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In situ gross nitrogen transformations differ between temperate deciduous and coniferous forest soils

Jeroen Staelens · Tobias Rütting · Dries Huygens · An De Schrijver · Christoph Müller · Kris Verheyen · Pascal Boeckx

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Abstract Despite long-term enhanced nitrogen (N) inputs, forests can retain considerable amounts of N. While rates of N inputs via throughfall and N leaching are increased in coniferous stands relative to deciduous stands at comparable sites, N leaching below coniferous stands is disproportionally enhanced relative to the N input. A better understanding of factors affecting N retention is needed to assess the impact of changing N deposition on N cycling and N loss of forests. Therefore, gross N transformation pathways were quantified in undisturbed well-drained sandy soils of adjacent equal-aged deciduous (pedunculate oak (*Quercus robur* L.)) and coniferous (Scots pine (*Pinus sylvestris* L.)) planted forest stands located in a region with high N deposition (north Belgium). In situ inorganic ^{15}N labelling of the mineral topsoil (0–10 cm) combined with numerical data analysis demonstrated that (i) all gross N transformations differed significantly ($p < 0.05$) between the two forest soils, (ii) gross N mineralization in the pine soil was less than half the rate in the oak soil, (iii) meaningful N immobilization was only observed for ammonium, (iv) nitrate production via oxidation of organic N occurred three times faster in the pine soil while ammonium oxidation was similar in both soils, and (v) dissimilatory nitrate reduction to ammonium was detected in both soils but was higher in the oak soil. We conclude that the higher gross nitrification (including oxidation of organic N) in the pine soil compared to the oak soil, combined with negligible nitrate immobilization, is in line with the observed higher nitrate leaching under the pine forest.

Keywords Forest type · Mineralization · Nitrification · ^{15}N · Nutrient cycling · Tracing model

J. Staelens (✉) · D. Huygens · P. Boeckx
Laboratory of Applied Physical Chemistry, ISOFYS, Ghent University, Coupure 653, 9000 Ghent, Belgium
e-mail: jeroen.staelens@ugent.be

J. Staelens · A. De Schrijver · K. Verheyen
Laboratory of Forestry, Ghent University, Geraardsbergsesteenweg 267, 9090 Gontrode, Belgium

T. Rütting
Department of Plant and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden

D. Huygens
Instituto de Ingeniería Agraria y Suelos, Universidad Austral de Chile, Casilla 567, Valdivia, Chile

C. Müller
Department of Plant Ecology, Justus-Liebig-University Giessen, Giessen, Germany

C. Müller
School of Biology and Environmental Science, University College Dublin, Dublin, Ireland

Introduction

Knowledge of the factors that determine nitrogen (N) retention in forest soils is crucial to assess the

impact of changing climate, anthropogenic N deposition and other perturbations on future N cycling and N loss of forests. For broadleaved deciduous and evergreen coniferous forests at comparable sites, a meta-analysis of 22 mainly European field studies showed that both atmospheric inorganic N deposition to the forest floor as well as inorganic N leaching below the rooting zone were significantly higher in coniferous forests (De Schrijver et al. 2007). The inorganic N deposition was on average 85% higher (95% confidence interval 64–107%) in coniferous forests than in adjacent deciduous forests, possibly related to the evergreen character, different vegetation structure and narrow needles of coniferous forests. Leaching of inorganic N, mainly occurring as nitrate (NO_3^-), was on average ten times higher (confidence interval 4–13) under coniferous forests (De Schrijver et al. 2007). Hence, N leaching beneath coniferous forests was disproportionally higher than expected from the increased N inputs, suggesting lower N retention in coniferous ecosystems than in deciduous ecosystems on comparable sites.

There is much interest in the conversion of coniferous forest plantations to forests with native broadleaved deciduous trees on sites where the latter would have dominated naturally (Spiecker et al. 2004; Van Herzele and Van Gossum 2009). In our study region (Flanders, northern Belgium), 30% of the forest cover consists of homogeneous plantations of Corsican pine (*Pinus nigra* subsp. *laricio* (Poiret) Maire), an exotic species, or Scots pine (*Pinus sylvestris* L.), which was reintroduced in the seventeenth century (De Schrijver et al. 2009). These forests, which are mostly located on sandy podzols with low buffering capacity, receive atmospheric N and sulphur (S) deposition inputs that exceed critical loads for acidification and eutrophication (Craenen et al. 2000). In addition to sylvicultural and ecological arguments for converting these coniferous plantations into forests with native deciduous tree species, environmental benefits with respect to soil and water protection have been demonstrated (von Wilpert et al. 2000; De Schrijver et al. 2007).

Nitrogen cycling in forest soils can vary among the dominant tree species (Christenson et al. 2009; Lovett et al. 2004; Yan et al. 2008; Zeller et al. 2007) because tree species affect physicochemical and biological characteristics of soils (Binkley and Giardina 1998). Soil microbial biomass, activity, and community structure can thus be tree species dependent (Hackl et al. 2005; Priha et al. 2001). With the exception of chemoautotrophic nitrifiers, most soil N transformations are carried out by heterotrophic microorganisms that depend on the supply of available organic carbon (C) (Hodge et al. 2000). Therefore, soil organic matter (SOM) is an influential factor for soil N dynamics (Priha et al. 2001). The quality and quantity of SOM in forests largely depends on the tree species composition due to differences in litterfall, root turnover, and root exudates (Brant et al. 2006). Compared to deciduous trees, litter of coniferous trees often contains a lower amount of nutrients and a higher proportion of recalcitrant compounds (Reich et al. 2005) and releases more organic acids (Howard and Howard 1990). Accordingly, soils developed under coniferous trees often have a higher C to N ratio and a lower pH compared to deciduous forest soils (Augusto et al. 2002).

Tree species effects on net soil N transformation rates have been reported more often (Yan et al. 2008; Zhong and Makeshin 2004) than effects on gross N rates (Christenson et al. 2009). However, a better understanding of N cycling in forest soils requires the quantification of gross N rates, since high gross ammonium (NH_4^+) and NO_3^- turnover rates have been found in forest soils with low or negative net mineralization and nitrification (Stark and Hart 1997; Verchot et al. 2001). Gross soil N dynamics are typically determined by ^{15}N pool dilution experiments, which often employ mixed and/or sieved soils. However, soil mixing has been reported to promote gross N mineralization and NH_4^+ consumption and to increase net nitrification by suppressing NO_3^- consumption (Booth et al. 2006). Moreover, when N transformations are assessed in intact cores, incubations often occur after cold storage and pre-incubation, which can change soils to such an extent that N cycling rates do not reflect field conditions (Arnold et al. 2008). Even if intact cores are studied under field conditions, roots are generally excised by inserting cylinders before ^{15}N addition (Frank and Groffman 2009). Total gross N production and consumption rates are calculated most commonly with analytical equations based on incubation periods of 1–2 days, assuming that remineralization of added ^{15}N is negligible (Hart et al. 1994).

Here we present an experimental approach with minimal soil disturbance that is commonly applied to

examine soil dynamics of amino acids (e.g. Andresen et al. 2008), but that to our knowledge has not been reported for examining gross N transformation rates. In this approach, after ^{15}N addition, soils are disrupted at sampling only, so that soil temperature, water and gas exchange, as well as plant root and mycorrhizal activity remain under field conditions during the experiment. Furthermore, a numerical data analysis based on a ^{15}N tracing model and a Monte Carlo optimization technique (Müller et al. 2007) was used to quantify process-related gross N transformation rates over 8 days. The advantage of numerical methods is that several simultaneously occurring gross N transformations can be estimated (Myrold and Tiedje 1986; Mary et al. 1998), at least when model parameters are optimized using a robust technique (Müller et al. 2007), while analytical equations quantify only total gross production and consumption rates of the labelled N pools (Schimel 1996). It has been shown that the applied numerical method can reliably quantify gross N transformation rates in soils of various grassland and forest ecosystems (Huygens et al. 2007; Laughlin et al. 2009; Müller et al. 2009; Rütting et al. 2010).

It is still not clear why N leaching losses in coniferous forests are disproportionally enhanced relative to the input compared to adjacent deciduous forests. The hypothesis tested in the present study is that gross N transformation rates differ between soils developed under adjacent deciduous and coniferous forests exposed to long-term atmospheric N deposition. The specific aims were (i) to examine gross N transformation rates in undisturbed well-drained sandy forest soils by in situ ^{15}N labelling and a numerical ^{15}N tracing model, and (ii) to compare gross rates between soils of adjacent deciduous and coniferous forest stands with a similar land use history.

Materials and methods

Study site

The study site is located in the nature reserve 'Heidebos' in northern Belgium (Wachtebeke-Moerbeke) (51°11′N, 3°55′E, 11 m a.s.l.). The mean (1980–2007) annual precipitation is 873 mm year^{-1} and the mean annual temperature is 10.4°C (Royal Meteorological Institute of Belgium). Adjacent monospecific forest stands of pedunculate oak (*Quercus robur* L.) and Scots pine (*P. sylvestris* L.) were selected with the same soil type, stand history, and tree age, and a flat surface area without gullies. The area has been continuously forested since at least 1775. In 1830, the study stands were registered as coppice wood, indicating the presence of deciduous tree species. All trees were harvested during WW I, after which Scots pine was planted in both stands. Harvesting occurred again during WW II, and in 1947 the current tree species were planted. In 2007, tree density was similar in both stands (823 oaks ha^{-1} and 920 pines ha^{-1}). However, basal area was more than twice as large in the pine stand (42 m^2 ha^{-1}) as in the oak stand (20 m^2 ha^{-1}) due to the higher mean diameter at breast height of pines (23.4 cm) compared to oaks (17.1 cm). Mean tree height was 15.6 and 18.2 m for oaks and pines, respectively. Ground vegetation covers the soil completely during summer, and consists in the oak stand of bracken (*Pteridium aquilinum*) while in the pine stand both bracken and broad buckler fern (*Dryopteris dilatata*) occur.

