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ORIGINAL PAPER

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

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Abstract
• ***Introduction*** We investigated changes in humus morphology and soil potential net N mineralization pathways along a pure beech even-aged forest chronosequence, composed of four stages (15, 65, 95, and 130 years old) on Luvisol.
• ***Objectives*** To quantify the respective contributions of bacteria and fungi to soil N processes, we used acetylene and a fungicide (captan). We analyzed the data in order to highlight correlation patterns between soil N cycling and humus morphology. We sampled the OL layer (unmodified leaf fragments), the FH layer (mixture of coarse plant fragments with fine organic matter) and the A layer.
• ***Results*** Within the organic layers, N mineralization was two times higher in mature stands than in younger ones. Nitrification decreased sharply within the OL and the A layers along the chronosequence but was stable in the FH layer. The relative contributions to ammonification by bacteria and fungi remained constant along the chronosequence in both the OL and the A layers. In the FH layer, fungal contribution to ammonium production increased along the chronosequence. Nitrification was mainly autotrophic. We observed strong correlations between morphological and soil N variables.
• ***Conclusions*** Our results suggest that pure beech forest maturation promotes soil ammonium production; its efficiency is probably controlled by fungal activity. They also suggest the potential use of selected macro-morphological variables as indicators of both soil N mineralization rates and the nature of N pathways.

Keywords *Fagus sylvatica* · Soil N cycle · Humus forms · Chronosequence · Loamy acidic soil

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1 Introduction

Nitrogen (N) cycling in forest soils can be described as an internal cycle including mineralization (i.e., conversion of organic N to mineral N), nitrification, immobilization by microorganisms, root uptake, and litter turnover (Hart et al. 1994). Mineral N production has long been recognized as a central soil process to tree N supply (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).

Ammonification and nitrification processes are performed by many groups of organisms including bacteria, fungi, and archaea (De Boer and Kowalchuk 2001; 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; Hayatsu et al. 2008).

Usually, autotrophic and heterotrophic nitrification pathways were distinguished. 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) 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 forest ecosystems (Hayatsu et al. 2008), the long-term changes in soil N transformations and their relationships with humus forms along forest maturation gradients have been rarely considered (Jussy et al. 2000). Usually, studies focusing on N cycle changes with forest stand aging are based upon a broad comparison between two contrasted silvicultural phases or stand ages (Pedersen et al. 1999; Coté et al. 2000; 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 tree N requirements, microbial communities and tree-microbe interactions, either competitive and/or mutually beneficial, are likely to vary with forest maturation and to influence soil N cycles (Brais et al. 1995; Gea-Izquierdo et al. 2010).

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). Successional patterns of humus forms have often been described along empirical forest chronosequences 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 feces and thin organo-mineral soil layer) have been frequently observed under old mature stands on acidic forest soils (Ponge 2003). 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. We can hence hypothesize 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). 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, 7,200 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 was used to empirically reconstitute a forest chronosequence. Sixteen pure beech (*Fagus sylvatica* L.) stands were selected within the Eawy forest (Table 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 four 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 (Laignel et al. 1998). Soil texture was similar between stands with 13.5% of clay, 69.5% of silt, and 17% of sand. Understory vegetation was defined as a characteristic *Endymio-Fagetum* according to the phytosociological classification of Durin et al. (1967). Herbaceous species included *Melica uniflora*, *Conopodium majus*, *Carex sylvatica*, *Oxalis acetosella* and a group of ferns *Dryopteris carthusiana*, *Dryopteris dilatata*, *Athyrium filix-femina*, *and Blechnum spicant* (Aubert et al. 2004). In each stand center, a 16 m² plot was delimited.

2.2 Organic and organo-mineral horizons morphology

Macro-morphological descriptions of organic and organo-mineral layers were previously done within frames (25 × 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). We distinguished mull (mainly dysmull) and moder (hemimoder+eumoder+dysmoder) humus forms on the base of morphological characters

Table 1 Main characteristics of stands used to reconstitute the 130-year-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	1.56	13.81	16.25	18.7
Basal area (m^2)	15	21	22	17	16	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)	x/(x)	x/(x)		x	x/(x)	x/(x)		x/(x)	x	x/(x)	x/(x)
					<1 cm	<1 cm	<1 cm		>1 cm	>1 cm	>1 cm		<1 cm	>1 cm	<1 cm	<1 cm
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 1-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)

