

Hypothèse H7. L'apport et la qualité de la litière influencent le cycle de l'azote dans le sol.

L'approche expérimentale nous a permis de tester l'effet de l'apport et de la qualité de la litière de hêtre sur le cycle de l'azote et les communautés microbiennes du sol. La litière de hêtre, indépendamment de sa qualité initiale, inhibe la nitrification autotrophe et favorise la communauté fongique. L'apport de litière affecte fortement le profil fonctionnel de la communauté bactérienne hétérotrophique. En revanche, les deux types de litière testés (20 ans *versus* 90 ans) ont des effets similaires sur le cycle de l'azote et les communautés microbiennes bien que leur composition chimique (fibres et éléments minéraux) soit différente. Ces résultats nous permettent donc **d'accepter partiellement l'hypothèse H7**.

Hypothèse H8. Les racines et la présence de champignons ectomycorhiziens influencent le cycle de l'azote.

L'approche expérimentale nous a également permis de tester l'effet de l'apport des racines de hêtre, mycorhizées ou non, sur le cycle de l'azote et les communautés microbiennes du sol. Les racines, mycorhizées ou non, favorisent l'ammonification potentielle et les racines ectomycorhizées inhibent la nitrification autotrophe. De plus, la présence de litière affecte l'effet des racines et vice-versa (interaction litière/racine). Par exemple, la teneur en azote minéral dans le sol est faible en présence de racines vivantes ectomycorhizées, excepté en présence de litière. Ce patron n'a pas été observé pour les racines non ectomycorhizées. Ces résultats nous permettent donc **d'accepter l'hypothèse H8**.

5. Bilan : Le cycle de l'azote le long de la maturation des peuplements forestiers

Les résultats issus de cette étude nous permettent de dresser un bilan annuel du cycle de l'azote pour chaque classe d'âge (Fig. 8.2) et de la variation des principaux flux d'azote au sein de l'épisolum humifère le long de la chronoséquence (Fig. 8.3).

5.1. Cycle de l'azote au sein des différents peuplements

15 ans – Les peuplements de 15 ans apportent environ 15 kg d'azote par hectare par an via la litière (Fig. 8.2). Au sein de l'épisolum humifère (horizons OL + OF + OH + A), le stock d'azote dans le sol est de 2.1 t par hectare incluant 5.1 kg d'ammonium et 2.6 kg de

nitrate. Seulement 9.7 kg d'azote sont dans la biomasse microbienne. Environ 130 kg d'ammonium sont produits, 2.3 kg sont lessivés et seulement 8,83 kg sont convertis en nitrate par hectare par an. La perte de nitrate par lessivage est élevée (*i.e.* 10.1 kg N ha^{-1} an^{-1}) comparé aux pertes par dénitrification (*i.e.* 1.5 kg N ha^{-1} an^{-1}). Environ 70 et 50 kg d'ammonium et de nitrate sont prélevés par les racines par hectare et par an, respectivement.

