Ann. For. Sci. 64 (2007) 151–158
© INRA, EDP Sciences, 2007
DOI: 10.1051/forest:2006099

Original article

Influence of tree species on gross and net N transformations in forest soils

Bernd ZELLER[a], Sylvie RECOUS[b], Morgan KUNZE[a], Judicaël MOUKOUMI[c], Micheline COLIN-BELGRAND[a], Séverine BIENAIMÉ[a], Jacques RANGER[a], Etienne DAMBRINE[a]*

[a] INRA Nancy, UR 1138 Biogéochimie des Écosystèmes Forestiers, 54280 Champenoux, France
[b] INRA, Unité d'Agronomie Laon-Reims-Mons, rue F. Christ, 02007 Laon Cedex, France
[c] LIMOS UMR 7137, CNRS-UHP Nancy I, BP 239, 54506 Vandœuvre-les-Nancy Cedex, France

(Received 23 May 2006; accepted 18 October 2006)

Abstract – We compared N fluxes in a 150-year-old *Fagus sylvatica* coppice and five adjacent 25-year-old plantations of *Fagus sylvatica*, *Picea abies*, *Quercus petraea*, *Pinus laricio* and *Pseudotsuga menziesii*. We measured net N mineralization fluxes in the upper mineral horizon (A1, 0–5 cm) for 4 weeks and gross N mineralization fluxes for two days. Gross rates were measured during the 48-h period after addition of $^{15}NH_4$ and $^{15}NO_3$. Mineralization was measured by the $^{15}NH_4$ dilution technique and gross nitrification by $^{15}NO_3$ production from the addition of $^{15}NH_4$, and by $^{15}NO_3$ dilution. Net and gross N mineralization was lower in the soil of the old coppice, than in the plantations, both on a soil weight and organic nitrogen basis. Gross nitrification was also very low. Gross nitrification measured by NO_3 dilution was slightly higher than measured by $^{15}NO_3$ production from the addition of $^{15}NH_4$. In the plantations, gross and net mineralization and nitrification from pool dilution were lowest in the spruce stand and highest in the beech and Corsican pine stands. We concluded that: (1) the low net mineralization in the soil of the old coppice was related to low gross rate of mineralization rather than to the concurrent effect of microbial immobilisation of mineral N; (2) the absence of nitrate in the old coppice was not related to the low rate of mineralization nor to the absence of nitrifyers, but most probably to the inhibition of nitrifyers in the moder humus; (3) substituting the old coppice by young stands favours nitrifyer communities; and (4) heterotrophic nitrifyers may bypass the ammonification step in these acid soils, but further research is needed to check this process and to characterize the microbial communities.

nitrogen / gross mineralization / gross nitrification / forest soils / ^{15}N techniques

Résumé – Influence de l'essence forestière sur la minéralisation brute et nette de l'azote du sol. Nous avons mesuré les flux de minéralisation nette d'azote au cours d'une incubation de quatre semaines et les flux bruts d'azote au cours d'une incubation de deux jours dans 6 sols prélevés dans une comparaison d'espèces forestières. Nous avons comparé les horizons A1 d'un taillis sous futaie (TSF) de *Fagus sylvatica* et de cinq plantations adjacentes de 25 ans de *Fagus sylvatica*, *Picea abies*, *Quercus petraea*, *Pinus laricio* et *Pseudotsuga menziesii*. Les taux bruts ont été mesurés 48 h après l'addition de $^{15}NH_4$ et $^{15}NO_3$. La minéralisation brute a été calculée à partir de la dilution de $^{15}NH_4$ et la nitrification brute à partir de la dilution de $^{15}NO_3$ mais aussi de la production de $^{15}NO_3$ à partir de l'apport de $^{15}NH_4$. La minéralisation brute et nette est la plus basse dans le TSF, exprimée par gramme de sol ou d'azote organique. La nitrification nette et brute mesurée par enrichissement en $^{15}NO_3$ est très faible, mais la nitrification brute est sensiblement plus élevée lorsqu'on l'évalue par dilution isotopique du $^{15}NO_3$. Dans les plantations, la minéralisation et la nitrification brute et nette sont plus faibles sous épicéa et plus élevées sous hêtre et pin Laricio. Nous en concluons que (1) la faible minéralisation d'azote dans le TSF est directement liée à une faible minéralisation brute et non à l'expression d'une immobilisation microbienne de l'azote minéral formé ; (2) l'absence de nitrate dans le TSF n'est pas liée à l'absence de nitrifiants mais plutôt à l'inhibition de leur activité sous le moder ; (3) la coupe rase du TSF et sa plantation entraîne une levée partielle ou totale de cette inhibition ; et (4) l'activité de nitrifiants hétérotrophes sans libération intermédiaire de NH_4 est possible dans ces sols acides. Des études plus approfondies devraient permettre de vérifier ce point et d'identifier ces populations.

azote / minéralisation brute / nitrification brute / sols forestiers / ^{15}N dilution

1. INTRODUCTION

In forest soils, mineral N is essentially provided by N deposition and soil N mineralization. Bacteria and fungi can transform organic N into ammonium (NH_4). Ammonium can be oxidized to nitrate (NO_3) by chemoautotrophic bacteria, using CO_2 as a carbon source, or by heterotrophs, using organic matter as C and N sources [7, 10]. Fungi in acid forest soils would be able to do such transformation, but the importance of this process is a matter of scientific debate. Net nitrate production in organic horizons is positively related to soil pH and negatively related to the C/N ratio [26]. But, it is very variable and poorly predicted from general soil characteristics in very acid soils with C/N ratio above 15.