The soil in both stands was a well-drained acid podzol (sandy texture) (Table 1) with a groundwater level below 1 m depth. The characteristics of the mineral topsoil differed significantly between the two stands. Moisture content, pH(KCl), and total C and N contents were higher ($p < 0.001$) in the topsoil of the oak stand than in the pine stand. Soil C:N ratio and bulk density were higher ($p < 0.01$) beneath pine. The amount of exchangeable cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) was higher ($p = 0.005$) in the oak soil. Extractable (1 M KCl) inorganic N was higher ($p < 0.001$) in the pine soil. Compared to oak, the NH_4–N concentration in the pine soil was twice as high while the NO_3–N concentration was 17 times higher. The field experiment was carried out from 6 to 14 June 2007, which was a dry period (only 3.7 mm of rainfall; Royal Meteorological Institute of Belgium). According to the classification of Jabiol et al. (1995) the organic layer was characterized as hemimoder in the oak stand and as mor in the pine stand. The mineral topsoil under oak was covered by a thin (2–4 cm) fermentation (F) layer with abundant fine roots, while the humified (H) layer was generally absent. Under pine, a thicker (6–9 cm) F layer was followed by a discontinuous H layer (1 cm) that was clearly separated from the mineral layer. Mean soil temperature (Eijkelkamp; e+Soil MCT sensor) at

Table 1 Properties (mean and SE) of the mineral topsoil (0–10 cm) of adjacent *Q. robur* and *P. sylvestris* forest stands

	Q. robur		*P. sylvestris*		*p* value
	Mean	SE	Mean	SE	
Moisture content (g g^{-1})	0.19	0.01	0.14	0.01	<0.001
Bulk density (g cm^{-3})	1.10	0.03	1.19	0.03	0.007
pH-KCl	3.28	0.02	3.12	0.02	<0.001
Total C (g kg^{-1})	26.6	0.7	21.5	0.9	<0.001
Total N (g kg^{-1})	1.36	0.04	1.03	0.04	<0.001
C:N	19.6	0.1	20.9	0.1	<0.001
Exchangeable Na^+, K^+, Ca^{2+}, and Mg^{2+} (cmol kg^{-1})	0.86	0.09	0.49	0.04	0.005
Extractable initial NH_4^+ (mg N kg^{-1})	1.32	0.11	2.66	0.28	<0.001
Extractable initial NO_3^- (mg N kg^{-1})	0.23	0.07	3.96	0.27	<0.001

p values of independent samples *t* tests, performed after evaluating the equality of variances with Levene tests (SPSS 15.0 for Windows, SPSS Inc.). Moisture content was determined by weighing fresh soil samples before and after oven-drying (48 h, 65°C). Bulk density was determined by weighing oven-dry soil in cylinders of known volume. Exchangeable Na^+, K^+, Ca^{2+}, and Mg^{2+} were determined by analyzing soil $BaCl_2$ extracts using atomic absorption spectrophotometry (Varian SpectrAA-220, USA). Total C and N, pH-KCl, and extractable initial NH_4^+ and NO_3^- (before ^{15}N addition) were determined as described in "Materials and methods". Bulk density and moisture content were measured using 50 samples per stand that were pooled to 10 samples for analysing pH-KCl, C, and N. Exchangeable cations, NH_4^+, and NO_3^- were determined using 15 samples per stand pooled to 5 chemical replicates

5 cm depth in the mineral soil was the same (16°C) in both stands during the study period.

The forest floor in both stands was exposed to a relatively high inorganic N input from atmospheric deposition, which was nearly twice as high in the pine stand as in the oak stand. Summed annual throughfall of NH_4^+ and NO_3^- (7 December 2007 to 3 December 2008; 15 collectors per stand) differed significantly ($p < 0.001$; *t* test) between oak (18.2 ± 0.8 kg N ha^{-1} $year^{-1}$; mean ± standard error (SE)) and pine (33.4 ± 1.6 kg N ha^{-1} $year^{-1}$), while open-field precipitation deposition next to the stands was 11.3 kg N ha^{-1} $year^{-1}$ (unpublished results). The N input was partly reflected in the N leaching beneath the pine stand, as indicated by the concentration of the soil solution sampled with suction cup lysimeters under the main rooting zone (12 lysimeters per stand; 60 cm depth). The annual average volume-weighted NO_3^- concentration was 18.6 ± 4.6 mg NO_3–N L^{-1} under the pine stand, which was eight times higher than under oak (2.3 ± 0.9 mg NO_3–N L^{-1}) ($p < 0.01$; unpublished results).

Plot installation and N addition

In both forest stands, five 1.5 × 1.0 m^2 plots were selected with an intermediate distance of 20 m, located more than 40 m from the forest edge (Fig. 1). In each of these ten plots, ferns were cut 1 week before the N addition. Four days later, within each plot ten soil locations of about 10 × 10 cm^2 were prepared, positioned in two rows of five locations with a distance of 20 cm within rows and 60 cm between the rows (Fig. 1). Per location, the fresh litter layer (L) and the fermentation and humified (FH) layer were carefully removed, each

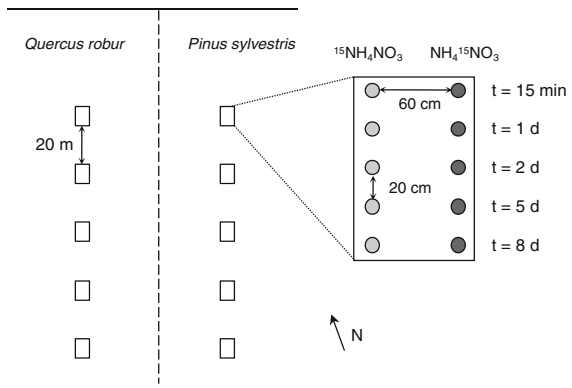

Fig. 1 Schematic overview of the study design in adjacent *Q. robur* and *P. sylvestris* forest stands in the Heidebos nature reserve, Belgium. The rectangles show the five experimental plots per stand. In each plot, the circles indicate the five locations per ^{15}N treatment where the mineral topsoil was injected at t_0 with solutions containing $^{15}NH_4NO_3$ or $NH_4{}^{15}NO_3$ (*t* = time of soil sampling)

place was marked out by four bamboo sticks, and a nylon mesh (1 mm mesh size) was put on top of the mineral soil layer before putting back the FH and L layers. The mesh allowed distinguishing the top of the mineral layer in the following injection and soil sampling steps.

After temporarily removing the FH and L layers, a 10 × 10 cm^2 template with four corner holes and seven central holes was slid over the bamboo sticks in order to apply the ^{15}N enriched solutions. Two ^{15}N treatments were applied to five 'virtual' soil cores (cylinders of 5.8 cm diameter and 10 cm high). Each treatment consisted of a water solution with NH_4^+ (0.188 mg NH_4–N L^{-1}) and NO_3^- (0.103 mg NO_3–N L^{-1}) in which one of the N moieties was labelled with ^{15}N at 99 at.% excess. The N was applied as NH_4Cl and $NaNO_3$. To assure an even distribution of the applied N in the mineral topsoil (0–10 cm), the solutions were injected through the seven equally distributed template holes using a 1-mL syringe and a 9-cm spinal needle that was inserted until 9 cm depth and pulled up during the injection. This approach is similar as described by Hart et al. (1994) for laboratory incubations. Seven millilitres of solution was injected in each 5.8-cm diameter soil core that contained on average 284 g dry soil in the oak stand and 319 g dry soil in the pine stand, which resulted in 4.65 and 4.13 μg ^{15}N–NH_4^+ per g dry soil as well as 2.55 and 2.26 μg ^{15}N–NO_3^- per g dry soil in the oak and pine stand, respectively. First, eight of the ten 'virtual core' locations in each plot were injected over a period of approximately 3.5 h in order to assess subsequently the N dynamics by sampling after 1, 2, 5, and 8 days (Fig. 1). The ^{15}N treatments were applied concurrently, after which the locations were immediately covered with the nylon mesh, FH and L layers. When finished, ^{15}N was injected into the two remaining locations per plot, leaving a 10-min time lag between the plots. These two locations were sampled 15 min after the ^{15}N injection.