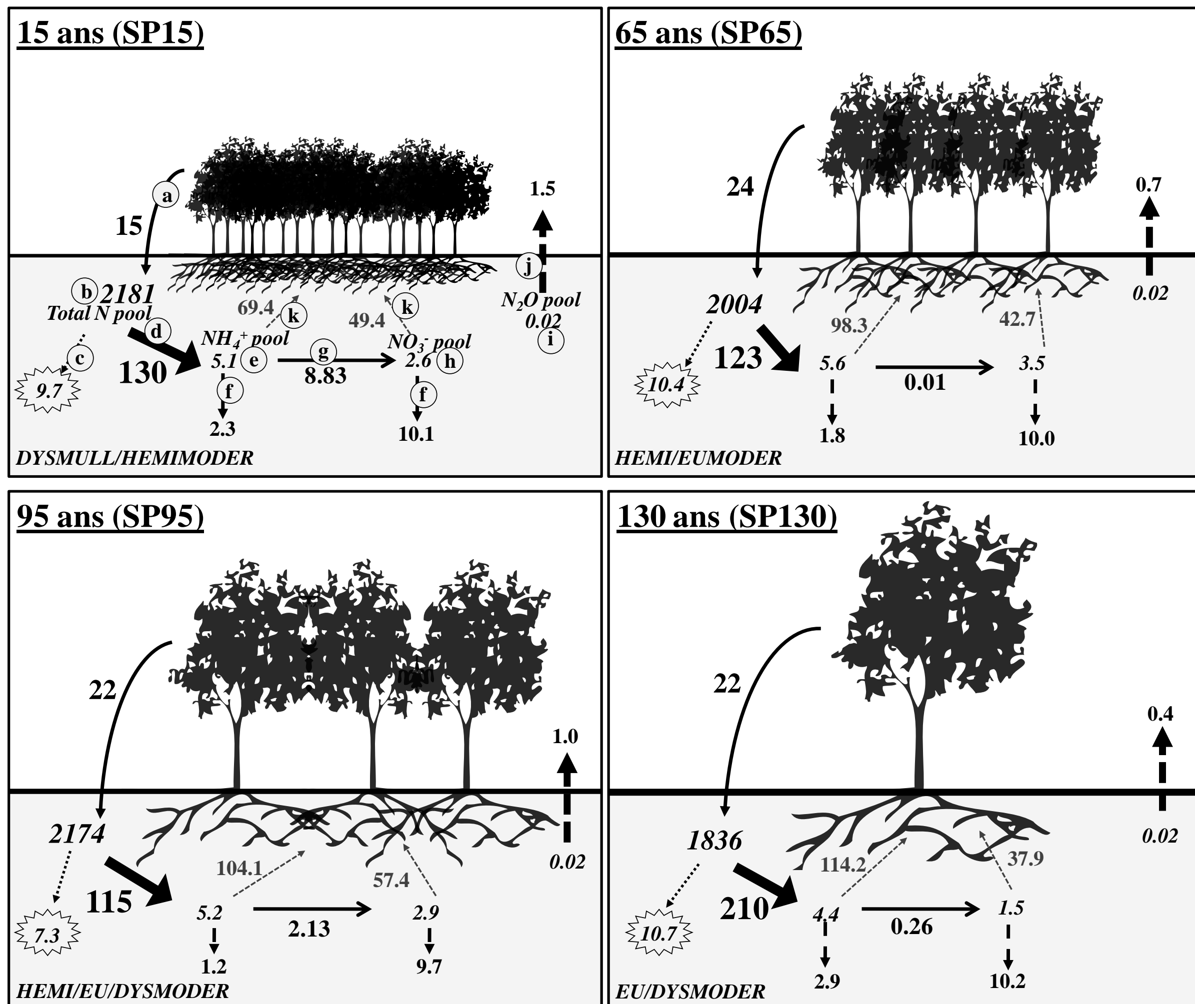

Figure 8.2. *Cycle de l'azote dans le sol (horizons OL+OF+OH+A) le long de la chronoséquence de 130 ans. Les nombres en italiques correspondent aux quantités d'azote total ou minéral dans le sol. (a) Apport d'azote par la litière en kg N ha^{-1} an^{-1} (cf. chapitre 4). (b) Azote total dans le sol en kg N ha^{-1} au mois de mai 2007 (cf. chapitre 2). (c) Azote contenu dans la biomasse microbienne en kg ha^{-1} au mois de mai 2008 (cf. chapitre 5). (d) Ammonification nette en kg N ha^{-1} an^{-1}. (e) Teneur en ammonium en kg N ha^{-1} (moyenne annuelle, cf. chapitre 3). (f) Lessivage d'ammonium et de nitrate en kg N ha^{-1} an^{-1}. (g) Nitrification nette en kg N ha^{-1} an^{-1} (cf. chapitre 3). (h) Teneur en nitrate en kg N ha^{-1} (moyenne annuelle, cf. chapitre 3). (i) Teneur en N_2O en kg ha^{-1}(moyenne annuelle, cf. chapitre 3). (j) Dénitrification nette en kg ha^{-1} an^{-1}. (k) Quantité d'ammonium et de nitrate prélevée par les racines en kg N ha^{-1} an^{-1}.*

65 ans – L'apport d'azote par la litière dans les peuplements de hêtres de 65 ans est plus élevé, avec 24 kg d'azote par hectare par an. L'ammonification est peu différente du peuplement de 20 ans alors que la nitrification est quasi nulle (0.01 kg de nitrate produit par hectare par an). Malgré une nitrification proche de 0, le lessivage des ions nitrate est élevé (10 *kg N* ha^{-1} an^{-1}) alors que la dénitrification est plus faible que celle

mesurée dans les peuplements de 15 ans. Les stocks d'azote dans le sol sont peu différents de ceux des peuplements de 15 ans. En revanche, la quantité d'ammonium prélevée par les racines est plus élevée, *i.e.* 98 kg d'ammonium sont prélevés par les racines par hectare et par an.

95 ans – Le cycle de l'azote au sein de l'épisolum humifère des peuplements de 95 ans est sensiblement similaire à celui précédemment décrit. Les seules différences sont une légère augmentation de la nitrification (2.1 kg de nitrate produits par hectare par an) et du prélèvement des ions ammonium (104.1 kg) et nitrate (57 kg) alors que l'azote dans la biomasse microbienne est plus faible (7.3 kg).

130 ans – L'apport d'azote par la litière dans les peuplements de hêtres de 65 ans est peu différent des peuplements plus jeunes. Cependant, la quantité d'ammonium produite est d'environ 210 kg d'azote par hectare par an, soit une augmentation de 36% depuis les peuplements de 15 ans. La nitrification nette et la dénitrification sont très faibles alors que le lessivage des ions nitrate est élevé (environ 10 kg d'azote par hectare par an). Contrairement aux ions nitrate, la quantité d'ammonium prélevée par les racines est élevée, *i.e.* 98 kg d'ammonium sont prélevés par les racines par hectare et par an, seulement 38 kg de nitrate sont prélevés. Au sein de l'épisolum humifère, le stock d'azote dans le sol est beaucoup plus faible que celui des peuplements plus jeunes, avec seulement 1836 kg d'azote par hectare incluant 4.4 kg d'ammonium et 2.6 kg de nitrate. Environ 10.7 kg d'azote sont dans la biomasse microbienne.

5.2. Variabilité temporelle du cycle de l'azote

Le résultat principal est le suivant: les processus en amont du cycle (apport d'azote, ammonification) sont favorisés au cours de la maturation des peuplements purs de hêtres alors que les processus en aval du cycle (nitrification, dénitrification) diminuent le long de la chronoséquence (Fig. 8.3). Le lessivage des ions nitrate varie peu le long de la chronoséquence alors que le prélèvement de l'azote minéral (surtout l'ammonium) et le lessivage des ions ammonium augmentent significativement. Les flux en rouge correspondent aux processus non caractérisés, dont l'étude pourrait certainement constituer la prochaine étape de cette thèse. De manière générale, les résultats issus du chapitre 2 (approche *ex situ*) confirment et accentuent les patrons observés *in situ*. L'ammonification potentielle nette au sein des horizons holorganiques (OL et FH) augmente le long de la chronoséquence alors que la nitrification nette potentielle chute le long de la chronoséquence au sein de l'OL et du A. Ces résultats vont dans le sens du paradigme proposé par Schimel et Bennett (2004) au sein duquel les processus en amont du cycle (dépolymérisation de l'azote organique) et non la nitrification, constituent les processus clefs de la régulation de l'ensemble du cycle de l'azote dans les systèmes forestiers. Afin de généraliser ce modèle de fonctionnement, d'autres peuplements forestiers doivent être considérés avec des conditions pédoclimatiques très contrastées.