Table 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 feces	OLnMfae	Number per square meter	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
	Macrofuana feces	OLvMfae	Number per square meter	21	25
	Bleach leaves	OLvBI	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: race, 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	Percentage	30	8
	Fine oraganic matter	OFfog	Percentage	23	26
	Macrofuana feces	OFMfae	Number per square meter	2	33
	Bleach leaves	OFBI	Percentage	10	13
	Recovery	OFrec	Percentage	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
	Maximal thickness	OHmt	Centimeters	15	12
	Fine organic matter from fauna feces	OHffog	Percentage	2	22
	Vegetables 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
	Aggregate 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

(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, (2) the OF horizon consisting of a mixture of coarse plant debris with fine organic matter, (3) the OH horizon characterized by an accumulation of decomposed organic matter, and (4) the A horizon or organo-mineral horizon.

2.3 Soil layer sampling

We sampled the OL, OF, OH, and A layers in May 2007 within frames (25×25 cm) in the same three corners of the central plot. Because the OH layer was sometimes discontinuous and not sufficiently abundant, we decided to mix the OF and OH layers in a single pool called the FH layer. A total of 144 samples (3 subsamples×3 soil layers×4 silvicultural phases×4 stands for each phases) was collected. Samples were stored at 4°C for transport. 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 introducing 15 g of OL, 15 g of FH, and 35 g of A in tightly sealed 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. 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 or 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 from heterotrophic nitrification (Hynes and Knowles 1978). Heterotrophic nitrification is not affected by acetylene at low concentrations (2% *v*/*v*) (De Boer and Kowalchuk 2001). The third and last treatment consisted of combining 2% acetylene with a fungicide (treatment AF) in order to inhibit both chemolitho-autotrophic nitrification and fungal activity simultaneously.

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. Captan is a broad-spectrum contact fungicide that has little effect on soil bacteria (Bailey et al. 2002).At low concentrations (1 gkg^{-1}), captan has few effects on bacterial biomass and activity (Piotrowska-Seget et al. 2008).

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 a gas chromatograph equipped with a flame ionization detector (Girdel 30 GC, Spherosil XOB 75 column, France). 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 115 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 (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 5 g (organic material) or 10 g (A layer) of each sample was placed in beakers with K_2SO_4 (0.2 M) solution (200 or 100 ml for organic and A material, respectively) and shaken for 1 h at 100 rpm (Alef 1995). The obtained extractions were filtered through Schleicher and Schuell 0790 ½ filter papers and frozen for analyzed mineral N pools. Filters were pre-leached with 0.2 MK_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 h for A layer and 65°C for 48 h for organic material to obtain the gravimetric water content. 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 the following equations:

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

$$\text{Potential net nitrification} = [(NO_3^- - N)_f - (NO_3^- - N)_i]/T_d$$

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

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 micrograms of N per gram of C per day (Hart et al. 1994). N mineralization rates were expressed as per mille f 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×100
Fungal ammonification=(A−AF)/A×100
Autotrophic nitrification=(C−A)/C×100
Heterotrophic nitrification=A/C×100
Bacterial heterotrophic nitrification=AF/C×100
Fungal nitrification=(A−AF)/C×100

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 the values to 0% or 100% of contribution, respectively.

2.7 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 variance homogeneity were checked using the Wilk–Shapiro and the Bartlett tests, respectively. When necessary, logarithmic transformations were applied and, if normality and/or homoscedasticity conditions were still violated, 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 arcsine transformation of percentage data to correct non-normality and heterogeneity of variance. 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. 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 (R Development Core Team 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 and minimal in SP95. C-to-N ratio did not change between silvicultural phases in both the FH and the A layer. Total N was maximal in SP95 and minimal SP65 in the OL layer (Table 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 in the OL layer and in SP15 in the FH layer. Nitrate content

Table 3 C-to-N ratio and N pools within the OL, the FH and the A layers according to silvicultural phases

Horizons	C-to-N ratio			Total N (g kg^{-1})			NH_4^+(mg N kg^{-1})			NO_3^-(mg N kg^{-1})		
Silvicultural phases												
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 Turkey HSD test (P=0.05 level, n=4)

tended to be higher in SP95 whatever the soil layers (Table 3).