Soil sampling and analysis

Before soil sampling, the L and FH layers and the nylon mesh were removed and another 10 × 10 cm^2 template with four corner holes and a central 6-cm diameter hole was placed over the bamboo sticks. This allowed sampling the ^{15}N labelled mineral soil with a PVC tube (5.8 cm inner diameter) that was pushed 10 cm into the soil. In the rare case of too heavy resistance to the sampling, thick roots were cut with a knife. The 0–10 cm mineral soil was sampled 15 min, 1, 2, 5, and 8 days after injection. After each sampling occasion, the 20 soil cores (2 stands × 5 plots × 2 treatments; Fig. 1) were left in their PVC tubes, packed in plastic bags and transported vertically to the laboratory to be processed, so that extraction as described below occurred 3.4, 31, 52, 124, and 195 h after ^{15}N injection. Care was taken to keep the same time lag between soil injection, sampling, and processing (within 3.5 h after the sampling) for each of the five spatial replicates per stand, treatment, and time step. In the laboratory, the fresh soil samples were weighed, sieved at 2 mm, separated from the roots, and split into two subsamples for further analysis. One week before the start of the experiment, three additional soil cores were sampled in each of the five plots per stand to determine the initial pool size and natural ^{15}N abundance of total soil N and extractable NH_4^+ and NO_3^-. The three samples per plot were mixed and analyzed in the same way as the ^{15}N enriched samples.

A first subsample of 40 g sieved fresh soil was dried at 65°C for 24 h to determine the gravimetric water content, and thereafter ground by a planetary ball mill (PM400, Retsch, Germany) for total N and ^{15}N analysis by an elemental analyzer (EA) (ANCA-SL, SerCon, UK) coupled to an isotope ratio mass spectrometer (IRMS) (20–20, SerCon, UK). These samples were then pooled to composite samples ($n = 5$) for measuring soil pH-KCl (1:5 ratio) and total C content (EA-IRMS). The roots were thoroughly rinsed in distilled water to remove adhering soil, dried at 65°C for 24 h, weighed, and grinded (Retsch ZM200) for N and ^{15}N analysis by EA-IRMS. A second subsample of 100 g sieved fresh soil of each individual core was extracted with 200 mL 1 M KCl, shaken for 1 h (150 rpm), and filtered (Schleicher & Schuell MicroScience 598½) prior to the analysis of N concentrations and ^{15}N contents of NH_4^+ and NO_3^-. Ammonium was determined colorimetrically by the salycilate-nitroprusside method (Mulvaney 1996) on an auto-analyzer (AA3, Bran & Luebbe, Germany). Nitrate was determined colorimetrically using the same auto-analyzer after reduction of NO_3^- to nitrite (NO_2^-) in a Cu-Cd column followed by the reaction of NO_2^- with

N-1-napthylethylenediamine to produce a chromophore. The ^{15}N contents of NH_4^+ and NO_3^- were analyzed after conversion to N_2O using a trace gas preparation unit (ANCA-TGII, SerCon, UK) coupled to an IRMS (20–20, SerCon, UK). Ammonium was converted by adding MgO to soil extracts and absorbing NH_3 into H_2SO_4, after which N_2O was produced by reaction with NaOBr (Hauck 1982; Saghir et al. 1993). Nitrate was reduced by Cd–Cu at pH 4.7 to produce nitrite and NH_2OH as intermediates of N_2O (Stevens and Laughlin 1994). Oak soil extracts with too low inorganic N concentrations for accurate conversion were spiked by adding NH_4^+ or NO_3^- solutions at natural ^{15}N abundance.

Calculations, data analysis, and ^{15}N tracing model

Total ^{15}N in the sampled soil cores was calculated using the ^{15}N measurements of total soil N and roots separated during soil sieving. To determine ^{15}N recovery, the initial amount of ^{15}N in the soil-root system was calculated based on the natural ^{15}N abundance of soil and roots and the added ^{15}N. The pool size and N isotope composition of soil organic N was calculated from the difference between total soil N and the sum of KCl-extractable NH_4^+ and NO_3.

Simultaneously occurring gross N transformation rates were quantified by a numerical ^{15}N tracing model via a Markov chain Monte Carlo Metropolis algorithm (Müller et al. 2007). This robust optimization technique is particularly useful for analyzing data with complex models and a large number of parameters (Rütting and Müller 2007, 2008). The model that was the starting point of the analysis consisted of five N pools connected by 12 N transformations. The N pools were ammonium (NH_4^+), nitrate (NO_3^-), organic N (N_{org}), and pools related to adsorption of NH_4^+ (NH_{4ads}^+) and storage of NO_3^- (NO_{3sto}^-) (Müller et al. 2004). These inorganic N storage pools were included since fast adsorption and desorption reactions of inorganic N commonly occur in natural soils (Roing et al. 2006; Russow et al. 2008). The N transformations could either be described by zero-order, first-order or Michaelis–Menten kinetics (Müller et al. 2007). The optimization was guided by minimizing a misfit function in the form of a quadratic weighted error between modelled and measured data for soil NH_4^+, NO_3^-, and total N (i.e. the size and ^{15}N content per pool and ^{15}N treatment). The optimization resulted in a probability density function for each model parameter, from which average parameter values and standard deviations (SD) were calculated (Müller et al. 2007). These parameter values were then used to calculate gross N rates.

In the present study, fluxes of NH_4^+ and NO_3^- were added to the tracing model (L_{NH_4} and L_{NO_3}; Fig. 2) to account for injected ^{15}N that was not recovered in inorganic and organic N pools of the sampled soil cores (Fig. 3). Loss of ^{15}N may have occurred via N leaching, gas emission, uptake and transport by roots and mycorrhizae, and diffusion to non-sampled soil. The current experiment did not allow distinguishing between these processes. The ^{15}N amount in the separated roots was not explicitly included in the model because of its negligible contribution to total ^{15}N in the soil-root system (<2%; Fig. 3). However, inorganic N uptake by roots was accounted for by the NH_4^+ and NO_3^- loss fluxes (Fig. 2). Possible root efflux of reduced ^{15}N compounds due to root turnover and exudation (Frank and Groffman 2009) was assumed to be negligible compared to the rate of other

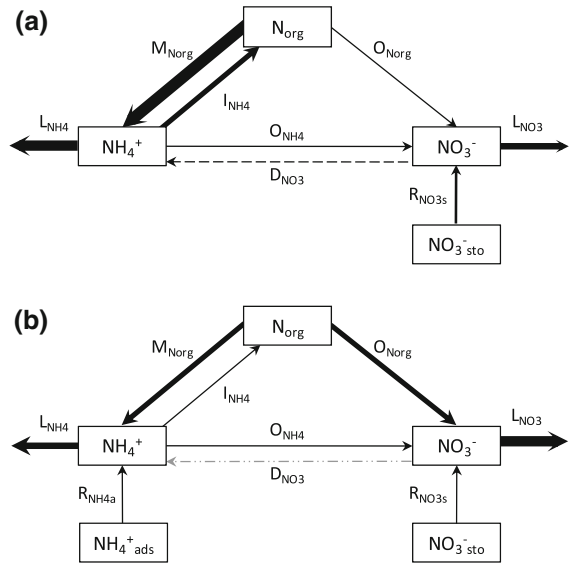

Fig. 2 Conceptual ^{15}N tracing model (model 4 in Table 4) of gross N transformations in the mineral topsoil of adjacent **a** *Q. robur* and **b** *P. sylvestris* forest stands. *Blocks* represent soil N pools (N_{org} = soil organic N, NH_4^+ = ammonium, NO_3^- = nitrate, NH_{4ads}^+ = adsorbed NH_4^+, NO_{3sto}^- = stored NO_3^-). The thickness of the *arrows* represents the relative importance of each flux (see Table 2 for a description and rates of the N transformations). *Dashed lines* indicate smaller fluxes than *full lines*

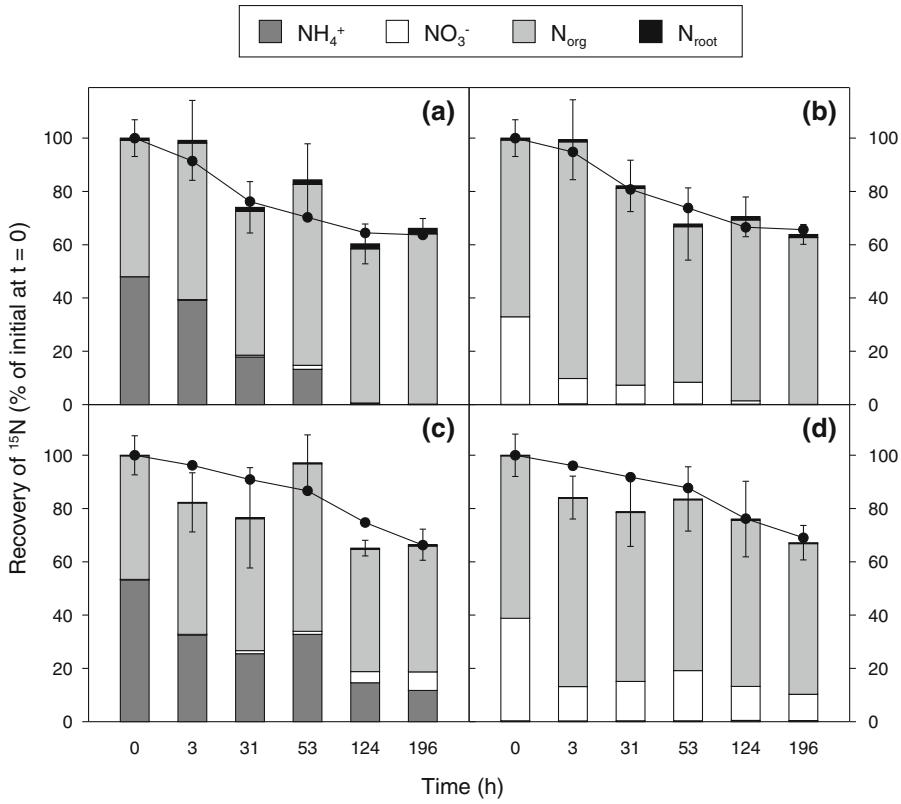

Fig. 3 Measured (*bars*) and modelled (*symbols*) recovery of ^{15}N (as a percentage of initial ^{15}N at $t = 0$) in the mineral topsoil of adjacent *Q. robur* (**a**, **b**) and *P. sylvestris* (**c**, **d**) forest stands after in situ application of $^{15}NH_4NO_3$ (**a**, **c**) or $NH_4{}^{15}NO_3$ (**b**, **d**). *Error bars* show the SE ($n = 5$) of total soil ^{15}N recovery based on the measured variation between replicates of the pool size and ^{15}N at.% of total soil N. Recovery of ^{15}N in total soil N, root N, NH_4^+, and NO_3^- is the mean of measured replicates ($n = 5$) per time step and ^{15}N treatment, while the recovery in organic N is calculated as total soil N minus NH_4^+ and NO_3^-