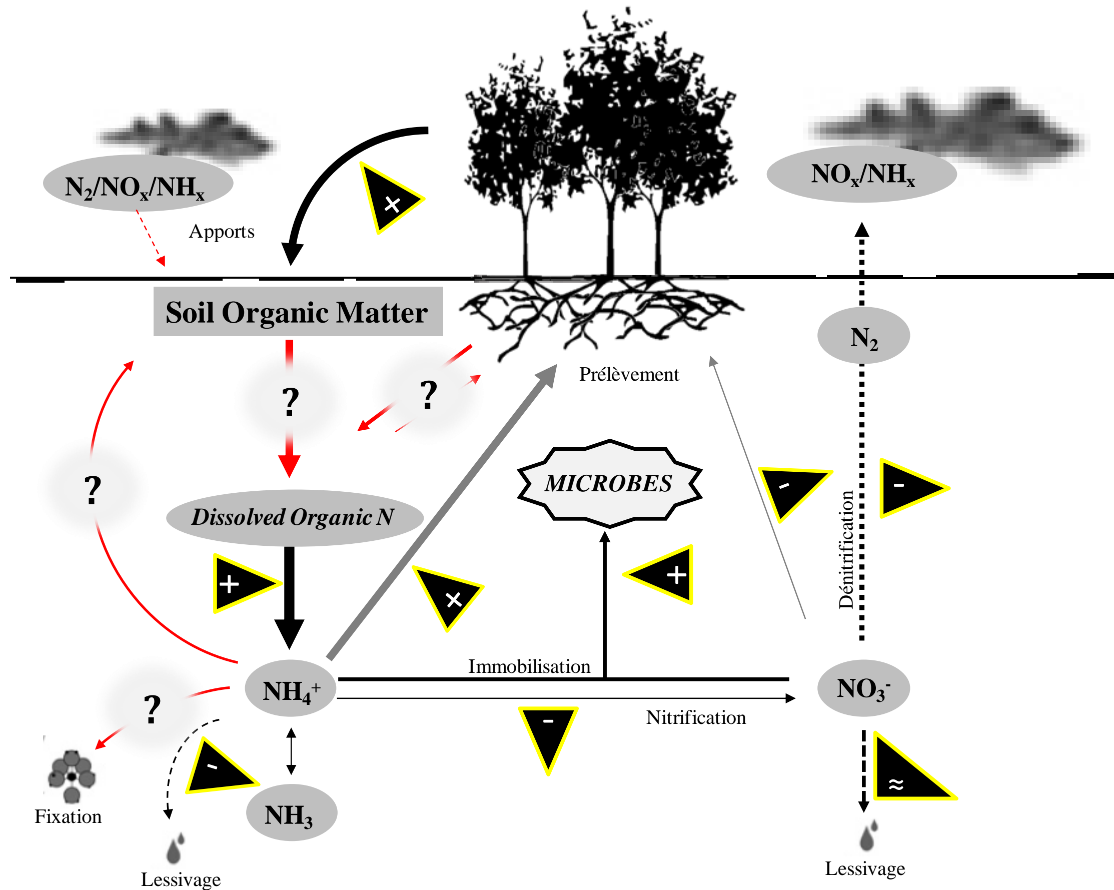

Figure 8.3. *Bilan du cycle simplifié de l'azote dans le sol le long de la chronoséquence de 130 ans de hêtraie pure. Les flèches noires à contour jaune indique si le flux augmente (+, pointe en avant), diminue (-, pointe en arrière) ou ne varie pas (≈) le long de la chronoséquence. Les flux en rouge n'ont pas été étudiés lors de cette thèse.*

5.3. Nature des processus de production d'azote

Nos résultats suggèrent que la nature du processus (fongique ou bactérien) peut certainement expliquer la quantité d'azote minéral produite. Par exemple, la contribution des champignons dans la production nette d'ammonium au sein des horizons OF+OH augmente à partir de 95 ans. En revanche, la contribution fongique dans la production d'ammonium au sein de l'horizon A est nulle. Or, l'exigence en azote par unité de carbone des champignons est beaucoup plus faible que celle des bactéries (Kooijman and Martinez-Hernandez 2009). L'augmentation de la production nette d'ammonium pourrait donc s'expliquer par une faible immobilisation microbienne, essentiellement fongique, malgré une production brute constante. L'analyse des flux bruts nous permettrait de valider ou non cette hypothèse.

5.4. Shift *mull-moder* synonyme d'augmentation de production d'ammonium

La trajectoire dynamique cyclique d'un écosystème forestier se décompose en deux phases principales : une phase autotrophe et une phase hétérotrophe (Bernier and Ponge 1994; Ponge *et al*. 1998). Durant la phase de croissance intense des peuplements (phase autotrophe), les formes d'humus évoluent depuis le mull vers les moders. Ce changement morphologique serait dû à l'intensité de l'absorption des nutriments nécessaires à la production primaire, qui conduirait à une acidification de l'épisolum

humifère. Cette acidification induirait une diminution du turn-over de la matière organique d'autant plus importante que les macro-organismes les plus efficients pour la décomposition (vers de terre anéciques et endogés) sont absents durant cette phase.

D'un point de vue fonctionnel, notre étude montre clairement que ce changement *mull-moder* n'est pas synonyme d'une baisse de production d'azote minéral, mais au contraire, indicateur d'une production importante d'ammonium. Ce résultat est d'autant plus incontestable lorsque les flux d'azote sont représentés par types d'humus (Fig. 8.4 et 8.5). Par exemple, les formes d'humus de type moder (hemimoder, eumoder et dysmoder) présentent une minéralisation de l'azote potentielle au sein des horizons organiques plus élevés que le dysmull (Fig. 8.4). En revanche, le dysmull présente une nitrification potentielle et *in situ* et une dénitrification bien plus élevée que les moders (Fig. 8.4 et 8.5).