Studies of soils beneath different tree species show that deciduous species tend to facilitate nitrification as compared to coniferous species [1, 31]. For herbaceous species, it has been shown that plants may positively or negatively influence nitrification, but no simple general mechanism or compound has been proposed to date, to explain this control [4, 12, 15]. The

* Corresponding author: dambrine@nancy.inra.fr

ammonium concentration is generally higher in rhizosphere than in bulk soil, but the trend for nitrate is less clear [9, 30]. Natural inhibitors of nitrification such as polyphenolic compounds have been identified in pine litter and in spruce forests [23, 24], but the influence of such compounds has not been shown for other coniferous species. Forest clear-cuts, as well as most large perturbations, generally increase the nitrate content of soils but the opposite has also been observed [13].

Net mineralization results from the balance between gross transformations of organic N into ammonium or nitrate and immobilisation of mineral N by living microorganisms. Net nitrification results from gross oxidation of ammonium and organic N minus nitrate immobilisation and denitrification. Gross mineralization can be directly quantified by measuring the dilution of an input of $^{15}NH_4$ by the $^{14}NH_4$ originating from the mineralization of organic N. Gross nitrification is quantified by measuring the formation of $^{15}NO_3$ from an input of $^{15}NH_4$ (potential gross nitrification) or by measuring the dilution of an input of $^{15}NO_3$ by nitrate originating from soil ^{14}N oxidation (actual gross nitrification). The interpretation of experimental data was originally developed by Kirkham and Bartholomew, [14] in Barraclough [2] and further improved by Mary and Recous [19], Mary et al. [20] and Müller et al. [22] using dynamic models. The difference sometimes found between gross nitrification rates measured by isotopic dilution of nitrate or by isotopic enrichment of nitrate can be explained by soil heterogeneities in the distribution of $^{15}NH_4/^{14}NH_4$ or $^{15}NO_3/^{14}NO_3$ and/or in the bacterial activity, or by the existence of heterotrophic nitrifyers, directly transforming organic N into nitrate, without the intermediate step of ammonium release in the soil [18, 22, 25]. This controversy is a matter of large scientific debate [10, 17].

The isotope dilution technique was used at the Breuil site, where different tree species were planted on the same soil after clear-cut of the original beech coppice. Preliminary measurement at this site show strong differences in nitrogen cycling between stands [27]. Nitrate is absent from soils below the old coppice, while nitrate is present in most of the plantations. The absence of nitrifyers or the microbial consumption of the nitrate formed are possible hypotheses explaining the absence of nitrate in the old coppice. The present study aims to measure gross and net fluxes in the different stands in order to understand the effects of tree species on soil N transformations.

2. MATERIALS AND METHODS

2.1. Site and sampling

The Breuil experimental site is located in the Morvan Mts, central France at 650 m. Annual rainfall is 1150 mm. Mean annual temperature is 6 °C. Annual atmospheric deposition of nitrogen in rain is 10 kg N ha^{-1} yr^{-1}. The soil is an alocrisol [29] with moder type humus and micro-podzolisation features in the upper mineral horizon. It is sandy (sand = 60%), acid (pH 4–4.7) and base saturation is below 10% (Tab. I). It is developed from the Granite de la Pierre qui Vire, locally covered by shallow, silty deposits. The original stand is an old coppice composed mainly of beech and oak [5]. In 1976, a part of the original stand located on a homogeneous soil type was clear-cut, trunk wood was harvested, stumps were mechanically extracted using bulldozers and piled with other debris (branches, humus…) in windrows. Beech (*Fagus sylvatica*), oak (*Quercus petraea*), spruce (*Picea abies*), Douglas fir (*Pseudotsuga menziesii*) and Corsican pine (*Pinus laricio*) were planted in 1976 [5]. Plantations are now between 10 to 20 m high. Density varies from 700 to 3 200 trees per ha as a result of forest management. Density is inversely related to stand development. The humus layer is a mull, with no H layer. The understory vegetation of the old coppice is sparse, dominated by acidophilus herbs such as *Deschampsia flexuosa*. Because of the high density of trees in the plantations, there is no understory vegetation, except below pine, where there are some spots dominated by *Deschampsia flexuosa*. On the 16th of January 2001, about 2 kg of the A1 horizon (0–5 cm depth) were sampled at four places in each stand; special care was taken to avoid contaminating the samples with humus or Bph horizon soil. The soil was sieved (4 mm), transferred to the laboratory, and stored in the dark at 4 °C in plastic containers, at field moisture. The litter OL layer was also sampled at the same time, dried at room temperature and milled. C and N concentrations were measured by dry combustion (Carlo Erba NC 1500).

Table I. Soil properties of the original old beech coppice (Ranger et al., 2004).

