Nitrate leaching from soil under different forest tree species is related to the vertical distribution of Nitrobacter abundance

A. Florio, A. Legout, M. Marechal, Margaux Clesse, A. Delort, C. Creuze Des Chatelliers, J. Gervaix, Y. Shi, G. van der Heijden, B. Zeller, et al.

Science of the Total Environment 967 (2025) 178776

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Nitrate leaching from soil under different forest tree species is related to the vertical distribution of *Nitrobacter* abundance

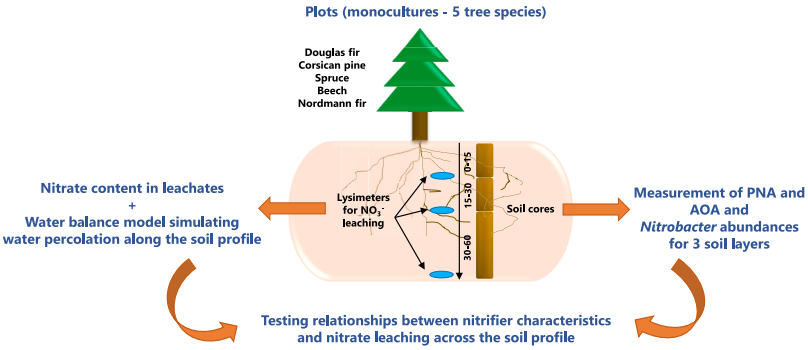
A. Florio [a,*], A. Legout [b], M. Marechal [a], M. Clesse [b], A. Delort [a], C. Creuze des Chatelliers [a], J. Gervaix [a], Y. Shi [c], G. van der Heijden [b], B. Zeller [b], X. Le Roux [a]

[a] INRAE, Univ Lyon, Université Claude Bernard Lyon 1, CNRS, VetAgro Sup, UMR 1418 LEM, Ecologie Microbienne, F 69622 Villeurbanne, France
[b] INRAE, BEF, F-54000 Nancy, France
[c] Institute of Grassland Science, Key Laboratory of Vegetation, Ecology of the Ministry of Education, Jilin Songnen Grassland Ecosystem National Observation and Research Station, Northeast Normal University, Changchun 130024, PR China

HIGHLIGHTS

- We evaluated the effects of trees with different N-uptake strategies on nitrifiers
- We coupled nitrates in soil solutions and modeling to obtain nitrate fluxes
- Nitrate fluxes explained by *Nitrobacter* abundance across the 0–60 cm soil profile
- *Nitrobacter* abundance as a proxy to predict the risk of nitrate leaching from soils

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Manuel Esteban Lucas-Borja

Keywords:
BNI
Nitrifying community
ammonia-oxidizers
Nitrite-oxidizers
Nitrate lixiviation
Forest ecosystems

ABSTRACT

Summary Forest tree species and their mineral N uptake strategies can influence the activity and abundance of nitrifying microorganisms in deeper soil layers and subsequent nitrate leaching. However, the role of nitrifier community from the topsoil or deeper soil layers for nitrate leaching below the rooting zone remains uncertain. We evaluated potential nitrification rates and the abundance of ammonia- and nitrite- oxidizers in soil profiles covered by different tree species having (i.e. spruce and Nordmann fir) or not (i.e. Douglas fir, Corsican pine and beech) the Biological Nitrification Inhibition, BNI, capacity. Concurrently, we calculated nitrate fluxes under each tree species by coupling nitrate concentrations in soil solutions with the hydrological model Watfor to simulate water percolation, and analyzed the relationships between nitrate fluxes and nitrifiers characteristics. We observed that nitrification rates under BNI species in the topsoil were lower than those under non-BNI species, and that these changes were associated to strong differences in the abundance of *Nitrobacter* (500-fold changes between tree species). Nitrification potentials drastically decreased with increasing soil depth and were strongly correlated with the abundance of *Nitrobacter*, not ammonia oxidizers. Furthermore, by computing weighted mean values of nitrifier activity and abundance, we showed that nitrate fluxes were explained by the abundance of *Nitrobacter* community across the 0-60 cm soil profile. In this context, the abundance of *Nitrobacter*

* Corresponding author.
E-mail address: alessandro.florio@univ-lyon1.fr (A. Florio).

https://doi.org/10.1016/j.scitotenv.2025.178776
Received 4 September 2024; Received in revised form 4 February 2025; Accepted 5 February 2025
Available online 16 February 2025

community seems an interesting proxy for evaluating water quality at the plot scale, and a promising tool to understand and predict the risk of nitrate leaching from soils in temperate forest ecosystems.

1. Introduction

Nitrification, i.e. the oxidation of NH_4^+ to NO_3^-, is a key pathway of the nitrogen (N) cycle in many terrestrial ecosystems. Nitrification is of crucial importance for soil quality, as it largely regulates the levels and types of mineral N forms available to plants such as NH_4^+ and NO_3^-, as well as NO_3^- leaching and N_2O emissions. Particularly, the leaching of NO_3^- may lead to soil acidification and soil nutrient depletion (Reuss and Johnson, 2012), which are major threats for tree growth and forest sustainability. Limiting NO_3^- leaching from soil is also a major concern for the preservation of water quality, which is particularly critical for forest ecosystems as they play an important role in hydrological processes such as surface and subsurface runoff (Sudduth et al., 2013). However, NO_3^- leaching from forest soils is a complex process resulting from (i) the sources of NO_3^-, i.e. the atmospheric deposition of NO_3^- (Alvarez-Cobelas et al., 2008) and the endogenous production of NO_3^- in the soil - which can be influenced by tree species, and (ii) the soil water regime that influences the nitrifier activity but also the transport of NO_3^- along the soil profile and below the rooting zone (Kozlowski et al., 2012).

In soils, NO_3^- produced through nitrification is mainly driven by nitrifying prokaryotes (Leininger et al., 2006; Prosser and Nicol, 2012). The first step of nitrification is the oxidation of NH_4^+, performed by ammonia oxidizing bacteria (AOB) and archaea (AOA), and the second step is the oxidation of NO_2^- to NO_3^- by nitrite-oxidizing bacteria (NOB), with two main genera present in soils (*Nitrobacter* and *Nitrospira;* Freitag et al., 2005). Both steps can be performed by the recently discovered complete ammonia oxidizers (comammox) (Daims et al., 2015). Trees thus depend on the activity of nitrifiers and can strongly compete with them for NH_4^+ (Kuzyakov and Xu, 2013). However, it is increasingly recognized that tree species can exert a major influence over soil N cycling rates (Chapman et al., 2006). For example, Douglas fir (*Pseudotsuga menziesii*) plantations are often associated with high soil NO_3^- concentrations and high NO_3^--to-NH_4^+ ratio in the topsoil (Jussy et al., 2004; Zeller et al., 2007, 2019; Paul et al., 2022). This is related to the high nitrification potential observed for soils under this species (Andrianarisoa et al., 2010; Mareschal et al., 2013) and likely to a capacity of Douglas fir to stimulate nitrification (Zeller et al., 2019). Other tree species like spruce (*Picea abies Karst*) and Nordmann fir (*Abies nordmanniana Spach.*) are associated with very low soil NO_3^- concentrations (Andrianarisoa et al., 2010; Legout et al., 2016). These species are able to regulate nitrifier activity and abundance (Laffite et al., 2020) through a process known as biological nitrification inhibition, BNI (Subbarao et al., 2015). By releasing specific organic compounds such as mono terpenes via root exudation or litter decomposition, these tree species have the capacity to inhibit the growth and abundance of the nitrite-oxdizing *Nitrobacter*, and to a lesser extent those of AOA, but not those of AOB, comammox or *Nitrospira* in topsoil (Laffite et al., 2020).