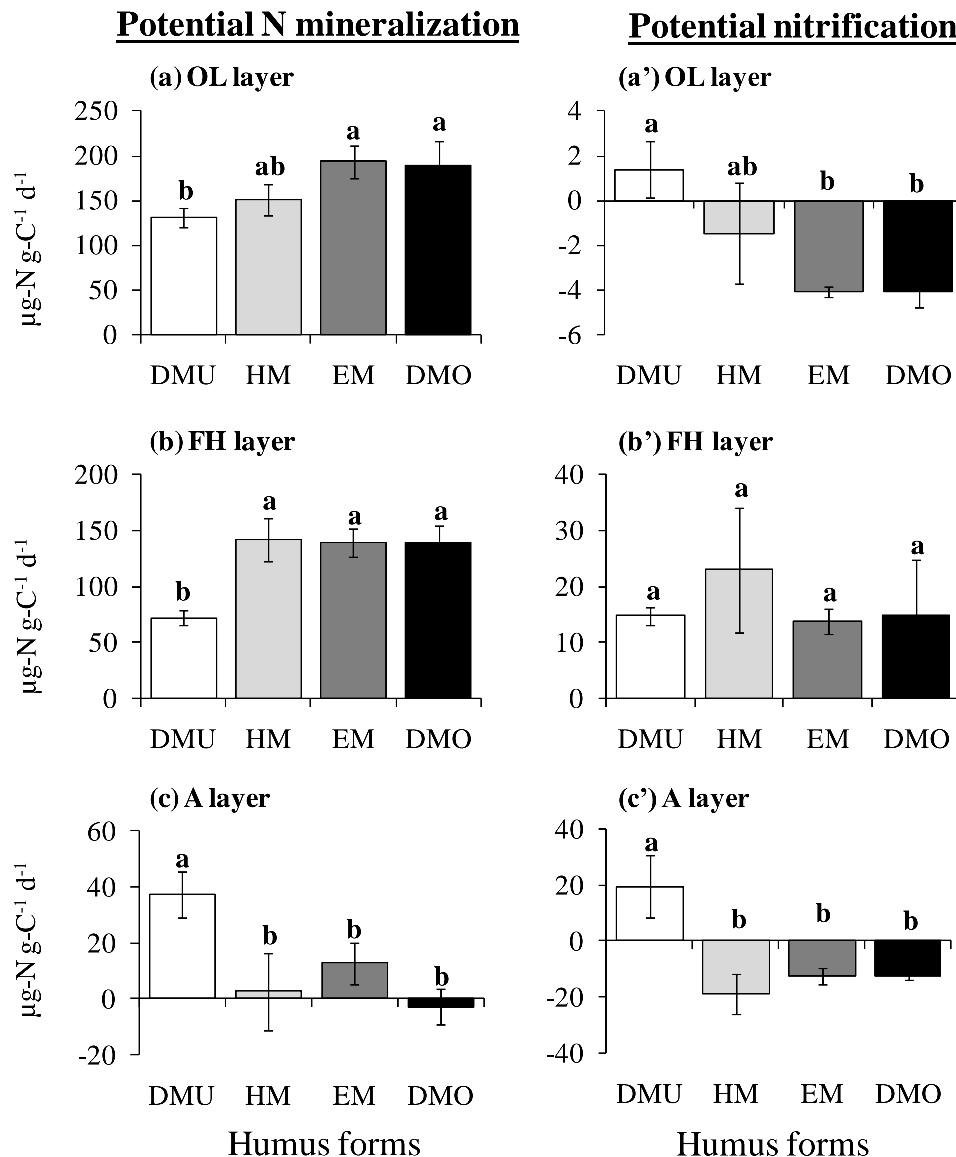

Figure 8.4. *Minéralisation potentielle de l'azote et nitrification potentielle dans l'OL (a et a'), le FH (b et b') et dans l'horizon A (c et c') en fonction des formes d'humus (DMU = Dysmull (n=3), HM = Hemimoder (n=3), EM = Eumoder (n=8), DMO = Dysmoder (n=2)). Les barres verticales correspondent à l'écart-type et les lettres aux différences significatives d'après le test de Kruskal-Wallis test à P < 0.05.*

De façon générale, les moders (surtout le dysmoder) sont indicateurs d'un cycle de l'azote en faveur de l'ammonium (ammonification élevée, nitrification faible) alors que le dysmull est indicateur d'un cycle en faveur des ions nitrate (nitrification élevée). Les moders (surtout le eumoder et le dysmoder) sont également caractérisés par un apport d'azote via la litière plus élevé que le dysmull.

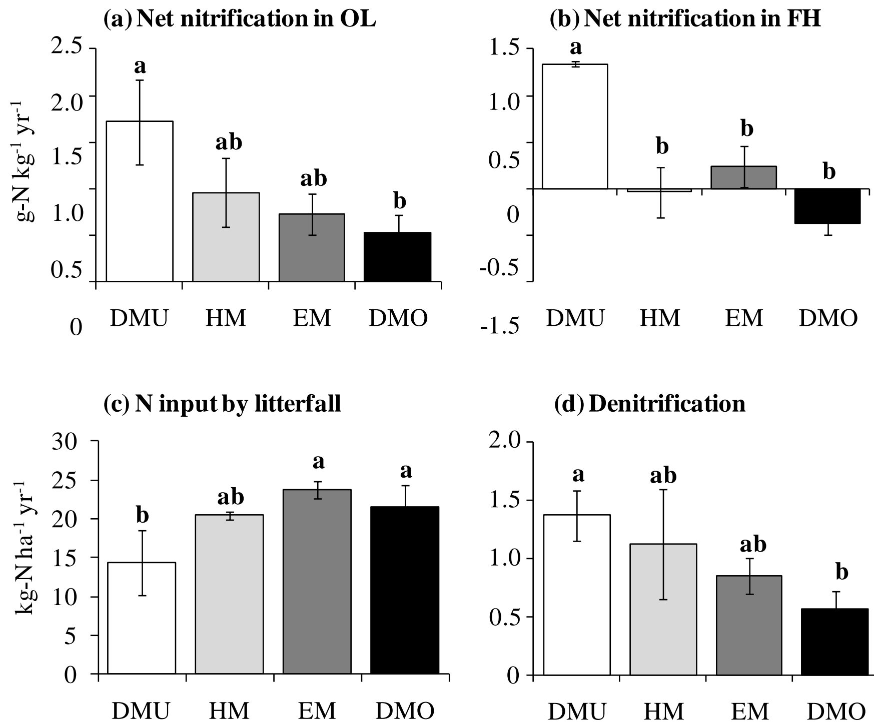

Figure 8.5. *Nitrification nette au sein des horizons OL (a) et FH (b), apport d'azote par la litière (c) et dénitrification (d) en fonction des formes d'humus (DMU = Dysmull (n=3), HM = Hemimoder (n=3), EM = Eumoder (n=8), DMO = Dysmoder (n=2)). Les barres verticales correspondent à l'écart-type et les lettres aux différences significatives d'après le test de Kruskal-Wallis test à P < 0.05.*