Depth (cm)	pH (H_2O)	CEC (cmolc.kg^{-1})	C (%)	N (%)	C/N	Clay (%)	Silt (%)	Sand (%)
0–5	3.8	9.2	7.4	0.39	19	18.9	20.4	60.7
5–10	4.2	6.9	4.0	0.21	20	16.1	20.0	63.9
10–15	4.5	5.1	2.3	0.17	20	14.2	20.8	65.0
15–25	4.6	4.0	2.4	0.13	19	15.5	22.5	62.0
25–40	4.5	3.0	1.5	0.08	18	15.5	23.5	61.0

Microbial biomass was measured using the fumigation-extraction technique [33]. Briefly, 12 g fresh soil was fumigated with alcohol-free chloroform over 24 h at room temperature in the dark and soluble N was extracted with 60 mL of 0.5 M K_2SO_4 [8]. The N concentration in fumigated and non-fumigated extracts was measured using continuous flow colorimetry (TRAACS, Bran and Luebbe) after mineralization of 50 mL of the extract (Kjeldahl method) followed by steam distillation. Microbial N was calculated from the difference between fumigated and non-fumigated extracts, using a correction factor $k_N = 0.48$.

2.2. Long term net mineralization and nitrification fluxes

The different soils (250 g) were put in plastic containers at 15 °C a week after sampling. In addition, in order to check if the availability of NH_4 was limiting nitrification in the old coppice, we added an ammonium sulphate solution $(NH_4)_2SO_4$ at a concentration of 50 mg N.kg^{-1} dry soil to a second soil sample from the old coppice. Soils were kept at constant temperature and field moisture for 4 weeks. Mineral nitrogen was extracted from 4 replicates of 12 g, before the incubation and after 2 and 4 weeks, by shaking 12 g soil with 60 mL of 0.5 M K_2SO_4 for 1 h. NH_4 and NO_3 concentrations were determined using continuous flow colorimetry (TRAACS, Bran and Luebbe).

2.3. Short term gross mineralization and nitrification fluxes

Soils in plastic containers were pre-incubated at 15 °C from the 2nd of April, a week before the gross mineralization experiment.

Table II. Concentration of carbon (C), nitrogen (N), C/N ratio, pH, microbial biomass N and soil moisture (H) in the humus layer (OL) and the mineral soil (0–5 cm depth) from an old coppice and in five pure stands of oak, beech, spruce, Douglas fir and Corsican pine planted after the clear-cut of the coppice in 1976. Means and standard deviation (n = 5). n.d = not determined.

Stands	Horizon	$N_{microbial}$ (mg N kg^{-1} soil)	C (%)	N (%)	C/N	pH (H_2O)	H (%)
Old coppice	O_L	n.d	50,4	1,1	46	n.d	n.d
	A	162.5 (± 5.5)	6.6 (± 0.56)	0.30 (± 0.03)	22	3.8	0.52
Oak	O_L	n.d	49,4	1,5	33	n.d	n.d
	A	119.0 (± 10.8)	3.5 (± 0.02)	0.16 (± 0.01)	22	3.8	0.38
Beech	O_L	n.d	50,0	1,5	33	n.d	n.d
	A	117.0 (± 13.0)	6.1 (± 0.06)	0.28 (± 0.01)	22	4.0	0.52
Spruce	O_L	n.d	49,8	1,6	31	n.d	n.d
	A	127.1 (± 9.8)	4.7 (± 0.13)	0.22 (± 0.01)	21	4.2	0.37
Douglas fir	O_L	n.d	48,1	2,0	24	n.d	n.d
	A	150.8 (± 20.3)	4.7 (± 0.17)	0.21 (± 0.01)	22	4.0	0.35
Corsican pine	O_L	n.d	50,7	1,1	46	n.d	n.d
	A	99.7 (± 15.6)	5.7 (± 0.25)	0.27 (± 0.01)	21	3.9	0.41

Samples of 120 g of soils from each stand were spread as a thin homogeneous layer of about 2 mm on plastic trays [20]. Soils were sprayed with 10 mL of either a 0.005 M $^{15}NH_4NO_3$ or $NH_4^{15}NO_3$ solution at 1% atom excess. These low amounts of N added were chosen in order to avoid artificially increasing the fluxes. Soils were incubated from 9th until 11th April.

In order to confirm the difference in gross fluxes between the old beech coppice and the young beech plantation, a second experiment with the same protocol was done. Only the soils of the young beech plantation and the old coppice were treated between the 23rd and 26th of April, after 3 weeks pre-incubation at 15 °C.

Mineral N (NH_4 and NO_3) was extracted immediately after spaying of the labelled solutions (n = 5 per soil), and after the incubation period (n = 5). As all extractions could not be processed the same day, soils were frozen in liquid nitrogen and stored at –20 °C before analysis. Mineral nitrogen was extracted after shaking 12 g soil with 60 mL of 0.5 M K_2SO_4 for 1 h. NH_4 and NO_3 concentrations were determined using continuous flow colorimetry (TRAACS, Bran and Luebbe). NH_4 and NO_3 were separated from 50 mL of the extract by steam distillation using MgO and Dewarda's alloy. After the extraction of mineral N, the soil recovered in the filters was extracted three times with 0.5 M K_2SO_4, then dried at 65 °C and milled. The isotopic excess of dry powders (soil extracts, soils) were measured with an elemental analyser (Carlo Erba NC 1500) coupled to a mass spectrometer (Finnigan Delta S). Results are expressed in atom % ^{15}N excess which is the percentage of ^{15}N above that of the atmosphere (0.3663%).