Despite the intensive efforts in understanding tree-nitrifier interactions, current research has mostly focused on topsoil layers where root biomass is greatest (Gill et al., 1999). However, the activity, abundance and diversity of nitrifier groups in deeper soil layers can significantly influence the quality of groundwater (Madsen, 1995). A decrease in nitrification with increasing soil depth has been observed in soils under Douglas fir (De Boer et al., 1992) or beech (Krieger, 2001), but other studies found that AOA were more abundant in forest subsurface soil layers than in topsoil of cypress and oak (Onodera et al., 2010). This indicates that forest tree species and their N uptake strategies might influence nitrifier activity and abundance in deeper soil layers and subsequent NO_3^- leaching. However, whether the environmental and biotic drivers of soil nitrification are similar or different between topsoil and deeper soil layers, and whether the activity and abundances of nitrifiers from the topsoil or deeper soil layers contribute to NO_3^- leaching below the rooting zone, remains uncertain.

Moreover, to our knowledge, no study has analyzed to what extent the vertical distribution of nitrifier activity and abundances along the soil profile determine the NO_3^- leaching flux below the rooting zone in forest ecosystems. Few sites in the world can address this research topic and the Breuil-Chenue experimental site is one of them: this common garden experiment (see details in material and methods section) was set up in 1976 and is composed of 6 monospecific plots (oak, beech, Norway spruce, Douglas fir, Nordmann fir and Laricio pine) which have been intensively monitored since 2001. At this site, it has been shown that NO_3^- concentrations in the soil solution and the NO_3^- leaching flux greatly varied between forest tree species (Legout et al., 2016). Particularly, higher NO_3^- concentrations in the soil solutions were observed for Douglas fir and Corsican pine (Legout et al., 2016), i.e. two non-BNI species, than BNI species such as Nordmann fir and spruce (Laffite et al., 2020). But to what extent the characteristics of the nitrifiers along the soil profile, which are likely influenced by the different tree species, explain the contrasted leaching NO_3^- levels remains unknown.

The objective of this present study was to analyse the relationships between NO_3^- leaching in the soil and nitrifier activity and abundance in soil profiles covered by different tree species with contrasting BNI capacities. We hypothesize that (i) the level of nitrifier activity and abundance in the rooting zone of tree species would influence NO_3^- fluxes along the soil profile and NO_3^- leaching below the rooting zone. Specifically, we assume that restricted nitrifier activity and abundance under BNI tree species would result in restricted NO_3^- fluxes, and that higher nitrification and nitrifier abundance under non-BNI species would result in higher NO_3^- fluxes along the soil profile. We also hypothesize that (ii) NO_3^- leaching below the rooting zone would be determined by the mass-weighted mean of nitrification and/or of nitrifier abundance along the soil profile. We compared the potential nitrification activity and the abundances of key nitrifier groups (AOA and *Nitrobacter* that determine nitrification in these forest soils according to Laffite et al. (2020) and Florio et al. (2021a), as well as AOB), along with environmental parameters (soil moisture, mineral N concentrations and pH) between soils from 43-year-old monoculture stands with BNI (i.e. spruce and Nordmann fir) or no BNI (i.e. Douglas fir, Corsican pine and beech) capacity. We distinguished 0–15 cm, 15–30 cm and 30–60 cm soil layers. We also collected soil solutions at three depths (15, 30 and 60 cm) under each monoculture plot during 6 months and measured the NO_3^- content of leachates. We coupled NO_3^- concentrations in soil solutions with the hydrological model Watfor to simulate water percolation and calculate NO_3^- fluxes under each monoculture stand. These data were used to analyse the relationships between nitrifier characteristics (i.e. PNA, AOA and *Nitrobacter* abundances along the soil profile) and NO_3^- leaching at the bottom of each soil layer.

2. Materials and methods

2.1. Study site

The study site is a long-term experimental site set up and managed by INRAE, located in the Breuil-Chenue forest (AnaEE France, In natura experimentation, Nièvre Morvan, France; 47°18′N and 4°44′; 650 m asl). From 2002 to 2019, mean temperature and precipitation in April were 12.2 °C and 81.4 mm, respectively, whereas temperature and precipitation in April 2019, i.e. corresponding to the sampling date, were 12.3 °C and 66.1 mm, respectively. In the 1970s, the native forest was a 150-year-old coppice dominated by beech (*Fagus sylvatica L.*) with

Quercus sessiliflora Smith, *Betula verrucosa,* and *Corylus avellana* (Cornelis et al., 2011; Mareschal et al., 2013). In 1976, a 10-ha flat area was clear-cut and planted in rows with tree monocultures of five tree species: beech (*Fagus sylvatica*), Corsican pine (*Pinus nigra* Arn. Spp laricio Poiret var. corsicana), Douglas fir (*Pseudotsuga menziesii* Franco), Nordmann fir (*Abies nordmanniana* Spach.) and spruce (*Picea abies* Karst) (1000 m^2 for each species) (Andrianarisoa et al., 2010). Soil homogeneity over the study site was assessed just before plantation in 1976 (Bonneau et al., 1977; Ranger et al., 2003). A weather station installed in 2001 and located at the Breuil-Chenue site monitored rainfall, wind speed, temperature, air humidity, and solar radiation on a daily basis. The Penman-Monteith equation was used to compute potential evapotranspiration (PET). Main physico-chemical characteristics of soils under these tree species are reported in Supplementary Table S1.

2.2. Monitoring of NO_3^- in the soil solution

All plots were equipped in 2000 to monitor the biogeochemical cycles of major elements, considering litterfall, throughfall, stemflow, forest floor and soil solutions. Soil solutions were collected in each plot at 15, 30 and 60 cm depths, with ceramic tension-cup lysimeters (5 replicates per depths, minimum distance between each lysimeter 2.5 m), with a continuous vacuum of −0.5 bars. Soil pits were dug and the lysimeters were inserted horizontally in the undisturbed soil (into holes previously drilled with an auger of the same diameter as the ceramic cups). Each ceramic cup was linked to a container located in a large pit dug in the soil, covered and insulated. Solutions were collected for each lysimeter every 28 days from 01/2002 to 06/2019. Samples were filtered at the laboratory with 0.45 μm Metriciel® membrane filters, grouped by season (3 or 4 28-days period are grouped to fit as possible with the calendar season) and analyzed for NO_3^- and Cl^- by molecular absorption spectrometry (San++ System, Skalar®). For this study, we only used the data of two periods around the soil sampling date: form 16 January to 12 March 2019 (considered as winter) and from 13 march to 4 June 2019 (considered as spring).

Sample contamination in the field or in the laboratory can cause incoherently elevated concentrations. A model was designed to highlight these outlier samples (Legout et al., 2016): concentrations higher than the criteria *C* were considered as abnormally high (Eq. 1):

$$Thresh_{x,y} = Q_{x,y}\ (75\%) + IQ_{x,y}$$

where:

$Thresh_{x,y}$ is the threshold concentration of element *x* in solution type *y* above which concentrations are considered abnormally high;

$Q_{x,y}$ *(75 %)* is the 75 % quantile of element *x* in solution type *y,* and $IQ_{x,y}$ is the interquartile of element *x* in solution type *y*.

All abnormally high concentrations were considered as outliers and removed from the database.

2.3. Soil sampling

On 26th April 2019, we collected four soil samples within each of the five monoculture plots at three soil depths (0–15 cm, 15–30 cm and 30–60 cm) with a corer (8 cm diameter). The soil samples were collected randomly at least 4 m away from the plot edges. The soil samples were sieved (4 mm) in the field and all visible roots were removed. Soil subsamples were stored a few days at +4 °C before assays for soil environmental parameters and microbial activities. A sub-sample was frozen at −20 °C before DNA extraction.

2.4. Measurement of soil environmental variables

For each fresh soil sample, a subsample of a few grams was weighed and dried at 105 °C during 24 h to determine gravimetric soil water content (SWC). We measured mineral N concentration using 5 g equivalent dry mass soil after extraction with 20 ml of 0.01 M $CaCl_2$. The solution was shaken at 10 °C for 2 h at 140 rpm, filtered at 0.2 μm and frozen at −20 °C until measurements of NO_3^-, NO_2^- and NH_4^+ concentrations with an ion chromatograph (Dionex ICS 900) equipped with a 4 × 50 mm column (IonPac AS22-Fast). Soil pH was determined on soil: water mixtures (1:5) using a pH analyzer (WTW InoLab multi 9420, Weilheim, Germany).