N fluxes, and hence, not included in the model. Potential remineralization of soil organic ^{15}N, in contrast, was accounted for in the model during the entire experiment. The model analysis did not include nitrite (NO_2^-) as an N pool and hence treated nitrification as a one-step process. Using a more complex numerical ^{15}N tracing model, nitrification has been modelled as a two-step process for other forest and grassland soils (e.g., Rütting et al. 2008; Rütting and Müller 2008). These studies, in which soil dynamics of $^{15}NH_4^+$, $^{15}NO_2^-$, and $^{15}NO_3^-$ were analyzed, indicated that the rate of NO_3^- production does not differ between a one-step and a two-step approach for cases in which the soil NO_2^- pool is small compared to NO_3^-, which generally holds.

Starting from the same complete model for both soils, several modifications in kinetic settings and included N transformations were tested to identify the model which best described the measured soil N dynamics in each stand. The selection of the final model was governed by the statistical Akaike Information Criterion (AIC) (Burnham and Anderson 2002). This criterion is based on information theory and tests the relative likelihood of models by taking into account the differences in goodness-of-fit and the number of model parameters. A model with a smaller AIC is more likely to be correct and, hence, only modifications decreasing the AIC were considered for the final data analysis. Furthermore, based on differences in AIC scores we calculated the probability (Akaike's weight) that a model setup was more likely compared to the other tested setups (Burnham and Anderson 2002). Although the optimization algorithm did not require a step-wise model analysis (Müller et al. 2007), different model setups were run to examine whether simpler models could describe the measured dynamics and to assess the robustness of the obtained gross N fluxes. A basic model (Table 4; model 1) considered only three N pools (N_{org}, NH_4^+, and NO_3^-) and seven N fluxes: mineralization (M_{Norg}), NO_3^- production by oxidation of NH_4^+ (O_{NH_4}) and N_{org} (O_{Norg}), immobilization (I_{NH_4} and I_{NO_3}) and losses (L_{NH_4} and L_{NO_3}) of inorganic N. This model was extended with the possibility of temporary inorganic N adsorption or storage (model 2) and dissimilatory NO_3^- reduction to NH_4^+ (D_{NO_3}) (model 3). Finally, a model without NO_3^- immobilization ($I_{NO_3} = 0$) was tested (model

4). The measured concentrations and ^{15}N enrichments of the NH_4^+ and NO_3^- pool were used in the model as the average and SE of the five field replicates per time step, ^{15}N treatment, and soil. First model runs indicated that the ^{15}N analysis tool had difficulties to simulate the average $^{15}NO_3^-$ patterns in the pine soil when the observed SE was accounted for, likely because of the variation in SE over time. To allow a reasonable fit between the modelled and the mean observed $^{15}NO_3^-$, for the pine soil the measured average values were used with a 10% SE. The measured total soil ^{15}N was used in the model to account for the ^{15}N mass recovery and allowed to quantify the N losses during the incubation.

The optimization algorithm was programmed in MatLab 7.9 (The MathWorks Inc.) and called the ^{15}N tracing model that was separately set up in Simulink 7.4 (The MathWorks Inc.). The initial size and ^{15}N content of the NH_4^+ and NO_3^- pools were obtained by extrapolating the data for 3.4 and 31 h back to 0 h (Müller et al. 2004). Based on the final kinetic settings and model parameters, mean gross N fluxes were calculated by integrating the rates over the 8-day period divided by the total time (Rütting and Müller 2007). The mean N fluxes were compared statistically between the forest soils using the 85% confidence interval, which is equivalent to testing at a significance level of 0.05 (Payton et al. 2000; Rütting et al. 2010). Model fit was evaluated for the size and ^{15}N enrichment of soil NH_4^+, NO_3^-, and organic N. The modelled values of the latter pool were calculated from the sum of the modelled $N_{org,}$ NH_{4ads}^+, and NO_{3sto}^- pools (Fig. 2), because this corresponded to the measured organic N pool determined from the total soil N minus KCl-extractable NH_4^+ plus NO_3^-. In addition to visual inspection, model performance was quantified by the coefficient of determination (R^2) of a linear regression between measured and modelled data. The deviation of R^2 from one can be a useful indicator for the model agreement to the data (Janssen and Heuberger 1995).

Results

Nitrogen pool sizes and ^{15}N enrichments

The size of the measured inorganic N pools decreased over time in the soil of the pedunculate oak stand, but not in the Scots pine stand (Figs. 4 and 5). The extractable NH_4^+ concentration in the oak forest soil (Fig. 4a) was on average 5.5 μg N g^{-1} dry soil 3.4 h after adding $^{15}NH_4$ and declined exponentially to a stable value of ~1.4 μg N g^{-1}. Similarly, the mean extractable NO_3^- in the oak soil (Fig. 5a) declined exponentially from a mean value of 2.8 to 0.3 μg N g^{-1}. For pine, the NH_4^+ pool differed between the ^{15}N treatments in the last two time steps (Fig. 4c), despite the fact that equal N amounts were injected. The NH_4^+ pool, however, remained approximately constant (~4.5 μg N g^{-1}) when averaged over both treatments. The NO_3^- pool in the pine forest soil (Fig. 5c) decreased only slightly over time. The organic N pool remained at the same level in both soils and was higher in the oak soil (Fig. 6a, c).

The observed ^{15}N enrichment of the inorganic N pools differed between the two forest soils. After adding $^{15}NH_4^+$, the ^{15}N enrichment of the NH_4^+ pool decreased asymptotically in the oak soil (Fig. 4b), while it declined linearly and more slowly in the pine soil (Fig. 4d). The ^{15}N enrichment of NO_3^- for oak (Fig. 5b) when the NH_4^+ pool was labelled first increased to 10 at.% and then declined, in contrast to the continuous increase to 13.6 at.% for pine (Fig. 5d). After $^{15}NO_3^-$ addition, a ^{15}N enrichment of the NH_4^+ pool was observed in the oak soil, with a maximum of 0.86 at.% (Fig. 4b, inset), while the increase in the pine soil was negligible (Fig. 4d, inset). Shortly after $^{15}NO_3^-$ application the total NO_3^- pool was more enriched in the oak soil (40 at.%, Fig. 5b) compared to the pine soil (14 at.%, Fig. 5d). However, at the end of the incubation this trend was reversed. In the organic N pools, negligible changes in ^{15}N content were observed, except for a slight increase in $^{15}N_{org}$ in the oak soil after $^{15}NH_4^+$ addition (Fig. 6b).

Soil and root ^{15}N recovery and model fit

The total ^{15}N amount in the two soils decreased during the experiment (Fig. 3), with a similar pattern for both ^{15}N tracers and both forest soils, and a recovery of 64–69% at the end of the experiment. The ^{15}N in the separated roots contributed <2% to the total ^{15}N in the sampled soil-root system. Root N amounted to 18 ± 2 g N g^{-1} oak soil and 4.5 ± 0.4 g N g^{-1} pine soil. The ^{15}N content (in at.%) increased from natural abundance (0.3653 ± 0.0009) to 0.97 ± 0.09