If the time interval is sufficiently short to allow processes to be described by zero order kinetics, the relationship between mineralization rate, isotope dilution, and variation in concentration can be expressed following Kirkham and Bartholomew [14]:

$$m = -\Delta A/\Delta t \times \ln(e_{a1}/e_{a0})/\ln(A_1/A_0) \quad (1)$$

A = exchangeable ammonium at time t_0 and t_1 (mg N kg^{-1} soil);
e_a = isotopic excess of the ammonium pool at time t_0 and t_1 (atom % ^{15}N excess);
t = time;
m = gross mineralization rate (mg N kg^{-1} soil day^{-1}).

In the same way for the nitrate compartment:

$$n = -\Delta N/\Delta t \times \ln(e_{a1}/e_{a0})/\ln(N_1/N_0) \quad (2)$$

N = nitrate at time t_0 and t_1 (mg N kg^{-1} soil);
e_a = isotopic excess of the nitrate pool at time t_0 and t_1 (at. % ^{15}N excess);
t = time;
n = gross nitrification rate (mg N kg^{-1} soil day^{-1}).

Ammonium and nitrate immobilization can be computed from the amount of ^{15}N remaining in the soil after extraction of nitrate and ammonium. It can also be computed from the difference between gross fluxes and $\Delta A = A_1 - A_0$ and $\Delta N = N_1 - N_0$.

In fact:

$$\Delta A = \text{gross mineralization} - \text{gross nitrification} - NH_4 \text{ immobilization} \quad (3)$$

$$\Delta N = \text{gross nitrification} - NO_3 \text{ immobilization.} \quad (4)$$

3. RESULTS

3.1. Soil properties

The C/N of the litter layers of the old beech coppice and Corsican pine plantation were the highest (46), and that of the Douglas fir plantation was the lowest (Tab. II). The highest contents of C and N in the A1 horizons were found in the old coppice. C and N contents decreased in the young plantations in the order: beech > Corsican pine > spruce = Douglas fir > oak. Nevertheless there was no difference between the C/N ratios of the A1 layer of all stands (21–22).

Amounts of nitrogen in the microbial biomass of the soils below Douglas fir (151 mg N kg^{-1} soil) and the old coppice (162 mg N kg^{-1} soil) were close and were higher than in the other plantations (Tab. II).

3.2. Net mineralization and nitrification

Net mineralization, calculated from the increase in mineral amounts (NH_4 + NO_3) during the 4 week incubation period was very low (0.12 mg N g^{-1} N day^{-1}) in the old coppice, and

Table III. Net N mineralization and nitrification rates in the mineral soil (0-5 cm depth) from an old coppice and in five pure stands of oak, beech, spruce, Douglas fir and Corsican pine planted after the clear-cut of the coppice in 1976. All soils were incubated during 4 weeks at 15 °C. Rates are calculated with respect to the N content of the soils. Means and standard deviation (n = 5).

Stands	Net mineralization	Net nitrification	Nitrification
	mg N g^{-1} N day^{-1}		% of mineralised N
Old coppice plantations	0.12 (± 0.006)	0.006 (± 0.001)	6
Oak	0.36 (± 0.005)	0.42 (± 0.002)	100
Beech	0.44 (± 0.010)	0.46 (± 0.013)	100
Spruce	0.43 (± 0.014)	0.08 (± 0.004)	19
Douglas fir	0.40 (± 0.007)	0.11 (± 0.002)	28
Corsican pine	0.39 (± 0.012)	0.36 (± 0.012)	94

was about 3 times higher in all plantations. No net nitrification occurred in the old coppice. Adding NH_4 to the old coppice stand increased NH_4 immobilization during the first two weeks, and mineralization during the two following weeks. But it did not induce any net nitrification (data not shown). Net nitrification was 19% and 28% of net mineralization in spruce and Douglas fir respectively, and close to net mineralization in Corsican pine, beech and oak.

3.3. Short term gross mineralization and nitrification fluxes

^{15}N remaining in the soil after mineral extraction was not detectable, because of the low isotopic excess and low input of nitrogen used. Therefore, ammonium and nitrate immobilisation could not be directly computed.

Gross mineralization, calculated after Kirkham and Bartholomew [14] from the dilution of $^{15}NH_4$ was lowest in the old coppice (0.46 mg N g^{-1} N day^{-1}). It was about four times higher than net mineralization measured over a month. Ammonium immobilisation, computed from Equation (3) was very low compared to mineralization. Gross mineralization increased in the plantations in the following order: beech (0.76 mg N g^{-1} N day^{-1}) < Corsican pine < spruce < Douglas fir < oak (4.9 mg N g^{-1} N day^{-1}).

Gross nitrification, calculated from the production of $^{15}NO_3$ after $^{15}NH_4$ addition, was very low (4% of gross mineralization) in the old coppice, but, when calculated from the dilution of $^{15}NO_3$ (Tab. III), was equal to 30% of gross mineralization. In the plantations, gross nitrification calculated from $^{15}NO_3$ production was below 3% of gross mineralization in spruce and Douglas fir, about 10% of gross mineralization in oak and Corsican pine, and 100% in beech (Tab. IV).

When calculated from the dilution of $^{15}NO_3$, gross nitrification was about 30% of net mineralization in oak and spruce, 56% to 76% in Douglas fir and beech, and exceeded 100% in Corsican pine (Tab. IV).