2.5. Potential nitrification assays

We measured potential nitrification activity (PNA) on all samples using the method described by Dassonville et al. (2011). Briefly, subsamples of fresh soil (3 g dry mass equivalent) were incubated for 10 h with 30 ml of $(NH_4)_2SO_4$ (1.25 mg N l^{-1}) using continuous shaking (140 rpm, 28 °C). Subsamples (1 ml) were collected at 2 h, 6 h, 10 h, 24 h, 48 h and 72 h, filtered (0.20 μm pore size) and stored at −20 °C. The NO_3^- and NO_2^- concentrations were analyzed using an ion chromatograph (ICS 900). PNA was computed as the linear rate of NO_3^- production over 24 h (no NO_2^- accumulation was observed). Activity measurements were performed at the AME platform (Microbial Ecology UMR1418, Lyon).

2.6. DNA extraction and quantification of nitrifier gene copy abundances

We extracted DNA from 0.5 g of soil for all soil samples using a PowerSoil DNA Isolation Kit (MO BIO laboratories, Inc., USA) according to the manufacturer's protocol. Extracted DNA was stored at −20 °C until use. The archaeal *amoA* coding for the ammonia monooxygenase, and the *nxrA* genes coding for the nitrite oxido-reductase of *Nitrobacter*, were quantified by real-time PCR, gene copy abundances being used as proxies for the abundances of AOA and *Nitrobacter* communities, respectively. In addition, the bacterial *amoA* as a proxy for the abundance of AOB was quantified by real-time PCR for the 0–15 cm soil samples. Amplification was performed as described by Laffite et al. (2020) by using amoA_1F/amoA_2R (Rotthauwe and Witzel, 1997) CrenamoA23f/CrenamoA616r (Tourna et al., 2011) and F1norA/R2norA (Wertz et al., 2008) primer sets for archaeal *amoA* and *nxrA*, respectively. All samples were run on a Lightcycler 480 (Roche Diagnostics, Meylan, France). Dilution series of the extracted DNA were performed to control for possible PCR inhibition by co-extracted compounds, and no inhibition was observed (data not shown). A melting curve analysis was performed to assess PCR product specificity after amplification. The average real-time PCR efficiency for each of these genes was 80 %, 82 % and 90 % for AOB, AOA, and *Nitrobacter*, respectively. Gene copy number per gram of dry soil was calculated. Molecular microbial analyses were performed at the PGE platform (Microbial Ecology UMR1418, Lyon).

2.7. Modeling of the soil water regime

The water drainage fluxes in the soil profile were calculated with the hydrological model WatFor (Legout et al., 2016; van der Heijden et al., 2019). WatFor is a daily water balance model and is composed of two modules that respectively compute the fluxes of water and a non-reactive solute such as chloride in forest ecosystems. Briefly, WatFor accounts for canopy rainfall interception, throughfall, soil water content change and water drainage in the soil profile. The model inputs are daily rainfall and daily potential evapotranspiration. The soil profile is divided into different layers (user-defined) and for each layer, the following parameters are required for each soil layer: layer thickness, fraction of stones (> 2 mm) (no unit), fraction of water uptake (no unit; water uptake distribution), volumetric water content ($m^3.m^{-3}$) at wilting point, field capacity and saturation. For each soil layer and at each time step, the variation of soil water content is calculated as follow (Eq. 2):

$$\Delta W(t) = Q_{in}(t) - ET(t) - Q_{out}(t)$$

where:

$\Delta W(t)$ is the daily variation of soil water content in the soil layer (l.m^{-2}.day^{-1}) at time step t;

$Q_{in}(t)$ is the daily water inflow flux to the soil layer as defined below (l.m^{-2}.day^{-1}) at time step t;

$ET(t)$ is the daily evapotranspiration flux from the soil layer (l.m^{-2}.day^{-1}) at time step t, and

$Q_{out}(t)$ is the water outflow draining from the soil layer (l.m^{-2}.day^{-1}) at time step t.

For each monospecific stand, the WatFor model was run for the 2002–2019 period and was calibrated using the monitoring data of the 2002–2019 period: the canopy rainfall interception parameters were calibrated to best reproduce the throughfall water flux (measured every 28 days) and the water uptake distribution parameters were calibrated to best reproduce the measured concentrations of chloride in the soil solution at 15, 30 and 60 cm depth. For this study, we only used the daily water drainage fluxes calculated from January 2019 to June 2019, around the soil sampling date.

2.8. Soil NO_3^- leaching fluxes

The NO_3^- leaching flux was estimated at 15, 30 and 60 cm depths for two periods around the soil sampling date and where the NO_3^- concentrations in the soil solution were measured: from 16 January to 12 March 2019 (considered as winter) and from 13 march to 4 June 2019 (considered as spring). The NO_3^- leaching flux was calculated for each stand and each depth by multiplying the measured concentrations in soil solutions every season with the cumulated daily water drainage fluxes (equivalent to each season) calculated with the WatFor model. The NO_3^- leaching flux was also estimated for shorter periods (30 and 60 days) using the same methodology: in this case, the daily water drainage was cumulated on these shorter periods and then multiplied by the corresponding seasonal NO_3^- concentration.

2.9. Data processing and statistical analyses

In this study, we did not test whether values observed for one tree species significantly differed from values for another species. Indeed, only one stand was studied per species and, strictly speaking, replicated soil samples corresponding to pseudo-replicates. We thus focused on the relationships between the different measured variables across the five stands studied, in particular the relationships between (i) PNA and nitrifier abundances, and (ii) NO_3^- leaching and nitrifier activity or abundances. Correlations were carried out with the JMP 17 software (www.jmp.com/fr). Pearson's correlation coefficients were achieved to explore the potential linear or non-linear correlations between NO_3^- fluxes, gene abundance and environmental factors. NO_3^- fluxes were expressed as kg NO_3^-/ha/0.5 y, i.e. the sum of NO_3^- fluxes computed for the period 01–03/2019 (winter) to 04–06/2019 (spring). We tested the relationships between NO_3^- fluxes at a given soil depth and nitrifier characteristics of the corresponding soil layer, i.e. NO_3^- fluxes at 15, 30 and 60 cm soil depths and nitrifier characteristics at 0–15, 15–30 and 30–60 cm soil layers, respectively. Furthermore, we computed NO_3^- fluxes over a range of 1, 2 and 3 months around the field campaign, and tested the relationships between these fluxes and nitrifier characteristics of the corresponding soil layer to evaluate the robustness of these relationships over time. In addition, we tested for relationships between NO_3^- fluxes at 60 cm and (i) PNA, AOA or *Nitrobacter* abundances in the 0–15 cm soil layer or (ii) the sum of PNA, AOA or *Nitrobacter* abundances (expressed per g soil) across the soil profile weighed for each soil layer, for example:

$$PNA_{weighed} = (PNA_{0-15cm} + PNA_{15-30cm} + 2\ x\ PNA_{30-60cm})/4$$

Finally, we tested the relationships between PNA, AOA or *Nitrobacter* abundances measured for the 0–15 cm soil layer in this study and measured by Laffite et al. (2020) in the same plots and for the same soil layer.