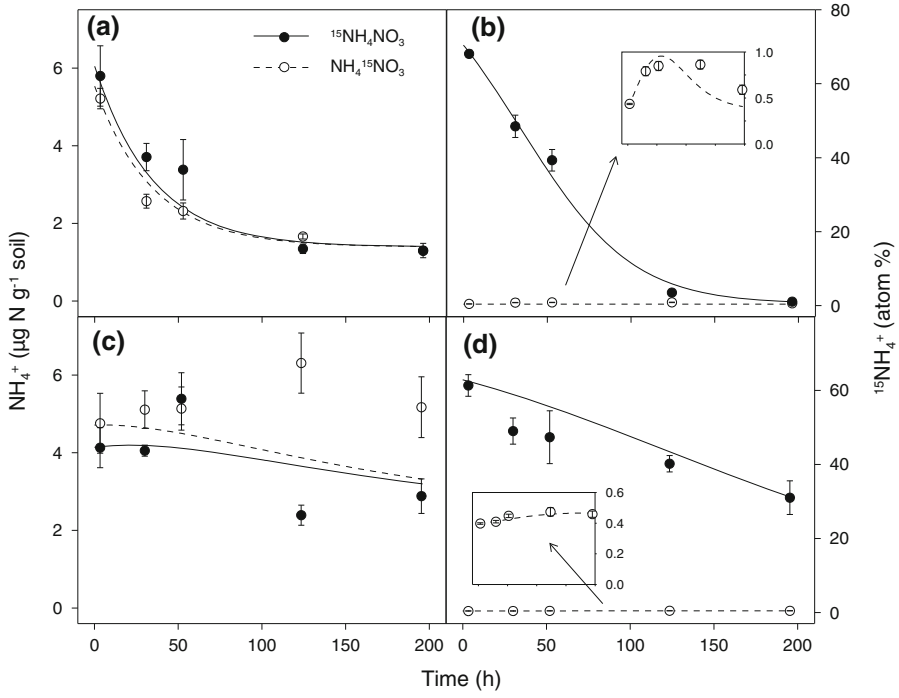

Fig. 4 Measured (*symbols*, mean ± SE) and modelled (*lines*) concentrations and ^{15}N contents of ammonium (NH_4^+) in the mineral topsoil of adjacent *Q. robur* (**a**, **b**) and *P. sylvestris* (**c**, **d**) forest stands after in situ application of $^{15}NH_4NO_3$ or $NH_4{}^{15}NO_3$

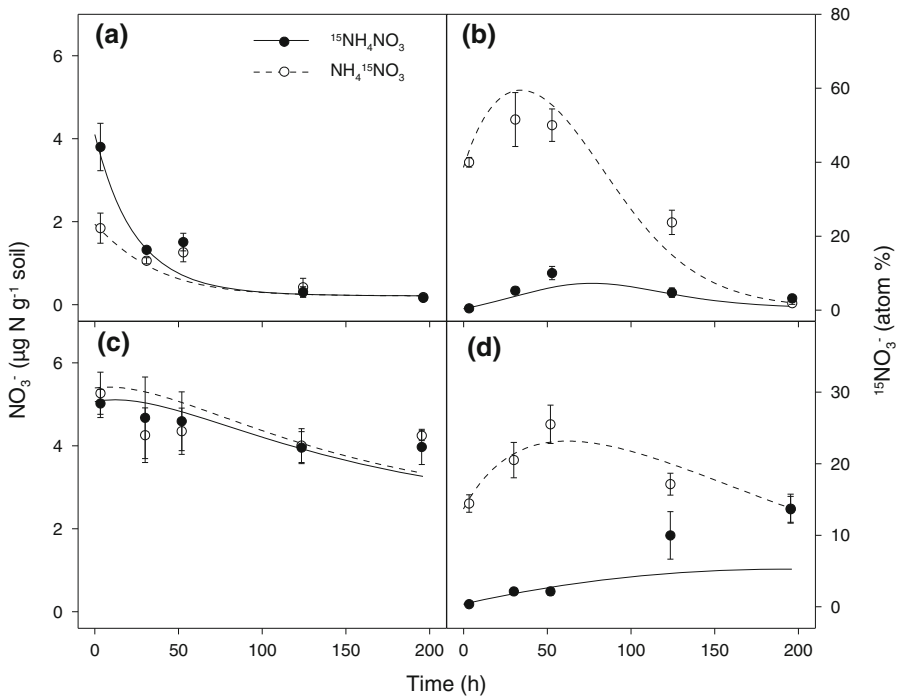

Fig. 5 Measured (*symbols*, mean ± SE) and modelled (*lines*) concentrations and ^{15}N contents of nitrate (NO_3^-) in the mineral topsoil of adjacent *Q. robur* (**a**, **b**) and *P. sylvestris* (**c**, **d**) forest stands after in situ application of $^{15}NH_4NO_3$ or $NH_4{}^{15}NO_3$

($^{15}NH_4^+$ labelling) and 0.59 ± 0.04 ($^{15}NO_3^-$ labelling) in the oak roots and to 0.77 ± 0.07 ($^{15}NH_4^+$ labelling) and 0.50 ± 0.03 ($^{15}NO_3^-$ labelling) in the pine roots after 8 days.

Different model setups were compared with respect to their fit to the measured N and ^{15}N data (Table 4) and the resulting N transformation rates (Fig. 7). This evaluation showed that most rates were

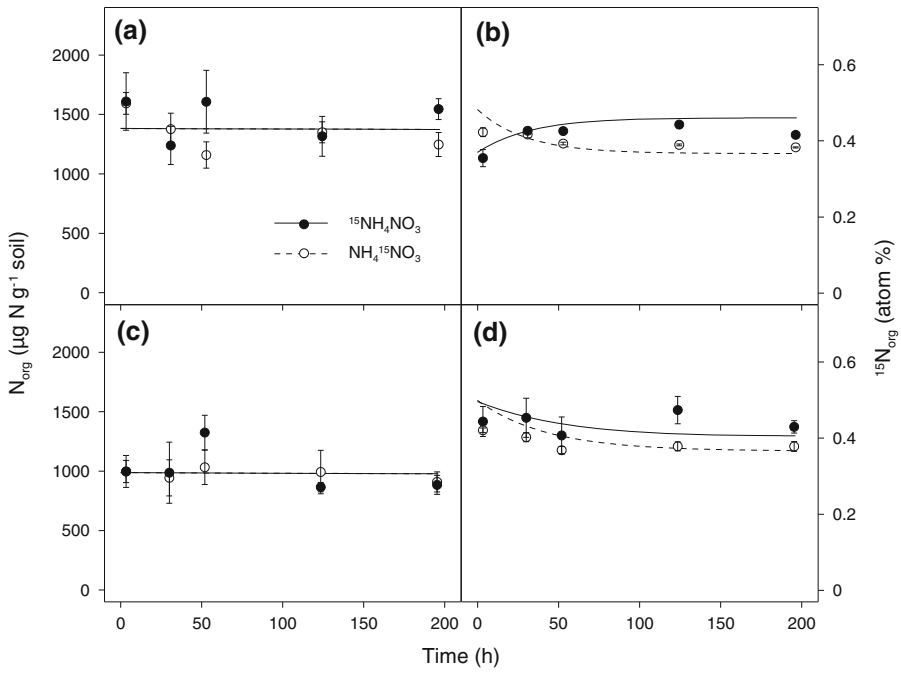

Fig. 6 Measured (*symbols*, mean ± SE) and modelled (*lines*) concentrations and ^{15}N contents of organic N (N_{org}) (determined as total soil N minus extractable NH_4^+ and NO_3^-) in the mineral topsoil of adjacent *Q. robur* (**a**, **b**) and *P. sylvestris* (**c**, **d**) forest stands after in situ application of $^{15}NH_4NO_3$ or $NH_4{}^{15}NO_3$. The modelled lines of the treatments overlap in (**a**) and (**c**)

not greatly affected by the model setup (Fig. 7). The largest difference was found between the basic model setup (model 1; Table 4) and the model setup accounting for temporary inorganic N storage (model 2; Table 4) for the rates O_{Norg} and L_{NO_3}. However, model 1 did not adequately describe all measurements, as indicated by the higher value of the statistical criterion (AIC; Table 4), so that the N rates according to model 1 were less representative for the measured soil N dynamics. Compared to model 2, including D_{NO_3} (model 3) in the optimization improved the model fit for both soils, as shown by the decrease in AIC. In model 3, the parameter describing I_{NO_3} did not differ significantly from zero for the oak soil, and had a relatively wide probability density function for the pine soil (results not shown), indicating that the model was insensitive to this parameter. Therefore, a model was optimized without I_{NO_3} (model 4), which still improved the fit for both soils. According to the Akaike's weights (Burnham and Anderson 2002), the final model 4 was the most probable model setup (Table 4), with a probability of 88% for the oak soil and 71% for the pine soil. There was no meaningful difference (<5%) in the obtained N rates between models 3 and 4, except for I_{NH_4} in the pine soil (Fig. 7).

The final model for the oak soil consisted of four N pools and eight N transformations (Fig. 2a). For the pine soil five N pools and ten gross N rates were included (Fig. 2b). The final N transformation rates and kinetic settings are presented in Table 2. Generally a good fit was obtained between the measured and modelled data, as indicated by visual evaluation (Figs. 3, 4, 5, 6) and the coefficient of determination (R^2; Table 3). For the inorganic N pools, model fit was good for the oak soil (Figs. 4a, 5a), but lower for pine due to the higher observed variation between field replicates (Figs. 4c, 5c). Inorganic ^{15}N enrichment was modelled well (Figs. 4b,d, 5b,d), except for $^{15}NO_3^-$ after adding $^{15}NH_4^+$ to the pine soil (Fig. 5d). While the trend of a continuous increase in $^{15}NO_3^-$ enrichment was correctly simulated in the pine soil ($R^2 = 0.90$), predictions at the last two time steps were less than half of the mean observations (Fig. 5d). For the size of the organic N pool, coefficients of determination were lower ($R^2 < 0.29$; Table 3) since the model correctly fitted the mean of the measured pool size without reflecting the variation between time steps that was due to the within-stand variability (Fig. 6a, c). Model agreement for the ^{15}N enrichment of the organic N pool was acceptable (Fig. 6b, d), except for an outlying value measured 124 h after