Gross flux measurements during the second period, after 3 weeks pre-incubation at 15 °C confirmed these first results, but gross mineralization and nitrification increased in the old coppice. In the beech plantation, fluxes were close to those measured during the first period.

Microbial consumption accounted for 30 to 90% of the mineralized N (Fig. 1). It was lowest in the old coppice and highest in the beech and pine stand whereas about 50% of the N was consumed by the microbes in the remaining stands. Almost all produced NO_3 was consumed by the microbes except in the beech plantation where microbial consumption of NO_3 was zero.

4. DISCUSSION

The topsoil in the old coppice differed from that in the young plantations. The initial old coppice soil had a moder humus and a thin Bph horizon, a sign of incipient surface podzolisation. These horizons were partly truncated and mixed during mechanical operations after logging of the stand and before plantation. These operations probably favoured organic matter mineralization [6, 36]. About two decades later, the C and N contents of soils in plantations are still lower than in the original coppice, and the former moder humus has not yet rebuilt. Since the C/N ratio of the A1 horizons has not changed, this implies an important loss/redistribution of nitrogen from the A1 horizon, probably as a result of soil mixing, leaching losses, and N immobilisation by the growing plantations.

For practical reasons, we used small additions of moderately labelled nitrogen to study gross mineralization and nitrification rates. This did not allow measurement of gross immobilisation of ^{15}N in organic matter and microbial biomass. We estimate that an enrichment of about 10 atom% ^{15}N would be needed to ensure a precise measurement of N immobilization in the bulk soil. Furthermore, these small additions of ^{15}N are probably the cause for the larger nitrification fluxes measured by $^{15}NO_3$ dilution compared to those measured by the production of $^{15}NO_3$ from $^{15}NH_4$ addition, especially when net N fluxes were low. However, gross nitrification measured by both methods in the beech plantation was close. A similar experiment, combined with the use of autotrophic nitrification inhibitors, has led Pedersen et al. [25] to suggest that heterotrophic nitrification was a main nitrification pathway in some acid forest soils. Other authors suggest that such features may come from heterogeneous distribution of native [35] or labelled NH_4 and NO_3, or heterogeneous distribution of nitrifying bacteria in the soil [35]. We cannot conclude on this point. Further experimental work as well as microbial identification should be done in order to evaluate these hypotheses, but heterotrophic nitrification may be considered in the future when assessing nitrification activities in acid forest soils. In the following, we will consider gross nitrification rates calculated from $^{15}NO_3$ dilution.

Short-term net mineralization fluxes were generally higher than long term net fluxes [3, 28]. This difference may be attributed to many factors which differed between the experiments, including the soil storage time at 4 °C, the pre-incubation period, and the soil preparation. Interpretation of these differences seems disputable. Verchot et al. [34], in a

Table IV. Net and gross nitrogen mineralization in the mineral soil (0–5 cm depth) from an old coppice and in five pure stands of oak, beech, spruce, Douglas fir and Corsican pine planted after the clear-cut of the coppice in 1976. Gross nitrogen fluxes in the soils were calculated according to Barraclough 1991. m = Gross mineralization, n = gross nitrification, N = gross nitrification from the addition of $^{15}NO_3$, ia = immobilisation of ammonium. Means and standard deviation (n = 5).

	Stands	Gross fluxes				Net fluxes	
		m	n	N	ia	m	N
		mg N kg^{-1} soil day^{-1}				mg N kg^{-1} soil day^{-1}	
Soils pre-incubated one week at 15 °C	Old coppice	1.33 (± 0.28)	0.06 (± 0.01)	0.40 (± 0.09)	0	0.98 (± 0.18)	0 (0)
	Oak	7.94 (± 0.98)	0.87 (± 0.21)	2.32 (± 0.95)	2.28	2.04 (± 0.68)	0.45 (± 0.08)
	Beech	2.05 (± 0.52)	2.10 (± 0.18)	1.57 (± 0.88)	0.11	0.28 (± 0.04)	2.02 (± 0.11)
	Spruce	6.43 (± 1.36)	0.11 (± 0.04)	2.17 (± 0.40)	1.08	3.05 (± 0.29)	0.10 (± 0.04)
	Douglas fir	8.50 (± 0.64)	0.31 (± 0.11)	4.77 (± 0.60)	0.42	2.83 (± 0.76)	0.31 (± 0.13)
	Corsican pine	4.19 (± 0.14)	0.56 (± 0.17)	5.83 (± 0.53)	0	0.65 (± 0.11)	0.47 (± 0.32)
Soils pre-incubated three weeks at 15 °C	Old coppice	2.23 (± 0.28)	0.04 (± 0.01)	1.32 (± 0.39)	0	0.70 (± 0.24)	0.11 (± 0.02)
	Beech	1.95 (± 0.48)	2.64 (± 0.22)	2.21 (± 0.49)	0.29	0.10 (± 0.02)	1.21 (± 0.23)