3. Results

3.1. Soil potential nitrification activity along the soil profile

The values of potential nitrification activity, PNA, were highest for the 0–15 cm soil layer, i.e. 0.054 μg N h^{-1} g^{-1} soil, intermediate at 15–30 cm depth, i.e. 0.024 μg N h^{-1} g^{-1} soil, and low at 30–60 cm soil depth, i.e. 0.014 μg N h^{-1} g^{-1} soil (Fig. 1, left). The PNA values in the upper soil layer ranged from 0.095 μg N h^{-1} g^{-1} soil for Corsican pine, i.e. a species without BNI capacity according to Laffite et al. (2020), to 0.024 μg N h^{-1} g^{-1} soil for Nordmann fir species with BNI capacity. The PNA values for Douglas fir (0.077 μg N h^{-1} g^{-1} soil; species without BNI capacity) were close to those of Corsican pine, intermediate for spruce (0.048 μg N h^{-1} g^{-1} soil; species with BNI capacity) and low for beech (0.025 μg N h^{-1} g^{-1} soil; species without BNI capacity; Fig. 1, left). The PNA values at 15–30 cm soil depth ranged from 0.039 μg N h^{-1} g^{-1} soil for Corsican pine to 0.010 μg N h^{-1} g^{-1} soil for Nordmann fir (Fig. 1, left). The PNA values of the other species with or without BNI capacity were intermediate and in the range of 0.022–0.026 μg N h^{-1} g^{-1} soil; Fig. 1, left). The PNA values for the 30–60 cm soil layer ranged from 0.021 μg N h^{-1} g^{-1} soil for spruce to 0.006 μg N h^{-1} g^{-1} soil for Nordmann fir (Fig. 1, left).

Soil NO_3^- concentration was strongly and positively correlated with PNA for the 0–15 and 15–30 cm soil layers ($R^2 = 0.91$ - $p = 0.011$, and $R^2 = 0.84$ - $p = 0.0275$, respectively; Fig. S1, top and middle), but not at 30–60 cm soil depth (Fig. S1, down).

3.2. Abundances of soil nitrifiers along the soil profile

The copy numbers of the archaeal *amoA* gene, used as a proxy for AOA abundance, were on average high for the 0–15 cm and 15–30 cm soil layers, i.e. 3.0×10^6 and 2.8×10^6 *amoA* copies g^{-1} soil, respectively, and lower for the 30–60 cm soil layer, i.e. 1.65×10^6 *amoA* copies g^{-1} soil (Fig. 1, middle). AOA abundance at 0–15 cm soil depth ranged from 5.4×10^6 to 9.5×10^5 *amoA* copies g^{-1} soil for Corsican pine and Nordmann fir, respectively (Fig. 1, middle). Conversely, AOA abundance at 30–60 cm soil depth ranged from 2.1×10^6 to 1.2×10^6 *amoA* copies g^{-1} soil for Nordmann fir and Corsican pine, respectively (Fig. 1, middle).

The abundance of bacterial *amoA* gene, used as a proxy for AOB abundance, ranged from 6.5×10^3 *amoA* copies g^{-1} soil for Nordmann fir to 2.2×10^5 *amoA* copies g^{-1} soil for Corsican pine. The abundance of bacterial *amoA* gene was below the detection limit for beech (i.e. $< 10^2$ gene copies g^{-1}; Fig. S3).

The copy numbers of the *nxrA* gene, used as a proxy for *Nitrobacter* abundance, were on average high at 0–15 cm soil depth, i.e. 1.5×10^5 *nxrA* copies g^{-1} soil, intermediate at 15–30 cm soil depth, i.e. 4.2×10^4 *nxrA* copies g^{-1} soil, and low at 30–60 cm soil depth, i.e. 8.7×10^3 *nxrA* copies g^{-1} soil (Fig. 1, right). *Nitrobacter* abundance for the 0–15 cm soil layer ranged from 3.7×10^5 to 6.3×10^3 *nxrA* copies g^{-1} soil for Corsican pine and Nordmann fir, respectively (Fig. 1, right). At 15–30 cm soil depth, it ranged from 9.9×10^4 to 7.9×10^2 *nxrA* copies g^{-1} soil for Douglas fir and Nordmann fir, respectively, whereas at 30–60 cm soil depth, it ranged 2.1×10^4 to 1.0×10^3 *nxrA* copies g^{-1} soil for Douglas fir and Nordmann fir, respectively (Fig. 1, right).

PNA was positively correlated with AOA abundance for the 0–15 cm soil layer ($R^2 = 0.87$, $p = 0.021$; Fig. 2, top left), but not for the deeper soil layers. PNA was positively correlated with *Nitrobacter* abundance for the 0–15 soil layer, ($R^2 = 0.876$, $p = 0.023$; Fig. 2, top right) but not for the 15–30 and 30–60 cm soil layers, although the relationship for the former was marginally significant ($R^2 = 0.68$, $p = 0.088$; Fig. 2, middle

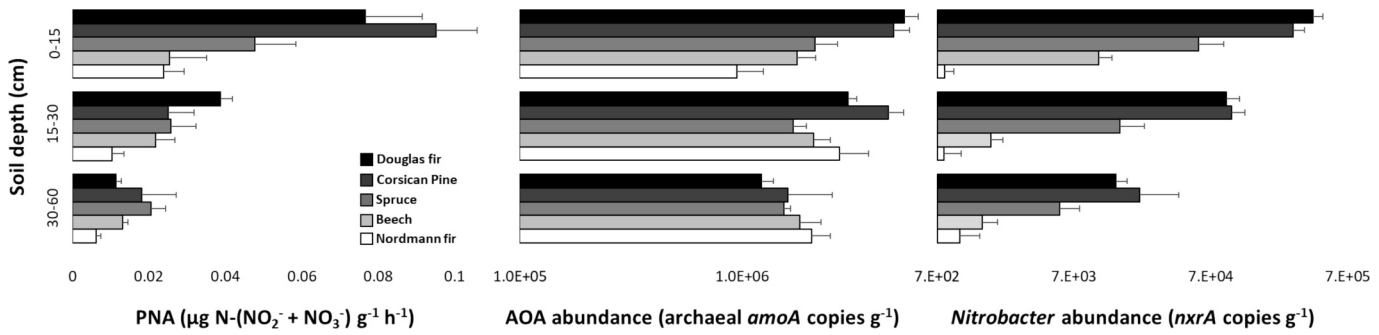


Fig. 1. Potential nitrification activity, PNA, and abundances of ammonia oxidizing archaea, AOA, and *Nitrobacter* in the different soil layers (0–15 cm, 15–30 cm and 30–60 cm depth) under the five forest tree species. One bar (colour) corresponds to one tree species.