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 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. 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 4), even if it tended to be higher in SP15. Within both the OL layer and the A layers, potential net nitrification was positive only for SP15, 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 4).

3.3 Changes in ammonification and nitrification pathways along the chronosequence

Fungal ammonification was significantly higher than bacterial ammonification in the OL layer whatever the silvicultural phase (Fig. 1). In contrast, potential net ammonification was exclusively bacterial in SP15 and SP65 in FH layers. In SP95, we found a positive fungal ammonification that was however significantly lower than bacterial ammonification within FH layers. 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 autotrophs, fungi, or by both types of microorganisms along the chronosequence (Fig. 2). Potential net nitrification was both autotrophic and fungal in the OL layer, and almost completely autotrophic in the FH layer. Potential net nitrification was exclusively autotrophic in SP15.

3.4 Humus forms changes along the chronosequence

The first two axes of the PCA explained 43% of the total inertia of humus morphological data (Fig. 3). The first axis of the PCA (relative inertia=29.8%) opposed the SP15 with negative coordinates to the three others phases (Fig. 3a). This axis opposed OLn and macrofauna-based variables (negative scores, group 1) 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. 3c). The first axis was interpreted as the changes in humus morphology during the shift mull-moder (Fig. 3a). The second axis of the PCA (relative inertia=11.7%) opposed the SP65 in negative coordinates to SP95 and SP130 in positives coordinates (Fig. 3a). The second axis

Table 4 Potential net N mineralization pathways expressed as micrograms of N per gram of C per day 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	(20.0)	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 Turkey HSD test (P=0.05 level, n=4)

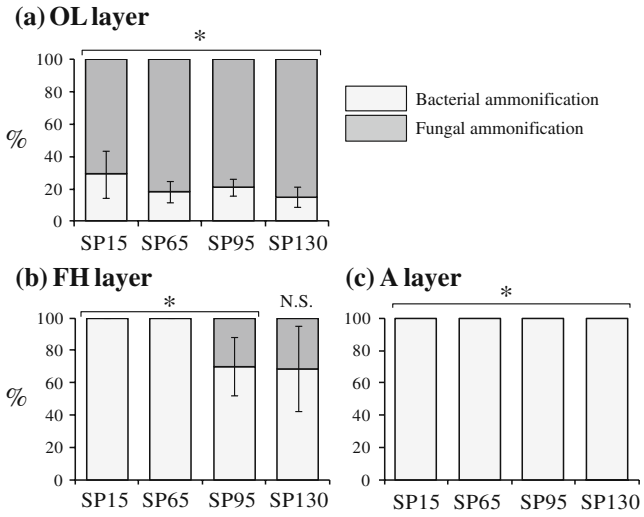

Fig. 1 Net ammonification pathways, i.e., bacterial and fungal, within the OL (**a**), the FH (**b**) and the A layers **c** expressed as percentage of contribution in total net ammonium production according to silvicultural phases. *Vertical bars* correspond to standard deviation. * indicates significant differences at $P<0.05$ between bacterial and fungal contributions while *N.S.* refers to non significant (Wilcoxon rank sum test, $n=4$)

opposed OLv-based variables (group 2 including OLvmt, OLvBl, OLvCom) against OH-based variables (group 3 including OHMt, OHmt, OHffog, OHvfog, and OHro; Fig. 3c).

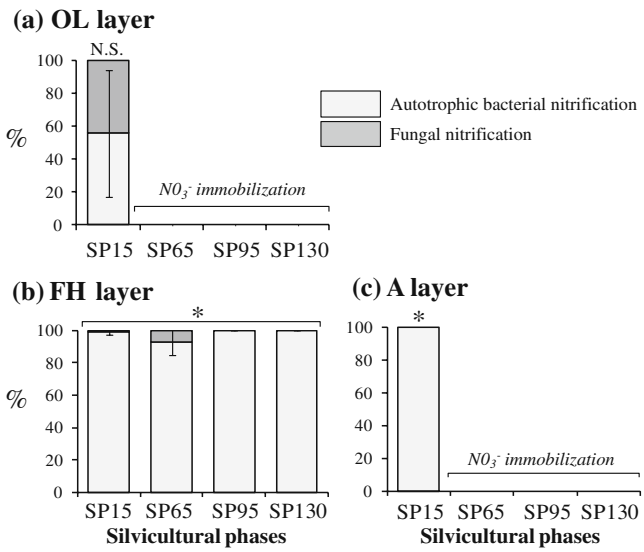

Fig. 2 Net nitrification pathways, i.e., autotrophic bacterial and fungal, within the OL (**a**), the FH (**b**) and the A layers (**c**) expressed as percentage of contribution in total net nitrate production according to silvicultural phases. *Vertical bars* correspond to standard deviation. * indicates significant differences at $P<0.05$ between bacterial and fungal contributions while *N.S.* refers to non significant (Wilcoxon rank sum test, $n=4$)