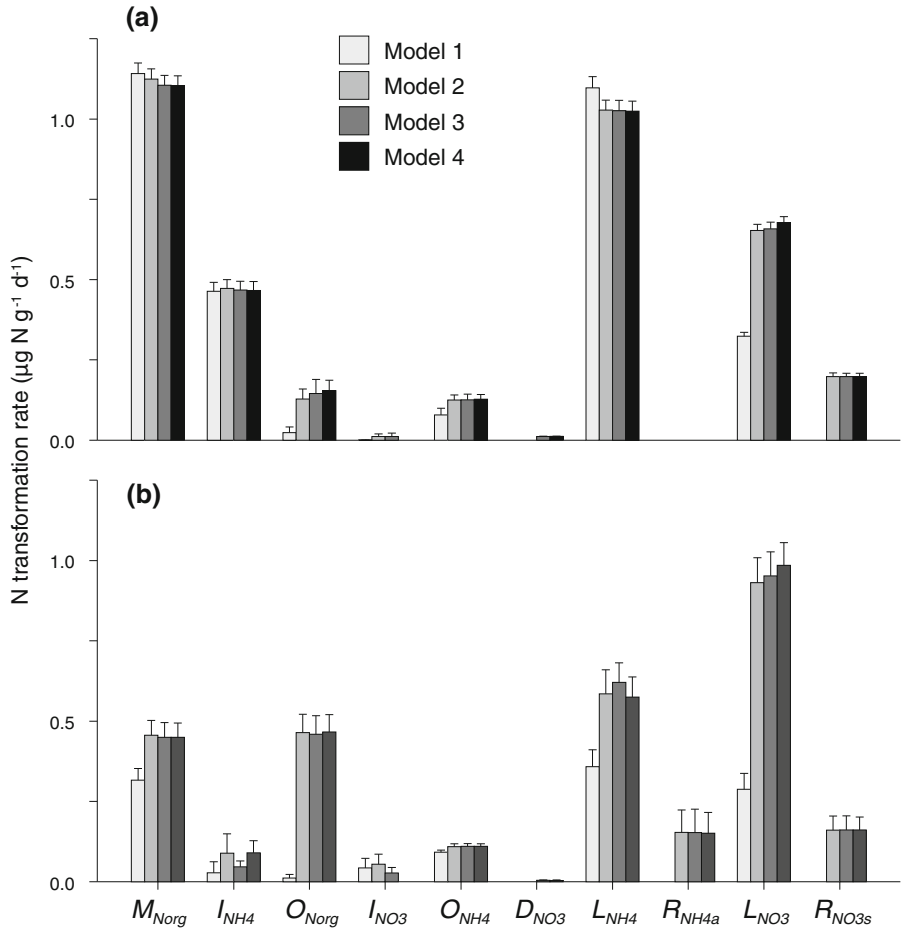

Fig. 7 N transformation rates (mean ± SD) in the mineral topsoil of adjacent **a** *Q. robur* and **b** *P. sylvestris* forest stands according to different model setups (see Tables 2 and 4 for a description of the N transformations and model setups, respectively)

$^{15}NH_4^+$ addition to the pine soil (Fig. 6d) that strongly affected R^2 (Table 3). By including NH_4^+ and NO_3^- loss rates in the model, the numerical data analysis accounted for the decline in ^{15}N recovery (Fig. 3). The coefficients of determination for total ^{15}N in the oak soil were 0.80 ($^{15}NH_4^+$ labelling; Fig. 3a) and 0.93 ($^{15}NO_3^-$ labelling; Fig. 3b) and for pine 0.42 ($^{15}NH_4^+$ labelling; Fig. 3c) and 0.81 ($^{15}NO_3^-$ labelling; Fig. 3d).

Gross nitrogen transformation rates

For both soils all optimized model parameters differed significantly from zero ($p < 0.05$). The resulting N fluxes all differed significantly ($p < 0.05$) between the two forest soils (Table 2). In the oak forest soil, mineralization (M_{Norg}), immobilization of NH_4^+ (I_{NH_4}), and NH_4^+ losses (L_{NH_4}) dominated the N dynamics (Fig. 2a), with rates of 1.1, 0.5, and 1.0 µg N g^{-1} day^{-1}, respectively (Table 2). Oxidation of NH_4^+ to NO_3^- (O_{NH_4}) was an order of magnitude slower than N mineralization and was slower than the NO_3^- production due to oxidation of organic N (O_{Norg}). Immobilization of NO_3^- to organic N (I_{NO_3}) was not significantly different from zero in model 3 (Table 4) and was therefore not considered in the final and most probable model (model 4). Release of stored NO_3^- (R_{NO_3}; 0.20 µg N g^{-1} day^{-1}) was estimated by the model as two-thirds of the total nitrification (O_{NH_4} plus O_{Norg}). In the oak soil a small but quantifiable flux of dissimilatory NO_3^- reduction to NH_4^+ (D_{NO_3}) occurred that accounted for 1.7% of total NO_3^- consumption. The potential efflux of $^{15}NH_4^+$ from roots after $^{15}NO_3$ uptake by roots was not included in the numerical data analysis, and could be an alternative pathway to D_{NO_3} for the conversion of $^{15}NO_3^-$ to $^{15}NH_4^+$ (Fig. 4b, inset). Therefore, root ^{15}N was compared with soil $^{15}NH_4^+$. After $^{15}NO_3^-$

Table 2 Description and rate of in situ gross N transformations (mean and SD) in the mineral topsoil of adjacent *Q. robur* and *P. sylvestris* forest stands

Abbreviation	Description	Kinetics[a]	N transformation rate (μg N g^{-1} day^{-1})			
			Q. robur		*P. sylvestris*	
			Mean	SD	Mean	SD
M_{Norg}	Mineralization of N_{org} to NH_4^+	0	1.104	0.031	0.450	0.045
I_{NH_4}	Immobilization of NH_4^+ to N_{org}	1	0.466	0.028	0.090	0.037
O_{Norg}	Oxidation of N_{org} to NO_3^-	0/1[b]	0.154	0.033	0.466	0.054
I_{NO_3}	Immobilization of NO_3^- to N_{org}	–	n.d.	n.d.	n.d.	n.d.
O_{NH_4}	Oxidation of NH_4^+ to NO_3^-	0	0.128	0.015	0.110	0.008
D_{NO_3}	Dissimilatory NO_3^- reduction to NH_4^+	1	0.012	0.001	0.004	0.002
L_{NH_4}	Losses of NH_4^+ during incubation	1	1.025	0.031	0.575	0.063
R_{NH_4a}	Release of adsorbed NH_4^+	1	n.d.	n.d.	0.151	0.065
L_{NO_3}	Losses of NO_3^- during incubation	1	0.678	0.018	0.985	0.071
R_{NO_3}	Release of NO_{3sto}^- to NO_3^-	1	0.198	0.010	0.161	0.040

All fluxes were determined using final model 4 (Table 4) and differed significantly ($p < 0.05$) between the two forest soils. All gross N fluxes were significantly ($p < 0.05$) different from zero. *n.d.* not determined; in previous model runs the rate was indistinguishable from zero so that it was excluded in the final run

[a] Kinetics: 0 = zero order, 1 = first order

[b] 0 for *Quercus*, 1 for *Pinus*

Table 3 Value of the coefficient of determination (R^2) of the final model (model 4; Table 4) for the size and ^{15}N enrichment of the measured N pools in the mineral topsoil of adjacent *Q. robur* and *P. sylvestris* forest stands

Pool	Treatment	Pool size		^{15}N enrichment	
		Quercus	*Pinus*	*Quercus*	*Pinus*
NH_4^+	$^{15}NH_4NO_3$	0.94	0.54	0.99	0.89
	$NH_4{}^{15}NO_3$	0.97	0.20	0.61	0.86
NO_3^-	$^{15}NH_4NO_3$	0.94	0.85	0.86	0.90
	$NH_4{}^{15}NO_3$	0.83	0.32	0.96	0.85
Organic N	$^{15}NH_4NO_3$	0.01	0.18	0.78	0.01
	$NH_4{}^{15}NO_3$	0.29	0.27	0.82	0.77

addition, the amount of $^{15}NH_4^+$ in the oak soil increased from 0.005 μg ^{15}N g^{-1} at the time of injection to 0.023 μg ^{15}N g^{-1} after 3.4 h (+0.018 μg ^{15}N g^{-1} in 3.4 h), which was more than three times greater than the net ^{15}N increase in the oak roots (from 0.056 to 0.061 μg ^{15}N g^{-1} soil, or +0.005 μg ^{15}N g^{-1} in 3.4 h).

In the pine forest soil, N transformations related to the NO_3^- pool and NH_4^+ pool were of equal importance (Table 2; Fig. 2b). The oxidation rate of organic N (O_{Norg}; 0.47 μg N g^{-1} day^{-1}) was similar to the rate of N mineralization (M_{Norg}; 0.45 μg N g^{-1} day^{-1}). In addition to M_{Norg}, release of NH_4^+ from adsorbed NH_4^+ (R_{NH_4}) was included in the pine model. The process of O_{Norg} occurred 4.2 times faster than O_{NH_4} in the pine soil (Table 4). Immobilization of NH_4^+ (I_{NH_4}) was relatively low (0.09 μg N g^{-1} day^{-1}). In model 3 (Table 4), a small immobilization rate of NO_3^- was detected (Fig. 7), amounting to <4% of the total nitrification in the pine soil. However, excluding I_{NO_3} in model 4 still increased model fit and resulted in a more likely model according to the Akaike's weights (Table 4). The model predicted that the highest N rate occurred by NO_3^- loss from the system (L_{NO_3}). The estimated D_{NO_3} rate differed significantly from zero but was 1–2 orders of magnitude smaller than the other N rates in the pine soil.