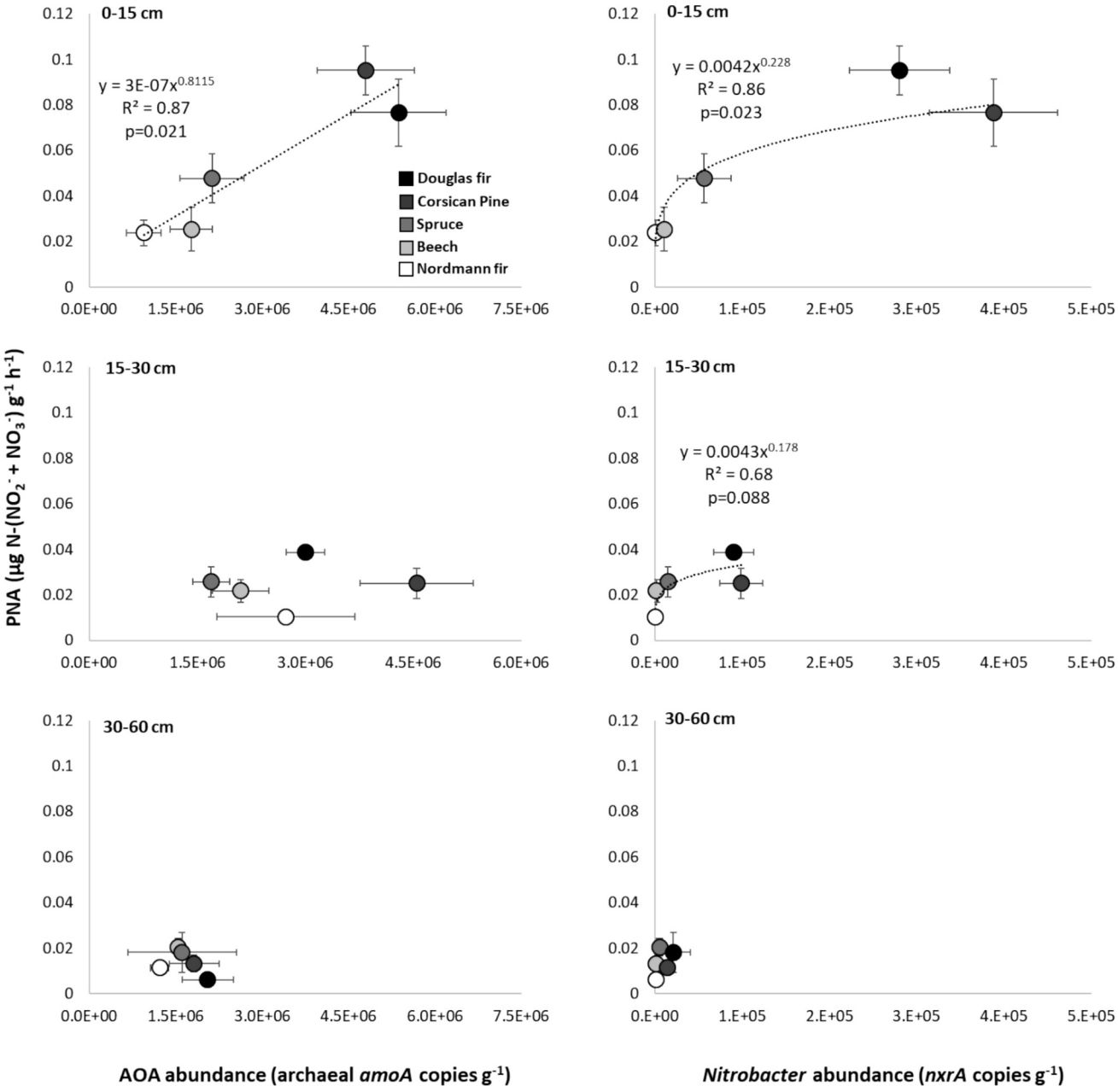


Fig. 2. Relationships between PNA and the abundances of AOA (left) or *Nitrobacter* (right) at 0–15 (top), 15–30 (middle) and 30–60 cm (down) soil depths. One point corresponds to one tree species.

and down right). AOB abundance was not correlated with PNA rates and no correlations were observed between PNA or nitrifiers abundances with soil pH (not shown).

3.3. Relationship between NO_3^- fluxes and nitrifier activity and abundance with increasing soil depth

NO_3^- fluxes over the monitoring period of 6 months were the highest at 15 cm soil depth, and the lowest at 60 cm soil depth, ranging from 17.9 Kg NO_3^-/ha/0.5 y under Douglas fir at 15 cm soil depth to 0.1 Kg NO_3^-/ha/0.5 y under beech at 60 cm soil depth. (Fig. 3). NO_3^- flux at 15 cm soil depth was strongly and positively correlated with PNA (R^2 = 0.84, p = 0.0298), AOA abundance (R^2 = 0.98, p = 0.0012) and *Nitrobacter* abundance (R^2 = 0.99, p = 0.0002; Fig. 3, top) for the topsoil. NO_3^- fluxes at 30 cm and 60 cm were strongly and positively correlated with *Nitrobacter* abundance (R^2 = 0.92, p = 0.0095 and R^2 = 0.82, p = 0.0342, respectively) at 15–30 and 30–60 cm soil layers, respectively (Fig. 3, middle and bottom right), whereas no correlation was observed between these fluxes and PNA or AOA abundance. The relationships between NO_3^- fluxes calculated for different time periods ranging from 30 to 90 days and nitrifier activity or abundance showed consistent results with those obtained when considering fluxes over the 6-months period (Table S2).

3.4. Relationship between NO_3^- leaching in the deeper soil layers and nitrifier activity and abundance in the topsoil

NO_3^- leaching flux at 60 cm soil depth was strongly and positively correlated with *Nitrobacter* abundance (R^2 = 0.88, p = 0.021; Fig. 4 top left) for the 0–15 cm soil layer. In addition, NO_3^- leaching flux at 60 cm soil depth was strongly and positively correlated with values of weighted mean PNA (R^2 = 0.82, p = 0.035; Fig. 4 down left) and *Nitrobacter* abundance (R^2 = 0.85, p = 0.026; Fig. 4 top right) calculated across the 0–60 cm soil profile. No correlation between the NO_3^- leaching flux at 60 cm and AOA abundance was observed (Fig. 4, down right).

4. Discussion

The main objective of our study was to test to what extent NO_3^- leaching in the soil profile is related to the vertical distribution of nitrifier activity and abundance by comparing monocultures of tree species with contrasting BNI capacities. This is an interesting approach because tree species with a BNI capacity exert a direct and strong effect on soil nitrification. No replicates of the monoculture stands at the Breuil-Chenue common garden experiment were setup in the 1970s to favor the size of the monoculture plots (1000 m^2 each). Therefore, the replications used here are pseudo-replicates. In ecology and forestry, technical and budgetary constraints often impose a trade-off between replication power and realism regarding biological complexity (Stewart et al., 2013; Osmond et al., 2004). Still, it was verified just before the plantation in 1976 that soil characteristics such as nutrients concentrations and pH did not significantly vary across the experimental site (Bonneau et al., 1977; Ranger et al., 2003).

4.1. Differences in potential nitrification activity between BNI and non-BNI species are higher for topsoil than for deeper soil layers

Previous work demonstrated that forest tree species have the capacity to strongly influence soil nitrification (Moukoumi et al., 2006; Zeller et al., 2007), which was confirmed here. 43 years after plantation, spruce and Nordmann fir were associated with potential nitrification rates in the topsoil lower than those associated with Corsican pine and Douglas fir. This is consistent with studies reporting the capacity of these tree species to inhibit nitrification for the same site (Laffite et al., 2020;