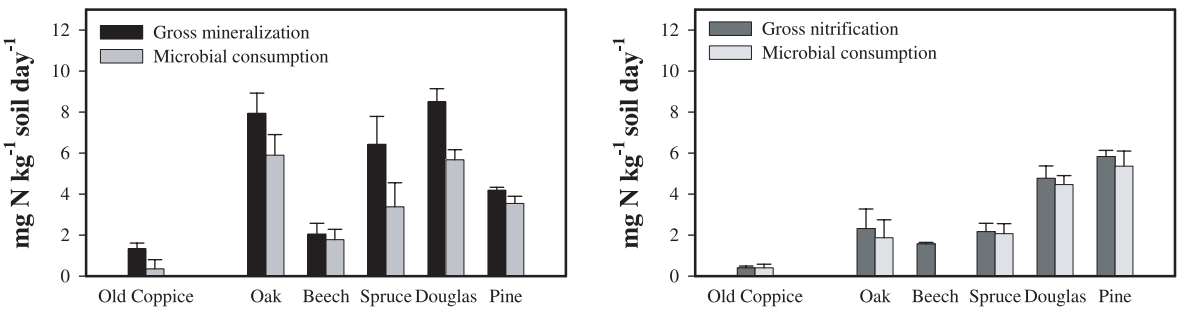

Figure 1. Gross mineralization (gross nitrification) and microbial consumption in soils from an old coppice and in five pure stands of oak, beech, spruce, Douglas fir and Corsican pine planted after the clear-cut of the coppice in 1976. Microbial consumption = gross mineralization – net mineralization. Means and standard deviation (n = 5).

comparison between net and gross fluxes measured with the same procedure in different stands in eastern New York State, also obtained diverging results from the two methods. In that study, gross rates were much lower and not systematically correlated to net rates. But the ecological differences between tree species were better predicted using net rates.

Gross and net mineralization of nitrogen was much lower in the old coppice than in the plantations. This indicates that the low net mineralization was not related to the combination of large fluxes of mineralization and immobilisation, but to an internal factor limiting mineralization. The very low gross and net nitrification rate was not limited by the ammonium availability in the soil, as shown for net fluxes by the addition experiment as well as by the second labelling experiment for gross fluxes. Nevertheless, although the net nitrification flux was zero, the gross flux of nitrification was measurable. Again, this suggests strong internal control of nitrifyer activity rather than the absence of nitrifyers. These results strongly differ from those obtained by Stark and Hart [32] in undisturbed primary forests, where net nitrification was zero (or negligible or not detected) while gross nitrification was large.

In the plantations, in comparison to the old coppice, the rates of long-term net and short- term gross mineralization and nitrification were much higher. Among the plantations, there was a strong positive relation between microbial biomass N (mg N g^{-1} N) and gross N mineralization (r^2 = 0.845).

The percent nitrification of gross and net mineralization fluxes were for both methods lower in spruce and higher in Corsican pine and beech. In oak and Douglas fir, the proportion of nitrate formed differed with the method.

High net nitrification rates in the plantations versus no net nitrification in the old coppice were confirmed by Moukoumi [21] after 4 weeks incubation of the same soils sampled in spring 2002. However he measured a much higher net nitrification rate in Douglas fir than the one measured in this study, which is consistent with the gross nitrification flux values obtained here, and with the high levels of nitrate in soil solutions measured by Ranger et al. [27] in this stand.

This author also showed that the highest rates of CO_2 respiration (mg CO_2 g^{-1} C day^{-1}; laboratory incubation) were observed in the old coppice, as well as in the spruce stand. Our interpretation is that microorganisms in these two stands mineralize organic matter with a high C/N ratio in order to satisfy their nitrogen requirements.

Reasons for this low nitrification rate in the old coppice soil versus high nitrification in the plantations are not clear, as no difference in C/N ratio or pH was detected between the incubated soils. But we analysed only bulk soils, while the changes

induced by the different tree species may affect a small, most active, part of the soil which was not identified.

Studying a network of spruce stands, Gundersen and Dise [11] showed that nitrate leaching was related to the composition of the humus (C/N) but not to that of the mineral soil. Persson et al. [26] also showed that nitrification could be predicted in humus from simple features such as pH and C/N ratio but not in mineral soils.

N mineralization in upper mineral soils of beech forest increased with increasing N content or decreasing C/N ratio [16]. The comparison between the old beech coppice and the young beech plantation shows that neither the litter itself nor root exudates play a direct role in beech stands, but rather that microbial degradation products from the humus layer are the drivers of mineralization and nitrification inhibition. The lower nitrification below spruce compared to beech, often reported in the literature [26] may be related to the relative closure of this stand, inhibition of nitrification by monoterpenes and high C/N ratio of the litter. The high nitrification measured in the Corsican pine plantation in spite of a relatively high C/N of the needle layer, could be related to the density of the understory layer linked to the very low density of the canopy. This understory layer is composed of herbaceous species such as *Deschampsia flexuosa*, which may release N-rich compounds in the soil. The higher nitrification measured in Douglas fir may be related to a relatively low C/N ratio of the litter layer.

We conclude that: (1) the low net mineralization in the soil of the old coppice is related to a low gross rate of mineralization rather than to the concurrent effect of microbial immobilization of mineral N; (2) the absence of nitrate in the old coppice is not directly related to the low rate of mineralization nor to the absence of nitrifyers, but most probably to their inhibition by microbial degradation products formed in the moder humus; (3) cutting of the old coppice and plantation of young stands favour nitrifyer communities, (4) litter composition (C/N) and degree of opening of the stand, allowing understory species to develop are likely factors favouring nitrification, and (5) the activity of heterotrophic nitrifyers bypassing the ammonium step in the soil is possible in these acid soils, but further tests should be developed in order to check this process and characterize the microbial communities.