Cette étude montre également que de nombreuses variables macro-morphologiques présentent un véritable potentiel indicateur de production d'azote minéral. Il s'agit par exemple du pourcentage de feuilles fragmentées dans l'OLv (indicateur de nitrification potentielle autotrophe dans l'OL et le FH), du pourcentage de recouvrement de l'OLv (indicateur de l'ammonification bactérienne dans l'OL), du nombre de turricules de vers de terre dans l'OLn et l'OLv (indicateur du pool de nitrate dans l'OL), l'épaisseur de l'OLv (indicateur de l'ammonification bactérienne dans l'OL), l'épaisseur de l'OF (indicateur du pool de nitrate dans l'OL et de l'ammonification fongique dans l'OL), la structure de l'horizon A (indicateur de nitrification dans l'OL), le pourcentage de matière organique fine dans l'OF (indicateur de nitrification)ou encore le pourcentage de feuilles blanchies dans l'OLv (indicateur du pool de nitrate dans l'OL). Il apparaît également que les variables morphologiques dont la variance contribue significativement à la dynamique temporelle des formes d'humus le long de la chronoséquence, ne sont pas assurément celles qui semblent être indicatrices de la production d'azote minéral. Par exemple, la production d'azote minéral est peu corrélée avec les variables morphologiques spécifiques à l'horizon OH. Or, ces dernières jouent un rôle majeur dans la dynamique de la morphologie de l'épisolum humifère le long de la chronoséquence. Ce résultat est également présent lorsque j'ai cherché à corréler les variables morphologiques avec les variables microbiennes. Ainsi, malgré une faible

réponse à la dynamique forestière, certaines variables morphologiques peuvent certainement constituer des indicateurs robustes de fonctionnement.

6. Perspectives

Nos travaux soulèvent beaucoup de questions auxquelles des études futures devraient répondre. Ci-après sont proposées quelques études qui pourraient compléter les résultats déjà acquis sur les facteurs responsables des changements morphologiques et fonctionnels (cycle de l'azote) le long de la maturation des peuplements forestiers.

Les formes d'humus : indicateurs potentiels de production d'azote minéral

La faible gamme des formes d'humus rencontrées dans cette étude (dysmull, hemimoder, eumoder et dysmoder), nous permet de mettre en avant la capacité des variables macro-morphologiques à prédire la production de l'azote minéral au sein de l'épisolum humifère. Cependant, la validation de ces résultats nécessite un échantillonnage exhaustif, incluant tous les types de formes d'humus (d'après la typologie de Jabiol *et al*. (2009) par exemple), représentées par un nombre important de répétitions. L'échantillonnage pourrait par exemple se concentrer sur les systèmes forestiers feuillus, purs ou mixtes, balayant un large spectre de conditions stationnelles. Pour chaque forme d'humus, un ensemble de variables morphologiques serait décrit sur le terrain, et la capacité potentielle des horizons à produire de l'azote minéral serait caractérisée au laboratoire. Cette approche permettrait de mieux comprendre les relations formes d'humus-production d'azote minéral au sein de l'épisolum humifère. Par ailleurs, les résultats opposent deux catégories de variables macro-morphologiques : celles dont la variance est significativement corrélée aux mesures potentielles (température et humidité très élevées) des flux d'azote, et celles dont la variance est significativement corrélée aux mesures *in situ* (température et humidité moyennes) des flux d'azote. Dans le contexte actuel du réchauffement climatique et de son impact sur le fonctionnement des écosystèmes, il peut être envisagé d'utiliser les variables de la première catégorie comme indicateur des changements fonctionnels potentiels de l'épisolum humifère en réponse aux changements climatiques. La caractérisation de la minéralisation potentielle de l'azote au sein des horizons de l'épisolum humifère (OLn , OLv, Olt, OF, OH, A), à l'aide de la méthode d'incubation *ex situ*, le long d'un gradient de température et d'humidité, pourrait permettre l'identification et la mise en place de variables macro-morphologiques prospectives des changements climatiques.

Horizons organiques versus horizon organo-minéral

Un résultat majeur qui émerge de cette thèse est la contribution élevée des horizons organiques dans la production d'azote minéral. Les horizons organiques sont véritablement le siège de la transformation biochimique de la matière organique et présentent une biomasse microbienne bien plus élevée que celle de l'horizon organo-minéral. Néanmoins, peu d'études, dont l'objectif était la caractérisation du cycle de l'azote dans le sol forestier, échantillonnent les horizons organiques. Il est vrai que la caractérisation du fonctionnement des horizons organiques est parfois difficile en raison de la teneur élevée en molécules récalcitrantes, tels que les acides humiques et fulviques. Bien évidemment, lorsque les flux sont calculés à l'hectare, la contribution de l'horizon organo-minéral est plus importante que les horizons organiques du fait de sa densité. Néanmoins, lorsqu'on raisonne en quantité d'azote minéral produit par gramme

sec de matériel ou de carbone, les horizons OL, OF et OH produisent plus d'azote minéral que l'horizon organo-minéral. La caractérisation de la dynamique de l'azote au sein des horizons OF et OH échantillonnés séparément, ainsi que dans l'OLn, l'OLv et l'OLt, nous permettrait certainement de mieux comprendre les relations morphologie-fonctionnement de l'épisolum humifère.

La dépolymérisation de l'azote et la dynamique du DON

L'utilisation des inhibiteurs sélectifs nous a permis d'évaluer la contribution des différents microorganismes dans la production nette potentielle d'ammonium et de nitrate. Or, les résultats suggèrent que les étapes clefs régulant le cycle de l'azote se situent en amont du cycle. La dépolymérisation de l'azote en fait partie. De plus, il est aujourd'hui admis que la plupart des espèces ligneuses sont capables de prélever directement l'azote sous forme organique (acides aminées ou peptides de faibles poids moléculaires) ou via leur partenaire fongique (Nasholm *et al*. 1998; Schimel and Bennett 2004; Nasholm *et al*. 2009). Cette capacité permettrait à l'arbre de court-circuiter la compétition avec les microorganismes du sol vis-à-vis de l'azote (Schimel and Bennett 2004). La caractérisation le long de la chronoséquence de (i) la dépolymérisation de l'azote (production de molécules azotées de faible poids moléculaire) au sein des différents horizons de l'épisolum humifère, (ii) la dynamique du DON et (iii) de l'ammonification en réalisant des profils enzymatiques exhaustifs (hydrolases, protéases, peptidases, urease, etc.), nous aurait peut être permis d'élargir le spectre des processus du cycle de l'azote quantifiés. De même, une mesure des flux bruts en utilisant la méthode de dilution isotopique de l'azote 15 permettrait d'affiner la caractérisation du cycle de l'azote et apporterait des réponses sur la diminution de la nitrification, *i.e.* s'agit-il d'une absence de production ou d'une forte immobilisation microbienne?