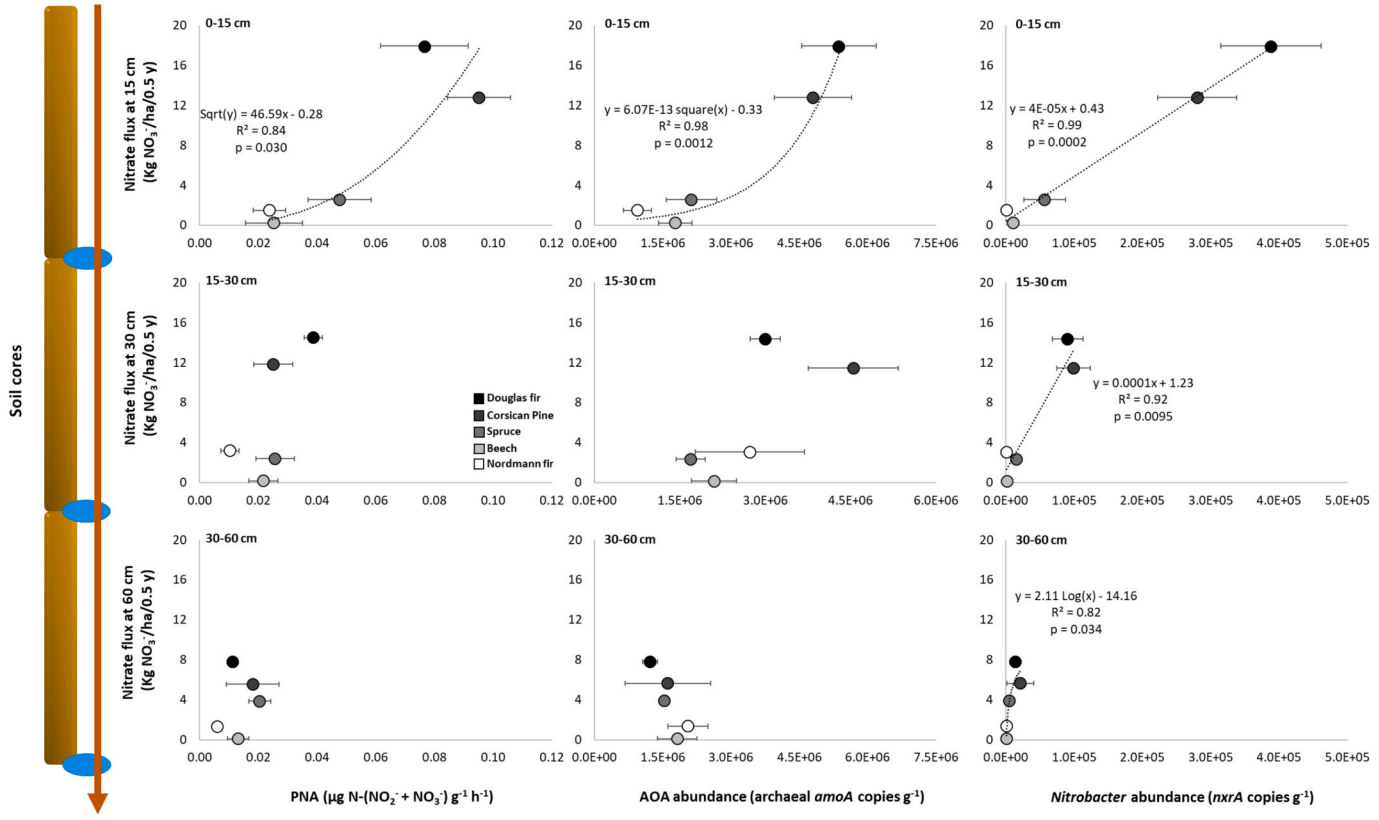


Fig. 3. Relationships between the NO_3^- flux measured at 15 (top), 30 (middle) and 60 cm soil depths (bottom), and PNA (left), AOA abundance (middle) or *Nitrobacter* abundance (right) at 0–15 (top), 15–30 (middle) and 30–60 cm (down) soil depths. One point corresponds to one tree species.

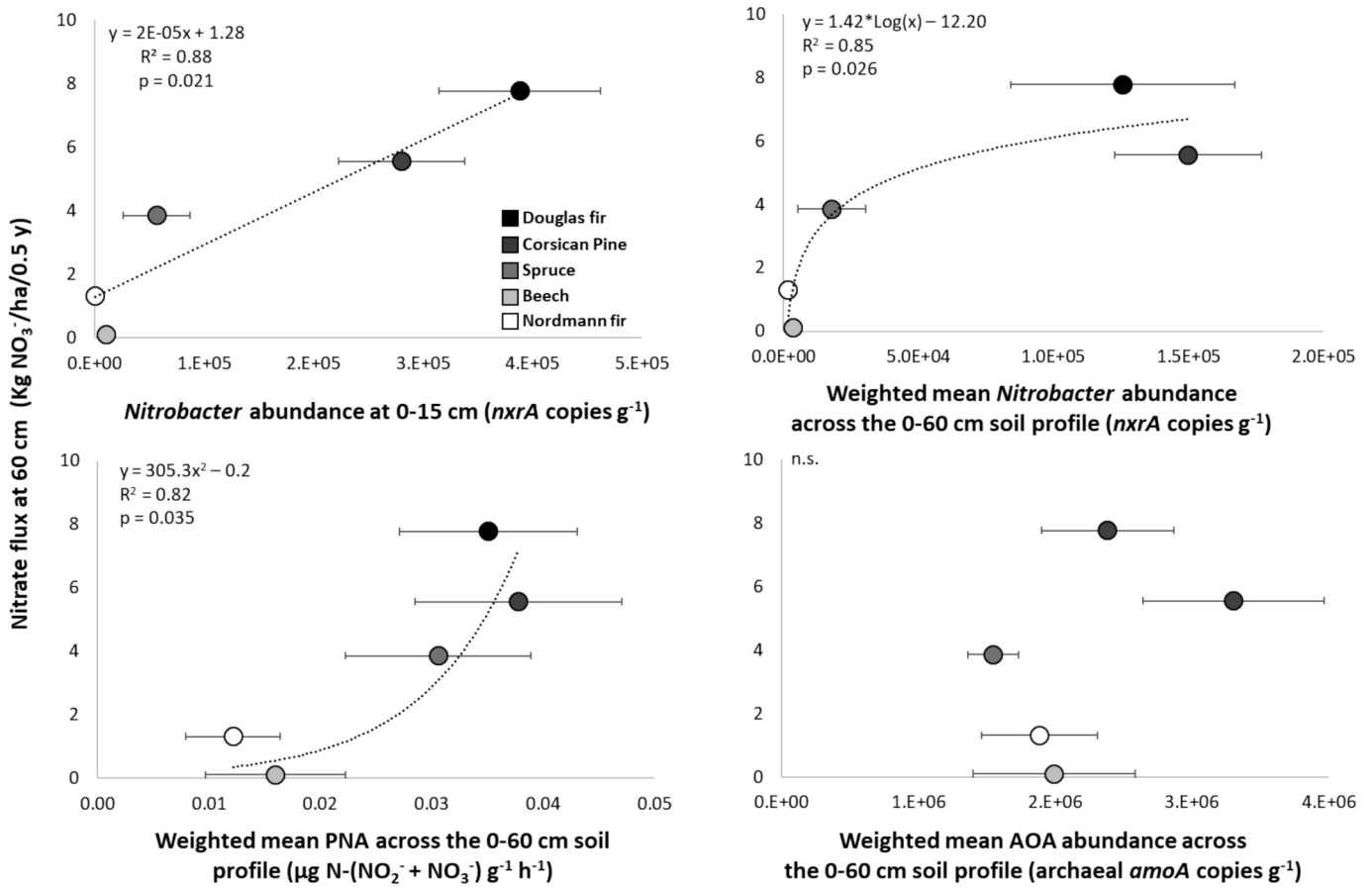


Fig. 4. Relationships between the NO_3^- flux at 60 cm depth and values of *Nitrobacter* abundance at 0–15 cm (top left), the mass weighted-mean values of PNA (down left), AOA abundance (down right) and *Nitrobacter* abundance (top right) across the 0–60 cm soil layer.

Florio et al., 2021a). However, beech species – with no BNI capacity according to Laffite et al. (2020) – exhibited PNA rates comparable or lower than those obtained for species with BNI capacities. Similarly, previous studies reported significantly low net nitrification for beech (Zak et al., 1989; Bárta et al., 2017; Florio et al., 2021a) and Verchot et al. (2001) observed highly variable net nitrification rates in beech stands, with some exhibiting near-zero rates. This indicates that beech influence on nitrification might not be stable with time, and this temporal variability would require further investigations. However, except for beech, values of PNA observed in this study for the four other tree monocultures were well correlated with those observed for soils collected in the same experimental site by Laffite et al. (2020).