In summary, compared to pine, the oak soil generally showed higher N transformation rates related to NH_4^+ dynamics but lower rates related to NO_3^- dynamics. Relative to pine, M_{Norg} was 2.5 times faster under oak (Fig. 2), as demonstrated by the more rapid $^{15}NH_4^+$ pool dilution in the oak soil (Fig. 4b) than in the pine soil (Fig. 4d). The rate of O_{Norg} was 3.0 times higher for the pine forest soil than for oak. Consequently, M_{Norg} was the main transformation pathway of organic to mineral N in

Table 4 Value of the Akaike Information Criterion (AIC) and probability (%) of four model setups simulating gross N transformations in the mineral topsoil of adjacent *Q. robur* and *P. sylvestris* forest stands (see Table 2 for a description of the N transformations; in all models O_{NH_4} has zero-order kinetics for *Quercus* and first-order kinetics for *Pinus*)

Setup	Model description	*Quercus*		*Pinus*	
		AIC	p (%)	AIC	p (%)
1	$D_{NO_3} = 0$; no NH_4^+ adsorption and NO_3^- storage pools	2226	$\ll$0.01	131	$\ll$0.01
2	$D_{NO_3} = 0$; NH_4^+ adsorption and NO_3^- storage pools included	474	$\ll$0.01	98	0.8
3	Full model	204	11.9	91	28.7
4	Full model with $I_{NO_3} = 0$ (final model setup)	200	88.1	89	70.5

the oak soil, yielding 88% of the total NH_4^+ and NO_3^- production, while in the pine soil M_{Norg} and O_{Norg} contributed equally to the production of NH_4^+ and NO_3^- (Fig. 2). In contrast to O_{Norg}, the rate of O_{NH_4} was similar in both soils, so that O_{Norg} accounted for 55% of total gross nitrification in the oak soil but for 81% in the pine soil.

Discussion

Interactions between rhizospheric microbial communities and growing plant roots are central to N dynamics in soils, as recently reviewed by Frank and Groffman (2009). The presence of live roots can strongly influence gross N transformations (Burger and Jackson 2004), and significantly faster N mineralization adjacent to roots than in bulk soil has been observed (Herman et al. 2006). By combining in situ ^{15}N isotope labelling over an 8-day period with numerical data analysis, the present study reports on gross N dynamics in intact temperate forest soils under field conditions, i.e. in the presence of live roots and their natural associated microbial community structure. Process-specific N transformations were considered in the ^{15}N tracing approach, which can provide more insight into soil N dynamics than the common dilution approach that quantifies total N production and consumption rates. Isotope dilution methods applied in situ to soils with intact roots have rarely been reported. One example is the in-growth core study of N dynamics in a tropical montane forest soil (Templer et al. 2008). However, in that study the soil in the in-growth cores was initially disrupted and then allowed to equilibrate over 11 months in the field before the 24-h dilution experiment was conducted, so that fine root biomass amounted to only 59% of the background level (Templer et al. 2008).

To account for the decline in ^{15}N recovery (Fig. 3), NH_4^+ and NO_3^- loss rates were considered in the ^{15}N tracing model (Fig. 2), which was feasible because of the determination of the total soil N concentration and its ^{15}N enrichment. The incomplete ^{15}N recovery can be due to N loss via leaching, gaseous forms, uptake by roots or mycorrhizae, or diffusion. Leaching of N seems unlikely as no rainfall reached the forest floor during the experiment, although the sandy soil may still have allowed dissolved N loss. A second reason could be gaseous N loss, but given the dry, acid, and sandy soil, minimal N gas emission is expected (Butterbach-Bahl et al. 2002). Thus uptake and transport of inorganic N by roots and mycorrhizae may possibly explain ^{15}N loss from the soil. The ^{15}N content of roots that were separated from the sampled soil increased throughout the experiment, but accounted for <2% of the total ^{15}N recovery (Fig. 3). While this is probably an underestimation of the actual ^{15}N uptake by roots because of N transport to non-sampled plant parts, the small contribution of roots to the total ^{15}N amount did not justify to explicitly include root uptake in the model, but to account implicitly for this pathway by the NH_4^+ and NO_3^- loss fluxes. A fourth and likely reason for the ^{15}N loss is diffusion of the injected $^{15}NH_4^+$ and $^{15}NO_3^-$ to non-labelled soil around and below the sampled virtual cores. Inflow of ^{14}N from the soil surrounding the sampled soil was not included in the model as the enhanced mineral N concentrations in the injected virtual core make outflow of N more important than inflow of ^{14}N. To minimize edge effects, however, future research with

a similar setup could inject ^{15}N into a larger area than the surface area of the soil core that is sampled later on (Rütting et al. 2011).

Production and consumption of ammonium

The gross N mineralization rates in the mineral soil of the oak and pine forest (1.1 and 0.5 μg N g^{-1} day^{-1}, respectively) are at the lower end of the 0–10 μg N g^{-1} day^{-1} range often reported for temperate forests ecosystems (Booth et al. 2005; Verchot et al. 2001; Zeller et al. 2007). Higher N mineralization rates in other field or laboratory studies may be related to site differences, e.g. in soil type, and in disturbance effects. Furthermore, the reported rates are from studies where N mineralization rates were quantified by pool dilution and analytical solutions that evaluate total production and consumption, while total NH_4^+ production in soils is not only due to mineralization. Nevertheless, the current N mineralization rates are similar to results for the sandy loam topsoil of a mixed deciduous forest in the same region (0.5–1.0 μg N g^{-1} day^{-1}; Vervaet et al. 2004). In the present study the rate of gross N mineralization was more than double in the topsoil of the oak forest than in the neighbouring pine forest. A review of literature data also indicated faster N mineralization in deciduous forest soils than in coniferous soils at similar C concentrations, which appeared to result mainly from differing C:N ratios of organic matter, with a mean ratio of 28 for coniferous forest soils and 18 for deciduous forest soils (Booth et al. 2005). However, in our study the C:N ratio was similar in both forest soils (Table 1), and hence cannot solely explain the observed difference in soil N mineralization rate.

Besides N mineralization, in the pine soil 20% of gross NH_4^+ production was supplied by release of previously adsorbed NH_4^+, as ammonium can be exchanged between free NH_4^+ and NH_4^+ adsorbed on clay minerals or organic matter. Added $^{15}NH_4^+$ can be adsorbed and be unextractable by KCl solution (Russow et al. 2008) and released later when the exchangeable NH_4^+ concentration in the soil decreases (Drury and Beauchamp 1991; Roing et al. 2006). Consumption of NH_4^+ occurred by immobilization to organic N, nitrification, and loss in both soils. Biotic and abiotic immobilization of all inorganic N forms can occur in forest soils (Johnson et al. 2000). In our study NH_4^+ immobilisation amounted to 42 and 20% of the mineralized N in the oak and pine soil, respectively, but immobilization in absolute rates was five times smaller in the pine soil. The latter result was supported by the negligible change of the organic ^{15}N pool in the pine forest soil (Fig. 6c, d). However, both soils showed rapid NH_4^+ immobilization within 3 h after ^{15}N addition (Fig. 4b, d), suggesting the occurrence of abiotic immobilization pathways (Fitzhugh et al. 2003).

Production and consumption of nitrate

The oxidation rate of NH_4^+ to NO_3^- was similar in both forest soils ($\sim$0.1 μg N g^{-1} day^{-1}) and four to nine times lower than the gross N mineralization rate. While higher nitrification rates have been reported for temperate forest soils (Booth et al. 2005; Christenson et al. 2009; Verchot et al. 2001), the current values exceed rates measured in the same region (Vervaet et al. 2004). At Harvard Forest, mineral soils of deciduous and pine forests had similar gross nitrification rates (Venterea et al. 2004). Another pathway for NO_3^- production is oxidation of organic N, which was slightly more important than NH_4^+ oxidation in the oak soil but occurred four times faster than NH_4^+ oxidation under pine. A wide range of bacteria and fungi posses the potential for heterotrophic nitrification by which both organic and inorganic N compounds can be oxidized to NO_3^- (De Boer and Kowalchuk 2001), but there is discussion on the importance of heterotrophic vs. autotrophic nitrification in acid soils. The review by De Boer and Kowalchuk (2001) concludes that autotrophic nitrifiers are likely the main nitrifying agents, even in acid soils, while other studies strongly support the idea that heterotrophs contribute largely to nitrification rates in mature forest soils mainly by oxidizing organic N (Brierley et al. 2001; Huygens et al. 2007; Jordan et al. 2005; Pedersen et al. 1999; Trap et al. 2009). It has been shown using genetic techniques that other microorganisms than autotrophic ammonia oxidizers contributed to high nitrification rates in Californian forest soils (Jordan et al. 2005). By ^{15}N labelling and numerical data analysis, approximately half of the gross nitrate production in a pristine forest soil was attributed to heterotrophic nitrification (Huygens et al. 2007). By incubating soils from a forest chronosequence with selective inhibitors, an

increase in net heterotrophic nitrification in the organic layers was found with decreasing fertility of the topsoil, suggesting a shift in nitrification towards heterotrophic activity with lower litter quality (Trap et al. 2009). Our results also indicate that heterotrophic nitrification of organic N is an important process of NO_3^- production in temperate acid forest soils. Heterotrophic nitrification is predominantly carried out by fungi, including ectomycorrhizae (Killham 1990) whose mycelium networks are fragmented by soil sieving (Bengtsson et al. 2003; de Vries et al. 2009) or delimiting soil cores. In this respect, the present gross N rates determined in undisrupted soils are more representative for the actual gross N dynamics.