La disponibilité des nutriments dans le sol

Les résultats des chapitres 4 et 6 supposent que la disponibilité des nutriments dans l'horizon A ne constitue pas un facteur explicatif des patrons de variations des formes d'humus et du cycle de l'azote dans le sol à l'échelle de la rotation sylvicole. Ce résultat est surprenant. Il est difficile de croire que l'environnement abiotique n'influence pas la dynamique morphologique de l'épisolum humifère et vice versa à l'échelle étudiée. Ce résultat est d'autant plus surprenant que plusieurs études ont montré une acidification de l'horizon A le long de chronoséquence forestière (Ponge and Delhaye 1995; Ponge 2003; Aubert *et al*. 2004; Hedde 2006). Il serait judicieux de compléter cette étude en caractérisant le budget minéral (teneur en éléments minéraux, CEC, taux de saturation, pH) de l'ensemble des horizons constitutifs de l'épisolum humifère mais également des horizons profonds. Cet objectif était initialement prévu dans la thèse mais finalement écarté par faute de temps. La biomasse racinaire élevée dans les horizons organiques supporte cette perspective. En effet, il est fort probable que le prélèvement des éléments minéraux par les racines au sein des horizons OF et OH soit élevé et que la disponibilité de ces nutriments dans ces horizons joue un rôle sur le fonctionnement de l'épisolum humifère. De plus, l'apparition des horizons OF et OH au cours de la maturation des peuplements, favorise cette distribution verticale des racines de hêtre. Cette approche permettrait de valider ou rejeter l'hypothèse selon laquelle la disponibilité des nutriments dans le sol serait responsable des changements morphologiques et fonctionnels de l'épisolum humifère à l'échelle de la rotation.

La qualité des litières

La qualité des litières est un trait bien connu pour son impact sur le fonctionnement des sols forestiers. Les résultats obtenus au cours du chapitre 6 montrent que la variabilité intra-spécifique de ce trait est considérable. Néanmoins, l'approche descriptive nous interdit de conclure sur l'impact de cette variabilité sur le fonctionnement de l'épisolum humifère. De même, nous ne pouvons pas affirmer qu'une espèce ligneuse est capable de produire une litière de qualité différente en fonction de son âge. Pour cela, il aurait fallu échantillonner la litière à l'échelle de l'individu. Cette perspective pourrait répondre aux questions que soulève cette thèse. Par exemple, quels sont les facteurs responsables de la variabilité de la qualité de la litière ? Ces changements sont-ils suffisamment importants pour induire un changement fonctionnel au sein de l'épisolum humifère ? L'approche expérimentale ne nous a pas permis de valider cette hypothèse alors qu'au cours du chapitre 6, nous avons observé des patrons de covariations très significatifs entre la qualité de la litière, la morphologie et le fonctionnement de l'épisolum humifère. Il serait peut être plus informatif de tester la qualité de la litière sur une période plus longue et en présence de vers de terre. A l'instar des champignons mycorhiziens qui influencent les effets des racines de hêtres sur le cycle de l'azote, les vers de terre pourraient accentuer les effets de la qualité de la litière via la fragmentation de la litière et le brassage des phases organiques et minérale. Il peut être également envisagé de tester la qualité de la litière de hêtre sur le cycle de l'azote en irriguant le sol avec des jus de litière concentrés.

Le rôle de la lignine et du manganèse

Un résultat important issu de cette thèse est l'augmentation de la biomasse fongique dans les horizons de fragmentation et d'humification. Il est difficile de croire que l'augmentation de la lignine et du manganèse ne jouent pas un rôle dans la promotion de la communauté fongique. Il est aujourd'hui connu que la lignine est principalement dégradée par les enzymes extracellulaires produites par les champignons saprophytes dont le fonctionnement est assuré par la présence de manganèse. L'absence de ce cofacteur induirait une baisse de l'activité lignolytique des champignons et donc une accumulation de lignine dans le sol. Par ailleurs, le manganèse est le seul cation qui augmente significativement le long de la chronoséquence. L'approche descriptive ne nous permet pas d'affirmer un rapport de causalité, mais il semblerait que l'augmentation de la teneur en lignine et en manganèse soit responsable de l'augmentation de la biomasse fongique au sein de l'épisolum humifère le long de la chronoséquence. Il serait intéressant de conduire une étude expérimentale dans laquelle la biomasse fongique serait mesurée dans un sol neutre, dépourvu de bactérie (utilisation d'un inhibiteur bactérien telle que la streptomycine), avant et après ajout de manganèse disponible et non disponible (chélaté) en présence de litière de hêtre et d'un semis de hêtre dont le taux de mycorhization serait connu.

Les effets des racines

Les effets des racines sur le cycle de l'azote ont été très peu appréhendés dans cette thèse. Or, les racines peuvent contrôler le cycle de l'azote via (i) les prélèvements racinaires, (ii) les exsudats racinaires, (iii) la présence du partenaire symbiotique fongique et (iv) le turnover racinaire et mycélien. Il est fort probable que la biomasse racinaire au sein des différents horizons de l'épisolum humifère, varie le long de la

chronoséquence, et que cette variabilité constitue un facteur explicatif des patrons de variations des transformations de l'azote. Par exemple, Palfner *et al*. (2005) ont montré une augmentation de la densité racinaire et de la diversité mycorhizienne (de 2 à 12 espèces fongiques ectomycorhiziennes) le long d'une chronoséquence de 40 ans d'épicea (*Picea sitchensis*) au nord de l'Angleterre. Par ailleurs, nous avons montré que la présence du partenaire fongique influence les effets des racines sur le cycle de l'azote. Il serait donc intéressant de caractériser (i) la biomasse racinaire au sein des différents horizons de l'épisolum humifère, (ii) les taux de mycorhization des racines, et (iii) la quantité et la qualité de la rhizodéposition des racines nues et ectomycorhizées le long de la chronoséquence. Ceci nous permettrait peut être d'expliquer la chute de la nitrification en présence de racines, observée dans le chapitre 7.