Nitrification potentials drastically decreased with increasing soil depth under both BNI and non-BNI species. Microbial activity is generally higher in the surface soil than at greater depths (Speir et al., 1984), because of the accumulation of litter derived soil organic matter which is a source of energy and nutrients for microorganisms, (Bauer et al., 2000). However, the observed decrease was particularly high for the non-BNI species Douglas fir and Corsican pine, where PNA decreased by up to 85 % in the deepest layer of the profile compared to the upper soil layer. In contrast, for beech and BNI species, PNA decreased only by about 50 %. In addition, values of PNA for non-BNI species at deep soil layers were comparable to those for BNI species at the same soil depth. In addition, soil NO_3^- concentrations were correlated with PNA rates for topsoil but not for deeper soil layers. These findings indicate that (i) BNI activity might be located mainly in the topsoil rather than at deeper soil layers, and (ii) the overall decrease in PNA along the soil profile might be mainly attributed to limitation by N. One explanation would be that BNI compounds released by root exudation and/or litter decomposition are characterized by a low mobility in soils. For agricultural and grassland ecosystems, it has been observed that hydrophilic or hydrophobic BNI compounds are released from plants into the soil (Subbarao et al., 2013). Difference in BNI compounds mobility in soil depends on their differential solubility and/or affinity to water. Whether hydrophilic BNI compounds are more likely to move out of their sources which may enhance their capacity to inhibit nitrification distant from compound sources, hydrophobic compounds may remain close to roots and litter as they are strongly adsorbed on soil mineral or organic particles (Subbarao et al., 2015). The hydrophobic compounds movement in soil occurs primarily via diffusion across concentration gradients (Subbarao et al., 2013). Yet, identification and characterization of the compounds responsible for BNI, which was beyond the scope of this study, remain to be investigated. In addition, the presumable exudation of BNI compounds from roots might depend on root density and biomass along the soil profile. A decrease in root density with soil depth is generally observed in temperate forest ecosystems (Persson, 1983; Burke and Raynal, 1994), which could result in a decrease in the exudation flux with depth, as already observed for the BNI grass species *Hyparrhenia diplandra* (Lata et al., 2000). Further studies on the relationships between BNI capacity by forest tree species and root functional traits (e.g. root density, biomass, root length…) considering the soil depth are needed.

4.2. Potential nitrification activity rates along the soil profile are mainly related to Nitrobacter abundance

The abundance of AOA and *Nitrobacter* in the topsoil followed the same pattern as that observed for PNA. Indeed, nitrification rates were significantly correlated with the abundances of AOA for soils at 0–15 cm depth. No correlation was observed between nitrification rates and AOB

(Florio et al., 2021a), *Nitrospira* or comammox bacteria (Laffite et al., 2020) for soils at 0–15 cm at the same experimental site. This is consistent with a large body of literature suggesting that AOA would be the main players of ammonia oxidation in forest soils (Stopnišek et al., 2010; Zhang et al., 2012). This would be due to a higher substrate affinity and a better adaptation of AOA to catalyze reactions in acidic and N-deficient soil environments, typical for forest soils, than AOB (Verhamme et al., 2011; He et al., 2012; Hu et al., 2015). As for potential nitrification, AOA and *Nitrobacter* abundances for non-BNI species decreased with increasing soil depth. However, AOA abundance for the beech and the BNI species did not decrease with depth, and sometimes even increased. Previous studies found that AOA increased with depth in oak and cypress forest soils (Onodera et al., 2010). Deeper horizons have much less plant inputs of N (Button, 2022) as compared to the topsoil. This is even more the case for BNI soils, whose deeper soil showed less total C and total N than non BNI soils (Table S1). The increasing abundance of AOA with depth observed for BNI soils could be attributed to their abilities to thrive in oligotrophic conditions as opposed to AOB (Florio et al., 2014). In addition, AOA abundance were correlated with PNA from both BNI and non-BNI species only for the topsoil. Further, we observed low nitrification rates for deeper soil layers despite high abundances of AOA, and changes in PNA were not explained by soil pH values (not shown). These results are thus not consistent with a tree species effect on nitrification mostly mediated by an effect on ammonia oxidizers and/or pH. However, ammonia oxidizer populations, here quantified by DNA-based quantitative PCR, might have been only partially functional. This could be further explored in future research by determining to what extent ammonia oxidizer populations are functional using RNA-based approaches.

Concurrently, the abundance of *Nitrobacter* strongly decreased along the soil profile for each tree monoculture, and was strongly correlated with PNA. This strong decrease of *Nitrobacter* abundance may be explained by the decrease in both oxygen availability and organic carbon sources with increasing soil depth, since *Nitrobacter* are particularly sensitive to these two parameters in soil (Le Roux et al., 2016). More specifically, *Nitrobacter* abundance showed 500-fold changes between soils under Nordmann fir and Douglas fir, i.e. species with and without BNI capacity, respectively, whereas AOA showed only 6-fold changes between these two tree species. In addition, *Nitrobacter* abundance, not AOA, was correlated with PNA rates for subsurface soils. Although temporal variability considered in our study was restricted, we observed consistent results on values of PNA, AOA and *Nitrobacter* abundances as those obtained by Laffite et al. (2020) on the same long-term experimental site, on the same plots and for the same soil layers (Fig. S2). Taken together, these results showed that nitrification was mainly related to *Nitrobacter* abundance for topsoil, and suggests that *Nitrobacter* may have a prominent role in nitrification from forest soils under tree species with BNI capacity.

4.3. *Nitrobacter* abundance across the soil profile is the main determinant of soil NO_3^- fluxes at deep soil layers

The NO_3^- flux over a period of 0.5 years under the BNI tree species were 8-fold lower than the flux associated with the non-BNI species (with the exception of beech). This was consistent with many other studies at the same experimental site (Andrianarisoa et al., 2010; Moukoumi et al., 2006; Zeller et al., 2007; Legout et al., 2016; Florio et al., 2021a) showing that values of NO_3^- concentrations in the soil solutions and NO_3^- leaching fluxes under Douglas fir and Corsican pine were higher than those under spruce and Nordmann fir. High NO_3^- concentrations in surface and groundwater cause eutrophication of natural ecosystems and are a major concern for water quality (Myrold, 1999), especially when exceeding the European legal drinking water limit set at 50 mg L^{-1} (Council Directive 98/83/EC). Excess NO_3^- production over the entire soil profile, i.e. when its production > its consumption, is a net source of H^+ in soil causing soil acidification (Reuss and Johnson, 2012). From a H^+ budget point of view, the excess NO_3^- production in the topsoil acidifies soil solution. Proton neutralization in soil is very fast and first leads to a desaturation process eliminating the nutrient cations (i.e. Ca, Mg, K), and then to a mobilization of Al, a toxic element for plant roots and to aquatic organisms (Cronan and Grigal, 1995; Igbokwe et al., 2019), which is transferred in the soil along with NO_3^- (Reuss and Johnson, 1986). At the Breuil-Chenue site, these processes are attested by the significant correlations between NO_3^- and Ca or Mg and Al in the soil solution (Legout et al., 2016), resulting in nutrient cations depletion from the topsoil of Douglas fir and Corsican pine and NO_3^- leaching into surface water or groundwater.

Changes in nitrate leaching with depth might be linked to increased NO_3^- consumption, e. g., by denitrification. In our study, differences in potential denitrification activity for the 0–15 cm soil samples were not related to PNA rates or to NO_3^- fluxes at 15 cm (not shown). Soil under the five tree species investigated at the Breuil-Chenue experimental site is well drained (Supplementary Fig. S4) and is developed on granite, its texture being quite constant over the soil profile (Supplementary Table S3), without any drastic change in porosity. Considering these findings, we may hypothesize that the redox conditions are fairly stable along the soil profile. However, further investigation on relationships between NO_3^- fluxes and denitrifier activity and abundance along the soil profile is needed.

To our knowledge, no study has attempted to relate differences in NO_3^- fluxes in the soil under tree species to differences in soil nitrifying communities. Here, by computing weighted mean values of nitrifier activity and abundance, we showed that NO_3^- fluxes are explained by the abundance of *Nitrobacter* community across the 0–60 cm soil profile. Specifically, we demonstrated that *Nitrobacter* abundance in the uppermost 10 cm had a significant role in explaining NO_3^- fluxes from soil. In this context, the abundance of *Nitrobacter* community seems an interesting proxy for water quality at the plot scale, and a promising tool to understand and predict the risk of NO_3^- leaching from soils in these temperate forest ecosystems.