Not all added NO_3^- could be recovered at the first soil extractions after 3.4 h, indicating rapid N immobilization, which was accounted for in the ^{15}N tracing model by including a storage pool for NO_3^- with the potential to release NO_3^- again (Müller et al. 2004). Possible explanations for such NO_3^- dynamics are abiotic NO_3^- immobilization or rapid biotic NO_3^- immobilization followed by release of unused NO_3^- (Ellis et al. 1996). The numerical analysis showed that other NO_3^- immobilization than the initial NO_3^- immobilization was unimportant in the studied forest soils. While NO_3^- immobilization may have occurred, the probability density functions of the corresponding model parameter (results not shown) indicated that this N transformation rate only negligibly contributed to the soil N dynamics measured during the experiment. In addition, the Akaike's weights (Burnham and Anderson 2002) showed that the final model 4 (Table 4) without NO_3^- immobilization was most likely for both soils. The small immobilization of inorganic N in the pine forest soil was supported by the fact that the ^{15}N enrichment of the organic N pool did not increase during the study (Fig. 6d). This result was unexpected, as ^{15}N addition may stimulate immobilization by increasing inorganic N pools, particularly for NO_3^- (Stark and Hart 1997).

Another NO_3^- consumption process that was evaluated with the tracing model was DNRA. Nitrate was transformed to NH_4^+ mainly under oak (Fig. 4b, inset), which may have occurred by (i) DNRA, i.e. direct conversion of $^{15}NO_3^-$ to $^{15}NH_4^+$, (ii) root efflux of $^{15}NH_4^+$ after $^{15}NO_3^-$ uptake, and/or (iii) microbial biomass turnover (Burger and Jackson 2004). Although root efflux of $^{15}NH_4^+$ was not accounted for in the analysis, a potential release of reduced ^{15}N by oak roots cannot explain the observed increase in oak soil $^{15}NH_4^+$ in the first hours after $^{15}NO_3^-$ addition because the net increase in root ^{15}N was much smaller than the increase in soil $^{15}NH_4^+$. The ^{15}N model accounted for remineralization of ^{15}N immobilized into soil organic matter. As $^{15}NO_3^-$ immobilization was negligible, microbial release of $^{15}NH_4^+$ can be considered to be also negligible during the experiment. Furthermore, remineralization of ^{15}N immobilized by microbes is unlikely to explain the fast increase (1–2 days) of $^{15}NH_4^+$ after $^{15}NO_3^-$ addition, since microbial turnover times are in the range of 7–18 days and therefore too slow (Burger and Jackson 2004; Schmidt et al. 2007). Until now, DNRA in forest soils has been reported mainly for N-limited ecosystems with high annual rainfall (Huygens et al. 2008; Pett-Ridge et al. 2006; Silver et al. 2005; Sotta et al. 2008; Templer et al. 2008). In the present study, the rate of DNRA under field conditions in a temperate forest soil with high N input was an order of magnitude smaller than in these humid tropical forests soils. In laboratory incubations, DNRA has also been observed in a fertilized boreal forest soil (Bengtsson and Bergwall 2000) and a temperate coniferous litter layer (Tietema and Van Dam 1996). Tiedje et al. (1982) concluded that DNRA increases as the ratio of soil organic C to extractable NO_3^- increases. In line with this finding, both the C to NO_3^- ratio and the DNRA rate were higher in the oak soil than in the pine soil.

Forest type as driver of N dynamics

According to the conceptual model by Schimel and Benett (2004), soil N cycling varies with increasing N availability and is based on the dynamics of microsite processes. The studied soils can be considered to be at the mid-zone to the higher end of the N-availability gradient. In the mid-zone of N availability, as described by Schimel and Benett (2004), the number of microsites with available N is larger than the number of N-limited microsites and a large fraction of the microbial community uses local organic N. In this case, NH_4^+ still prevails over NO_3^- because the demand of both microbial and plant uptake limit the supply of NH_4^+ to nitrifiers. At higher relative N availability, the competition for NH_4^+ between plants

and microorganisms is reduced so that the rate of NH_4^+ oxidation can increase and NO_3^- becomes the dominant mineral N form. According to Schimel and Benett (2004), this case can occur in agricultural systems and N-rich forests at increased levels of mineral soil N. Compared to the oak soil, the extractable NH_4^+ concentration was twice as high in the pine soil and the NO_3^- concentration was even an order of magnitude higher (Table 1; Figs. 4, 5). Considering overall gross N dynamics, N mineralization was more than two times faster in the oak forest soil while combined autotrophic and heterotrophic nitrification was two times faster in the pine forest soil (Table 2). Taken together, these findings suggest that the oak soil is situated in the mid-zone of N availability described by Schimel and Benett (2004), while the pine soil represents the high N-availability conditions. As the two forest stands are located on the same soil type, had the same history, tree age, and topsoil temperature, this indicates the dominant effect of vegetation cover on microbial and plant N availability. In contrast to the conceptual model by Schimel and Benett (2004) the present results show that the production of NO_3^- is not only due to NH_4^+ oxidation but also due to organic N oxidation, as also postulated by Huygens et al. (2007), and that the latter pathway depends on the forest type.

The inorganic N input to the forest floor by atmospheric deposition is more enhanced in the pine forest than in the oak forest (33 and 18 kg N ha^{-1} $year^{-1}$, respectively). This is in line with the general effect of coniferous vs. deciduous forest types on atmospheric N deposition as reviewed by De Schrijver et al. (2007). Since N deposition in the study region has been chronically enhanced for several decades (Schöpp et al. 2003), the question arises whether the observed difference in N cycling between the two soils is related to the different N inputs to the stands. Experiments indicate that N additions do not affect or even decrease gross N mineralization rates in mineral forest soils, while gross nitrification is increased (Barnard and Leadley 2005; Corre et al. 2003; Venterea et al. 2004). The results of the Harvard forest chronic N amendment study (Venterea et al. 2004; 50–150 kg N ha^{-1} $year^{-1}$ added over 13 years) suggested that enhanced gross nitrification is the fundamental process driving other symptoms of N saturation. Following Aber et al. (1998), the pine stand with enhanced N leaching below the rooting zone is in a later stage of N saturation than the adjacent oak stand, where inorganic N leaching is relatively low. Consequently, the observed higher gross nitrification rate ($O_{Norg} + O_{NH_4}$) in the pine forest soil than in the oak forest soil supports the idea that this process is key to N leaching and N saturation. The production of NO_3^- was higher in the pine soil than in the oak soil, and NO_3^- immobilization was negligible. This difference in soil N dynamics between the two forests is in line with the higher NO_3^- leaching under the rooting zone of the pine forest. However, gross N dynamics were studied in the mineral topsoil during an eight-day period, while N leaching integrates processing occurring over longer periods in the forest floor and mineral soil layers, so that no direct relationship between soil N dynamics and N leaching can be concluded from the present study.

The type of forest and tree species may affect microbial N cycling through a difference in atmospheric N deposition, but also by the quality and quantity of released organic material, and the latter effect is probably more important in the present study. Gross N mineralization rates have been found to be negatively correlated both with the fungi to bacteria ratio as with the C:N ratio in boreal forest soils in Sweden (Högberg et al. 2007). In deciduous forests in north-eastern US, gross N mineralization and nitrification rates in mineral topsoils were negatively related to the C:N ratio, but less than half of the variance was explained and the relationship did not hold for organic horizons (Christenson et al. 2009). Bengtsson et al. (2003) concluded that differences in gross N mineralization rates in three deciduous forests were related more to microbial activity than to the soil C:N ratio. In the present study, the topsoil pH and C:N ratio were similar in the two stands (Table 1). Nevertheless, gross N dynamics differed significantly between the two forest soils. Tree species exert control on the composition of soil organic matter and microbial community structure through litterfall, root turnover, and root exudates (Brant et al. 2006; Priha and Smolander 1999). Oak litter contains less lignin and is less recalcitrant to decomposition than pine litter (Reich et al. 2005), as demonstrated by the thicker organic horizon in the pine stand; even though slower needle decomposition may be partly related to

negative effects of high N input as well (Knorr et al. 2005). Thus, it can be hypothesized that the fungi to bacteria ratio is lower in the oak soil than in the pine soil, as this could explain both the faster gross N mineralization in the oak soil (cf. Högberg et al. 2007) and the faster heterotrophic nitrification by fungi in the pine soil.

Overall, our findings agree with earlier indications (Lovett et al. 2004; Christenson et al. 2009) that gross N cycling in the mineral topsoil of temperate forests is affected more by the quality of soil organic matter, controlling microbial activity (Compton and Boone 2002), than by a simple measure such as the soil C:N ratio. This underlines the importance of considering the interaction between tree species and soil N and C turnover when modelling the response of forest ecosystems to global change scenarios. In situ determination of gross soil N transformation rates contributes to a better understanding of the actual N cycling processes.

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