3.5 N mineralization pathways and humus forms changes

Four humus form variables were significantly correlated with N mineralization pathways (Table 5). The other macro-morphological variables were not significantly related with N variables. The percentage of leaf 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 correlated 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 5).

Each macro-morphological variable was ranked from 1 to 33 according to its R^2 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^2, i.e., "living roots in OH" for the first axis and "OLv cover" for the second one of the PCA (Table 2). Conversely, the 33th rank corresponded to the lower R^2, i.e., "OF cover" for the first axis and "A aggregate size" for the second one of the PCA (Table 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.

4 Discussion

4.1 Changes in soil N pathways along the forest chronosequence

The temporal patterns observed in our study highlight the existence of two main functional changes in mineral N production during forest aging. The first one corresponds to the gradual increase in potential net ammonification in the organic layers with stand aging. 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 nitrifica-

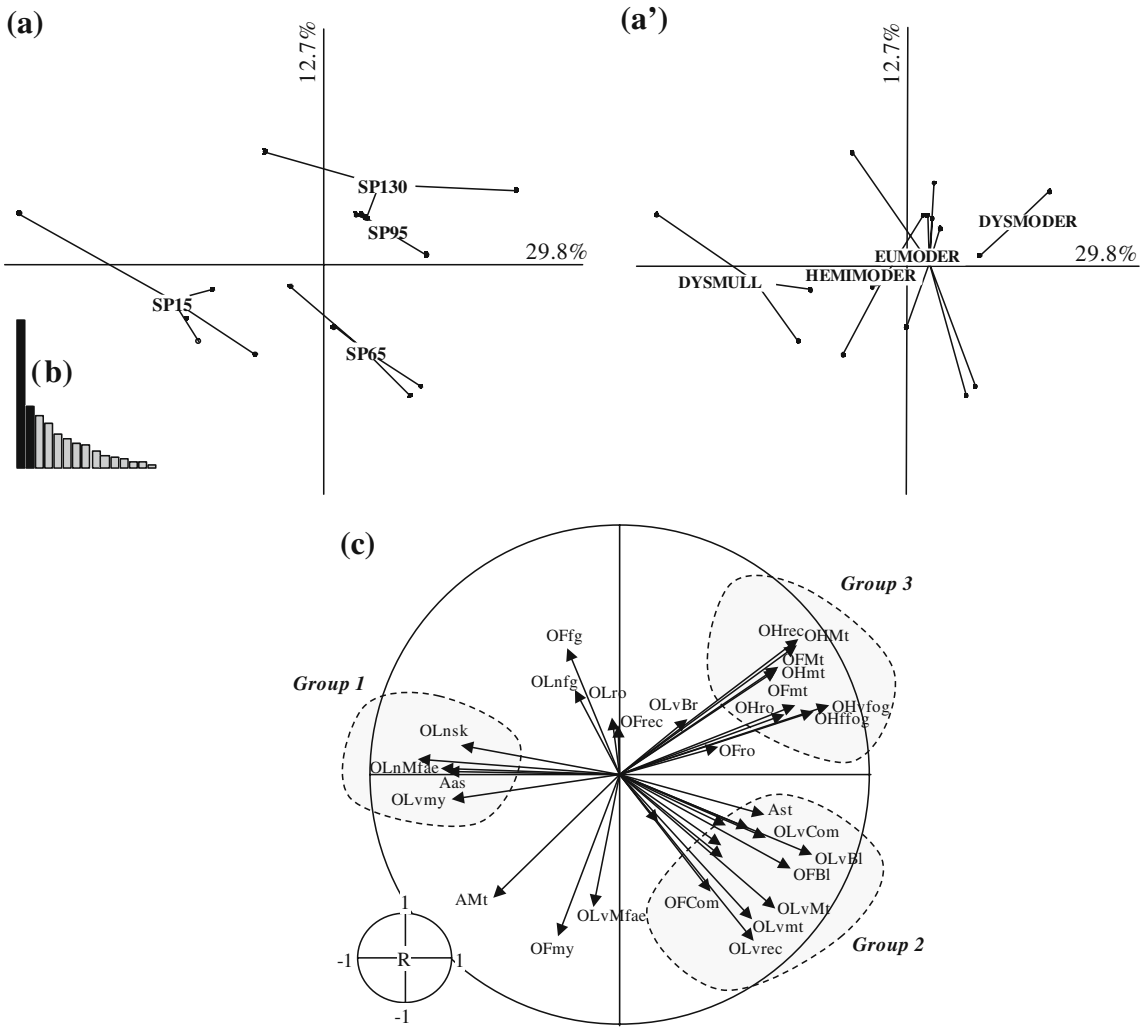

Fig. 3 Principal Component Analysis performed on humus forms data set. Age class ordination (**a**) and humus forms ordination (**a'**) represented by ellipse of dispersion, i.e., 11–18 years (SP15), 65–66 years (SP65), 91–103 years (SP95) and 121–135 years (SP130); each *black point* corresponds to a stand. Eigenvalue diagram (**b**). Correlations circle (**c**); only labels of the more relevant variables are indicated; variable codes are given in Table 2

tion along stand development was also observed by other authors (Bauhus et al. 1997; Trap et al. 2009).

We found that changes in mineral N production were clearly paired with substantial variations in the relative

Table 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 ammonification in the OL layer	OLv cover (%)	19	1	−	0.63	0.01
Fungal ammoniffication 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^2 value on the first axis of the PCA

[b] The rank of the variable according to its R^2 value on the second axis of the PCA

contribution of bacteria and fungi in soil N mineralization pathways along the chronosequence. Fungal contribution in ammonium production within the FH layer increased after 65 years of stand aging. It may be related to high production of fungal extracellular enzymes such as peroxidases or polyphenol-oxidases, that are efficient to degrade complex molecules (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 (95 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 (unpublished data), we investigated soil microbial community structure using DNA fingerprints. We found that fungal biomass was five times higher in the OL layer and five times lower in the A layer than bacterial biomass. Those results support fungal and bacterial contributions in net ammonium production.