Acknowledgements: We thank the Office National des Forêts, the GIP Ecofor and the Parc National du Morvan for funding the Breuil research site, L.H. Pardo for thinning operations and two anonymous referees for helpful suggestions.

REFERENCES

[1] Augusto L., Ranger J., Impact of tree species on soil solutions in acidic conditions, Ann. For. Sci. 58 (2001) 47–58.

[2] Barraclough D., The use of mean pool abundances to interpret ^{15}N tracer experiments. I. Theory, Plant Soil 131 (1990) 89–96.

[3] Bengtsson G., Bengtson P., Mansson K.F., Gross nitrogen mineralization, immobilization-, and nitrification rates as a function of soil C/N ratio and microbial activity, Soil Biol. Biochem. 35 (2003) 143–154.

[4] Berendse F., Effects of dominant plant species on soils during succession in nutrient-poor ecosystems, Biogeochemistry 42 (1998) 73–88.

[5] Bonneau M., Modification de la fertilité des sols sous boisement artificiels de résineux purs, Compte rendu de fin d'étude, 1977, 88 p.

[6] Bonneau M., Evolution of the mineral fertility of an acid soil during a period of ten years in the Vosges mountains (France). Impact of humus mineralization, Ann. For. Sci. 62 (2005) 253–260.

[7] Brierley E.D.R., Wood M., Shaw P.J.A., Influence of tree species and ground vegetation on nitrification in an acid forest soil, Plant Soil 229 (2001) 97–104.

[8] Chaussod R., Houot S., Guiraud G., Hetier J.M., Size and turnover of the microbial biomass in agricultural soils: laboratory and field experiments, in: Jenkinson D.S., Smith K.A. (Eds.), Nitrogen efficiency in agricultural soils, Elsevier, Amsterdam, 1987, pp. 323–338.

[9] Colin-Belgrand M., Dambrine E., Bienaimé S., Nys C., Turpault M.P., Influence of tree roots on nitrogen mineralization, Scand. J. For. Res. 18 (2003) 260–268.

[10] De Boer W., Kovalchuk G. A. Nitrification in acid soils: microorganisms and mechanisms, Soil Biol. Biochem. 33 (2001) 853–866.

[11] Gundersen P., Callesen I., de Vries W., Nitrate leaching in forest ecosystems is related to forest floor C/N ratios, Environ. Pollut. 102 (1998) 403–407.

[12] Hamilton, E.W., Frank D.A., Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass, Ecology 82 (2001) 2397–2402.

[13] Jussy J.H., Ranger J., Bienaimé S., Dambrine E., Effects of a clear-cut on the in situ nitrogen mineralisation and the nitrogen cycle in a 67-year-old Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) plantation, Ann. For. Sci. 61 (2004) 397–408.

[14] Kirkham D., Bartholemew D.W., Equations for following nutrient transformations in soil utilizing tracer data, Soil Sci. Soc. Am. Proc. 18 (1954) 33–34.

[15] Lata J.C., Durand J., Lensi R., Abbadie L., Stable coexistence of contrasted nitrification statuses in a wet tropical savannah ecosystem, Funct. Ecol. 13 (1999) 762–768.

[16] Leuschner C., On the niche breadth of *Fagus sylvatica*: soil nutrient status in 50 Central European beech stands on a broad range of bedrocks types, Ann. For. Sci. 63 (2006) 355–368.

[17] Luxhoi J., Nielsen N.E., Jensen L.S., Influence of ($^{15}NH_4^+$)-application on gross N turnover rates in soil, Soil Biol. Biochem. 35 (2003) 603–606.

[18] Matheson F.E., Nguyen M.L., Cooper A.B., Burt T.P., Short-term nitrogen transformation rates in riparian wetland soil determined with nitrogen-15, Biol. Fertil. Soils 38 (2003) 129–136.

[19] Mary B., Recous S., Measurement of nitrogen mineralisation and immobilisation fluxes in soil as a means of predicting net mineralisation, Eur. J. Agron. 3 (1994) 291–300.

[20] Mary B., Recous S., Robin D., A model for calculating nitrogen fluxes in soil using ^{15}N tracing, Soil Biol. Biochem. 30, (1998) 1–17.

[21] Moukoumi J., Munier-Lamy C., Berthelin J., Ranger J., Effect of tree species substitution on organic matter biodegradability and mineral nutrient availability in a temperate topsoil, Ann. For. Sci. 63 (2006) 763–771.

[22] Müller C., Stevens R.J., Laughlin R.J., A ^{15}N tracing model to analyse N transformations in old grassland soil, Soil Biol. Biochem. 36 (2004) 619–632.

[23] Northup R.R., Yu Z., Dahlgren R.A., Vogt K.A., Polyphenol control of nitrogen release from pine litter, Nature 377 (1995) 227–229.

[24] Paavolainen L., Kitunen V., Somlander A., Inhibition of nitrification in forest soil by monoterpenes, Plant Soil 205 (1998) 147–154.

[25] Pedersen H., Dunkin K.A., Firestone M.K., The relative importance of autotrophic and heterotrophic nitrification in a conifer forest soil as measured by ^{15}N tracer and pool dilution techniques, Biogeochemistry 44 (1999) 135–150.