Interactions litière-mycorhize-azote à l'échelle de la rotation sylvicole

Récemment, les résultats de Wurzburger *et al*. (2009) suggèrent un recyclage complexe de l'azote faisant intervenir la qualité de la litière, la rétention de l'azote dans le sol et le symbionte ectomycorhizien des racines de Rhododendron (*Rhododendron maximum* L.). L'azote des litières de Rhododendron est fortement complexé, via les tanins, à la matière organique du sol, ce qui augmente sa rétention dans le sol et limite son recyclage par les décomposeurs. Toutefois, en contribuant significativement à la dégradation de la matière organique du sol, les mycorhizes des racines de Rhododendron semblent avoir facilement accès à cette ressource azotée, contrairement aux racines mycorhizées des espèces de plantes co-existantes. Au cours du chapitre 6, nous avons montré que le pourcentage de l'azote total dans la lignine augmentait de plus de 11% au début de la chronoséquence. L'azote dans la lignine serait principalement rendu disponible par les champignons. A partir de la bibliographie et de l'ensemble des résultats obtenus dans cette étude, nous pouvons donc formuler l'hypothèse suivante : a*u cours de son vieillissement, le peuplement de hêtres influence les flux d'azote dans le sol de manière à maximiser l'acquisition de l'azote via les ectomycorhizes et minimiser son immobilisation par les microorganismes du sol (bactéries et champignons saprophytes). Ce contrôle des flux s'opère directement sur la disponibilité et les transformations de l'azote (favoriser les processus en amont du cycle) et indirectement via le compartiment microbien (favoriser la communauté fongique de manière à maximiser la colonisation des racines par les champignons). Les mécanismes de contrôle font donc intervenir (i) le système symbiotique mycorhizien et (ii) la qualité de litière de hêtres.* Le suivi de l'azote de chaque fractions carbonées dans les différents compartiments de l'épisolum humifère (fixation au complexe, assimilation par les racines, immobilisation microbienne, etc.) pourrait certainement nous permettre de mieux comprendre le cycle de l'azote dans le sol.

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Annexe 1

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Variables	Codes	Unities
Ca	Ca	Concentration in leaf litter in mg/g
Mg	Mg	Concentration in leaf litter in mg/g
K	K	Concentration in leaf litter in mg/g
Na	Na	Concentration in leaf litter in mg/g
Mn	Mn	Concentration in leaf litter in mg/g
Total cation	T-Ca	Concentration in leaf litter in mg/g
Soluble	Sol	% of fraction in leaf litter on ash free basis
Hemicellulose	Hem	% of fraction in leaf litter on ash free basis
Cellulose	Cell	% of fraction in leaf litter on ash free basis
Lignin	Lig	% of fraction in leaf litter on ash free basis
Soluble N	Sol-N	% of N in fraction
Hemicellulose N	Hem-N	% of N in fraction
Cellulose N	Cell-N	% of N in fraction
Lignin N	Lig-N	% of N in fraction
Total C	C	% of C in fraction
Total N	N	% of N in fraction
Soluble C/N	Sol-C/N	-
Hemicellulose C/N$	Hem-C/N	-
Cellulose C/N$	Cell-C/N	-
Lignin C/N	Lig-C/N	-
Relative N amount in soluble	Sol-RN	Relative amount in % of leaf litter N contained in soluble
Relative N amount in hemicellulose$	Hem-RN	Relative amount in % of leaf litter N contained in hemicellulose
Relative N amount in cellulose$	Cell-RN	Relative amount in % of leaf litter N contained in cellulose
Relative N amount in lignin	Lig-RN	Relative amount in % of leaf litter N contained in lignin
C/N$	C/N	-
Lignin/N	Lig/N	-

"$" indicates that the variable was excluded from the multiple regression analysis due to autocorrelation ($R^2 > 0.90$).

Annexe 2

Codes of each variable from the table “Humus forms” or “H” used in the Multiple Co-Inertia Analysis and the multiple regression analysis.

Soil layers	Variables	Codes	Unities/Modalities
OLn layer	Leaves fragments	OLnfg	Percentage
	Skeletonized leaves	OLnsk	Percentage
	Earthworms casts$	OLnEc	Nomber per square meters
OLv layer	Maximal thickness	OLvMt	Centimeters
	Minimal thickness	OLvmt	Centimeters
	Leaves fragments	OLvfg	Percentage
	Cover	OLvcov	Percentage
	Skeletonized leaves	OLvsk	Percentage
	Earthworms casts	OLvEc	Nomber per square meters
	Bleach leaves	OLvBl	Percentage
	Brown leaves	OLvBr	Percentage
	Compacted leaves	OLvCom	From 1 to 4 (1: no compact, 2: slightly compact, 3: moderately compact, 4: strongly compact)
	Living roots	OLro	From 0 to 3 (0: absent, 1: rare, 2: moderately present, 3: abundant)
OF layer	Maximal thickness	OFMt	Centimeters
	Minimal thickness	OFmt	Centimeters
	Leaves fragment	OFfg	Percentage
	Fine organic matter	OFfog	Percentage
	Earthworms casts	OFEc	Nomber per square meters
	Bleach leaves	OFBl	Percentage
	Cover	OFcov	Percentage
	Compacted leaves	OFCom	From 1 to 4 (1: no compact, 2: slightly compact, 3: moderately compact, 4: strongly compact)
	Living roots	OFro	From 0 to 3 (0: absent, 1: rare, 2: moderately present, 3: abundant)
OH layer	Maximal thickness	OHMt	Centimeters
	Minimal thickness$	OHmt	Centimeters
	Fine organic matter from fauna faeces	OHffog	Percentage
	Vegetable fine organic matter	OHvfog	Percentage
	Cover	OHcov	Percentage
	Living roots	OHro	From 0 to 3 (0: absent, 1: rare, 2: moderately present, 3: abundant)
A	Maximal thickness	AMt	Centimeters
	Minimal thickness	Amt	Centimeters
	Agregate size	Aas	Millimeters
	Structure	Ast	From 1 to 4 (1: without aggregate, 2: slightly aggregate, 3: moderately aggregate, 4: strongly aggregate)
	Living roots	Aro	From 0 to 3 (0: absent, 1: rare, 2: moderately present, 3: abundant)

"$" indicates that the variable was excluded from the multiple regression analysis due to autocorrelation.