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 Kooijman 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 five and seven) (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. In contrast to ammonification, the nature of nitrification did not seem to change significantly along the chronosequence. Indeed, 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 (1) a decrease in ammonia availability (Booth et al. 2005), (2) an inhibition of ammonia monooxigenase of autotrophic bacteria by allelopathic compounds or by competition with fungi for ammonium (De Boer and Kowalchuk 2001; Zeller et al. 2007), and (3) an decrease of autotrophic bacteria in mature stands. Conversely to what is currently acknowledged (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. 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 it variation along stand aging (Li et al. 2010) should be done in order to evaluate theses hypotheses.

4.2 Humus forms—potential indicators of soil N changes?

Macro-morphological variables discriminated clearly the four silvicultural phases and appeared robust as temporal indicators of forest functioning at the rotation scale. Among the morphological variables, the percentage of fragments in OLv appeared as an important indicator of soil N cycle, especially of autotrophic net nitrification in the FH layer. The development of the OLv layer is mainly due to high fungal activity by leaf bleaching (Lavelle and Spain 2001) but the percentage of organic fragments in this layer may be directly due to faunal activity. It is possible that the presence of macroinvertebrates favors autotrophic bacteria activity in the organic layer and thus probably locally enhances autotrophic net nitrification. Besides the percentage of fragments in OLv, OF thickness appeared as a variable potentially indicative of fungal net ammonification. It is possible that the development of fragmented litter layers in mature stands provides large pools of dissolved organic C and N from fine organic matter (Park et al. 2002). Dissolved organic matter may be mineralized under optimal conditions (high temperature and moisture) leading to higher net ammonification in the mature stands. The presence of fungi in organic layers could enhance net ammonification, probably due to the production of exoenzymes that efficiently depolymerize and mineralize organic N.

Our results finally allowed us sorting the morphological variables in two groups. The first one 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). The second group gathers morphological variables significantly correlated with soil N mineralization pathways at the rotation scale. 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 cycles.

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