[26] Persson T., Rudebeck A., Jussy J.H., Colin-Belgrand M., Priemé A., Dambrine E., Karlsson P.S., Sjoberg R.M, Soil nitrogen turnover – mineralization, nitrification and denitrification in European forest soils, in: Schulze E.D. (Ed.), Carbon and nitrogen cycling in European forest ecosystems, Ecological Studies 142, Springer, Berlin, 2000, pp. 297–331.

[27] Ranger J., Andreux F., Bienaimé S., Berthelin J., Bonnaud P., Boudot J.P., Bréchet C., Buée M., Calmet J.P., Chaussod R., Gelhaye D., Gelhaye L., Gérard F., Jaffrain J., Lejon D., Le Tacon F., Lévèque J., Maurice J.P., Merlet D., Moukoumi J., Munier-Lamy C., Nourrisson G., Pollier B, Ranjard L., Simonsson M., Turpault M.P, Vairelles D., Zeller B., Effets des substitutions d'essence sur le fonctionnement organo-minéral de l'écosystème forestier, sur les communautés microbiennes et sur la diversité des communautés fongiques mycorhiziennes et saprophytes (cas du dispositif de Breuil – Morvan), Rapport final contrat INRA-GIP Ecofor 2001–24, 2004, 202 p.

[28] Recous S., Aita, C., Mary B., In situ changes in gross N transformations in bare soil after addition of straw, Soil Biol. Biochem. 31 (1999) 119–133.

[29] Référentiel pedologique, Baize D., Girard M.C. (Eds.), Editions INRA, Paris, 1995, 332 p.

[30] Schöttelndreier M., Falkengren-Grerup U., Plant induced alteration in the rhizosphere and the utilization of soil heterogeneity, Plant and Soil 209 (1999) 297–309.

[31] Son Y., Lee I.K., Soil nitrogen mineralization in adjacent stands of larch, pine and oak in central Korea, Ann. Sci. For. 54 (1997) 1–8.

[32] Stark J.M., Hart S.C., High rates of nitrification and nitrate turnover in undisturbed coniferous forests, Nature 385 (1997) 61–64.

[33] Vance E.D., Brookes P.C., Jenkinson D.S., Microbial biomass measurements in forest soils: The use of the chloroform-fumigation-incubation method in strongly acid soils, Soil Biol. Biochem. 19 (1987) 697–702.

[34] Verchot L.V., Holmes Z., Mulon L., Groffman P.M., Lovett G.M., Gross vs. net rates of N mineralization and nitrification as indicators of functional differences between forest types, Soil Biol. Biochem. 33 (2001) 1889–1901.

[35] Wattson C.J., Travers G., Kilpatrick D.J., Laidlaw A.S., Riordan E.O., Overestimation of gross N transformation rates in grassland soils due to non-uniform exploitation of applied and native pools, Soil Biol. Biochem. 32 (2000) 2019–2030.

[36] Zerva A., Mencuccini M., Carbon stock changes in a peaty soil profile after afforestation with Sitka spruce (*Picea sitchensis*), Ann. For. Sci. 62 (2005) 873–880.

To access this journal online:
www.edpsciences.org/forest

Annex 1. Mineral N contents and atom % excess 1 h and two days after labelling of the soil with $^{15}NH_4$ or $^{15}NO_3$. The soils from the old coppice and the plantations were incubated at 15 ˚C and field moisture.

		Labelling with $^{15}NH_4$				Labelling with $^{15}N0_3$			
		$N\text{-}NH_4^+$	$N\text{-}NO_3^-$	NH_4	NO_3	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$	NH_4	NO_3
		(mg N kg^{-1})		(atom% ^{15}N excess)		(mg N kg^{-1})		(atom% ^{15}N excess)	
Old coppice	T_O = 1 h	11.77	4.23	0.2782	0.0077	11.67	4.36	0.0089	0.3645
	T_2 = 2 d	14.26	4.26	0.2264	0.0146	13.10	4.02	0.0133	0.3012
Oak	T_O = 1 h	15.07	18.92	0.1942	0.0079	19.01	18.00	0.0056	0.1296
	T_2 = 2 d	21.78	19.87	0.0808	0.0179	20.47	17.91	0.0069	0.0968
Beech	T_O = 1 h	6.03	39.47	0.3648	0.0046	6.76	39.12	0.0107	0.1197
	T_2 = 2 d	6.76	43.43	0.1929	0.0299	6.32	44.28	0.0141	0.1146
Spruce	T_O = 1 h	31.31	7.90	0.1289	0.0051	31.73	7.87	0.0030	0.2434
	T_2 = 2 d	37.67	7.86	0.0886	0.0079	37.56	8.00	0.0033	0.1395
Douglas fir	T_O = 1 h	32.53	17.99	0.1311	0.0018	37.51	19.41	0.0033	0.1513
	T_2 = 2 d	39.15	18.59	0.0815	0.0053	42.22	19.56	0.0044	0.0923
Corsican pine	T_O = 1 h	5.93	35.87	0.2505	0.0086	5.32	33.70	0.0026	0.1023
	T_2 = 2 d	7.32	35.14	0.0708	0.0126	6.53	34.60	0.0058	0.0728