Annexe 3

Codes of each variable from the table "N cycle" or "N" used in the Multiple Co-Inertia Analysis and the multiple regression analysis.

Soil layers	Variables	Codes	Unities
OL layer	Maximal N immobilization	MaxN	mg-N/g initial litter
	Final litter C/N	FC/N	-
	Total N	TNL	%
	In situ ammonium pools	NH4-L	µg-N g dry matter
	In situ nitrate pools	NO3-L	µg-N g dry matter
	Net N mineralization	MinL	µg-N per g-C per d
	N mineralization rates$	MRL	‰ of total N mineralized after 28 days
	Net nitrification	NitL	µg-N per g-C per d
	Net autotrophic nitrification	ANL	µg-N per g-C per d
	Net heterotrophic nitrification$	HNL	µg-N per g-C per d
FH layer	Total N	TNFH	%
	In situ ammonium pools	NH4-FH	µg-N g dry matter
	In situ nitrate pools	NO3-FH	µg-N g dry matter
	Net N mineralization	MinFH	µg-N per g-C per d
	N mineralization rates	MRFH	‰ of total N mineralized after 28 days
	Net nitrification	NitFH	µg-N per g-C per d
	Net autotrophic nitrification$	ANFH	µg-N per g-C per d
	Net heterotrophic nitrification	HNFH	µg-N per g-C per d
A	Total N	TNA	%
	In situ ammonium pools	NH4-A	µg-N g dry matter
	In situ nitrate pools	NO3-A	µg-N g dry matter
	Net N mineralization	MinA	µg-N per g-C per d
	N mineralization rates$	MRA	‰ of total N mineralized after 28 days
	Net nitrification	NitA	µg-N per g-C per d
	Net autotrophic nitrification	ANA	µg-N per g-C per d
	Net heterotrophic nitrification	HNA	µg-N per g-C per d

"$" indicates that the variable was excluded from the model due to autocorrelation ($R^2 > 0.90$).

Annexe 4

Codes of each variable from the table “A quality” or “A” used in the Multiple Co-Inertia Analysis and the multiple regression analysis.

Variables	Codes	Unities
Total C$^{\$}$	TC	g per kg dry soil
Total N$^{\$}$	TN	g per kg dry soil
C/N	C/N	g per kg dry soil
Phosphorus	P	g per kg dry soil
Cation Exchange Capacity	CEC	cmol+ per kg dry soil
Proton	H^+	cmol+ per kg dry soil
Calcium	Ca	cmol+ per kg dry soil
Magnesium	Mg	cmol+ per kg dry soil
Sodium	Na	cmol+ per kg dry soil
Potassium	K	cmol+ per kg dry soil
Fer	Fe	cmol+ per kg dry soil
Manganese	Mn	cmol+ per kg dry soil
Aluminium	Al	cmol+ per kg dry soil
pH_{water}	pH_{water}	-
pH_{KCl}	pH_{KCl}	-
ΔpH	ΔpH	-

"$" indicates that the variable was excluded from the multiple regression analysis due to autocorrelation.

Annexe 5

Review of 5 references focused on Fagus sylvatica leaf quality trait variability

References	Sariyildiz and Anderson		Cortez et al.	Sariyildiz and Anderson$^{\$}$		Tyler$^{\$}$		Hobbie et al.	This present study
Year	2003		1996	2005		2005		2006	2010
Site/Forest	Blean Woods National Nature Reserve		Temperate forest	Temperate forest		Temperate forest		Common garden	Temperate forest/Eawy
Region	South East England		Southern France	South West England		South Sweden		Biadaszki, Poland	North East France
Variable	Tree species/Leaf category		Species	Soil fertility		Senescence and decomposition		Tree species	Ageing
Soil pH$_{water}$	4.2		5.6	4.1-7.1		3.2-3.6		4.3	3.8
Leaf category	Shade leaf litter	Sun leaf litter	Leaf litter	Green leaves	Leaf litter	Green leaves	Leaf litter	Leaf litter	Leaf litter
Litter quality									
Nutrients (%)									
Ca	…	…	…	0.77-0.89	0.87-1.36	0.68-0.71	0.71-0.87	1.29	0.58
Mg	…	…	…	0.17-0.25	0.13-0.25	0.16-0.20	0.09-0.12	0.11	0.09
K	…	…	…	0.65-1.63	0.17-0.26	0.57-0.83	0.19-0.25	0.42	0.33
P	…	…	…	0.11-0.18	0.06-0.17	0.11-0.13	0.07-0.09	0.14	…
Mn	…	…	…	0.07-0.23	0.05-0.14	0.08-0.09	0.18-0.20	…	0.37
Total N	1.01	0.92	0.70	2.39-2.66	1.01-1.35	…	…	0.84	
C fractions (% ash-free)									
Hemicellulose	…	-	23.7	…	…	…	…	13.8	11.5
Cellulose	35.1	29.2	32.4	25.4-29.0	28.9-32.8	…	…	23.1	20.4
Lignin	30.4	38.7	31.5	17.4-20.6	37.7-42.9	…	…	24.5	31.9
Ratio									
C/N	45.2	51.1	64.0	17.0-19.5	33.0-44.6	…	…	55.9	48.4
Lignin/N	30.1	42.3	43.3	7.07-7.87	30.4-37.3	…	…	29.7	32.9

Note: Ellipses (…) indicate that data are not available.

$^{\$}$minimal-maximal values