4.4. Conclusions

In this study, we investigated tree species effect on soil processes by monitoring soil solution and element fluxes, which are one of the most reactive components of the ecosystem (Zabowski and Ugolini, 1990), and thus represent a dynamic and integrative indicator (Robertson et al., 2000). Although monitoring soil solution is a powerful tool to address these ecological questions in forest ecosystems, the associated difficulty and cost are often a barrier to this approach at a large scale. In our study, we showed that (i) differences in NO_3^- fluxes under different tree species were related to their BNI (or no BNI) capacities, and that (ii) NO_3^- fluxes depend on the abundance of *Nitrobacter* community across the soil profile at the plot scale, thus validating our two hypotheses. It has recently been shown that excess in NO_3^- production under species stimulating nitrification like Douglas fir drives NO_3^- concentration in the stream water at the catchment scale (Paul et al., 2023). Furthermore, NO_3^- production and consumption, i.e. via denitrification leading to the emissions of the potent greenhouse gas N_2O, might be decoupled in soils under Douglas-fir (Florio et al., 2021b), thus exacerbating the risk of NO_3^- leaching through the soil profile. In this context, studies are needed to evaluate whether and how forest tree species with contrasting capacity regarding N uptake influence NO_3^- leaching from soil and subsequent NO_3^- concentrations in streams at the forest catchment scale and over longer periods.

CRediT authorship contribution statement

A. Florio: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **A. Legout:** Writing – review &

editing, Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **M. Marechal:** Methodology, Data curation. **M. Clesse:** Methodology, Data curation. **A. Delort:** Methodology, Data curation. **C. Creuze des Chatelliers:** Methodology, Data curation. **J. Gervaix:** Methodology, Data curation. **Y. Shi:** Validation, Formal analysis. **G. van der Heijden:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. **B. Zeller:** Writing – review & editing, Writing – original draft, Resources, Methodology, Conceptualization. **X. Le Roux:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was funded by the French Agency for Research, ANR, to the FORBES project (ANR-23-CE02-0026). The Breuil-Chenue site belongs to French national research infrastructure ANAEE-France (ANR-11-INBS-0001). We gratefully acknowledge the financial support received successively from GIP ECOFOR, AllEnvi, ANAEE France, the LTSER Zone Atelier Bassin Moselle, Office National des Forêts (ONF) and INRAE (DISC, ECODIV). The UR BEF is supported by the French National Research Agency through the Cluster of Excellence ARBRE (ANR-11-LABX-0002-01).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.178776.

Data availability

Data will be made available on request.

References

Alvarez-Cobelas, M., Angeler, D.G., Sánchez-Carrillo, S., 2008. Export of nitrogen from catchments: a worldwide analysis. Environ. Pollut. 156, 261–269. https://doi.org/10.1016/J.ENVPOL.2008.02.016.

Andrianarisoa, K.S., Zeller, B., Poly, F., Siegenfuhr, H., Bienaimé, S., Ranger, J., Dambrine, E., 2010. Control of nitrification by tree species in a common-garden experiment. Ecosystems 13, 1171–1187. https://doi.org/10.1007/s10021-010-9390-x.

Bárta, J., Tahovská, K., Šantrůčková, H., Oulehle, F., 2017. Microbial communities with distinct denitrification potential in spruce and beech soils differing in nitrate leaching. Sci. Rep. 7, 9738. https://doi.org/10.1038/s41598-017-08554-1.

Bauer, G.A., Persson, H., Persson, T., Mund, M., Hein, M., Kummetz, E., Schulze, E.D., 2000. Linking plant nutrition and ecosystem processes. Carbon and nitrogen cycling in European forest ecosystems 63–98.

Bonneau, M., Brethes, A., Lacaze, J.F., Lelong, F., Levy, G., Nys, C., Souchier, B., 1977. Modifications de la fertilité des sols sous boisements artificiels de résineux purs. Fin d'Etude D.G.R.S.T, C.R, p. 88.

Burke, M.K., Raynal, D.J., 1994. Fine root growth phenology, production, and turnover in a northern hardwood forest ecosystem. Plant Soil 162, 135–146.

Button, E., 2022. Deep soil: investigating carbon sequestration potential and greenhouse gas behaviour in agricultural subsoil. Bangor University (United Kingdom).

Chapman, S.K., Langley, J.A., Hart, S.C., Koch, G.W., 2006. Plants actively control nitrogen cycling: uncorking the microbial bottleneck. New Phytol. 169, 27–34. https://doi.org/10.1111/j.1469-8137.2005.01571.x.

Cornelis, J.T., Titeux, H., Ranger, J., Delvaux, B., 2011. Identification and distribution of the readily soluble silicon pool in a temperate forest soil below three distinct tree species. Plant Soil 342, 369–378. https://doi.org/10.1007/s11104-010-0702-x.

Cronan, C.S., Grigal, D.F., 1995. Use of calcium/aluminum ratios as indicators of stress in forest ecosystems. J. Environ. Qual. 24, 209–226.

Daims, H., Lebedeva, E.V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., Jehmlich, N., Palatinsky, M., Vierheilig, J., Bualev, A., Kirkegaard, R.H., von Bergen, M., Rattei, M., Bendinger, B., Nielsen, P.H., Wagner, M., 2015. Complete nitrification by *Nitrospira* bacteria. Nature 528, 504–509.

Dassonville, N., Guillaumaud, N., Piola, F., Meerts, P., Poly, F., 2011. Niche construction by the invasive Asian knotweeds (species complex *Fallopia*): impact on activity, abundance and community structure of denitrifiers and nitrifiers. Biol. Invasions 13, 1115–1133. https://doi.org/10.1007/s10530-011-9954-5.

De Boer, W., Tietema, A., Klein Gijnnewiek, P.J.A., Laanbroek, H.J., 1992. The chemolithotrophic ammonium-oxidizing community in a nitrogen-saturated acid forest soil in relation to pH-dependent nitrifying activity. Soil Biol. Biochem. 24, 229–234.

Florio, A., Bréfort, C., Creuze des Chatelliers, C., Gervaix, J., Poly, F., Le Roux, X., 2021. How tree species with contrasting biological nitrification inhibition capacity influence denitrifier activity and abundance? Insights from reciprocal transfers of soil. Biol. Fertil. Soils 57, 1089–1101.

Florio, A., Clark, I.M., Hirsch, P.R., Jhurreea, D., Benedetti, A., 2014. Effects of the nitrification inhibitor 3, 4-dimethylpyrazole phosphate (DMPP) on abundance and activity of ammonia oxidizers in soil. Biol. Fertil. Soils 50, 795–807.

Florio, A., Marechal, M., Legout, A., Creuse Des Chatelliers, C., Gervaix, J., Didier, S., Zeller, B., Le Roux, X., 2021b. Influence of biological nitrification inhibition by forest tree species on soil denitrifiers and N_2O emissions. Soil Biol. Biochem. 155, 108164. https://doi.org/10.1016/j.soilbio.2021.108164.

Freitag, T.E., Chang, L., Clegg, C.D., Prosser, J.I., 2005. Influence of inorganic nitrogen management regime on the diversity of nitrite-oxidizing Bacteria in agricultural grassland soils. Appl. Environ. Microbiol. 71, 8323–8334. https://doi.org/10.1128/AEM.71.12.8323-8334.2005.

Gill, R., Burke, I.C., Milchunas, D.G., Lauenroth, W.K., 1999. Relationship between root biomass and soil organic matter pools in the shortgrass steppe of eastern Colorado. Ecosystems 2, 226–236.

He, J.Z., Hu, H.W., Zhang, L.M., 2012. Current insights into the autotrophic thaumarchaeal ammonia oxidation in acidic soils. Soil Biol. Biochem. 55, 146–154.

Hu, H.W., Zhang, L.M., Yuan, C.L., Zheng, Y., Wang, J.T., Chen, D., He, J.Z., 2015. The large-scale distribution of ammonia oxidizers in paddy soils is driven by soil pH, geographic distance, and climatic factors. Front. Microbiol. 6, 938.

Igbokwe, I.O., Igwenagu, E., Igbokwe, N.A., 2019. Aluminium toxicosis: a review of toxic actions and effects. Interdiscip. Toxicol. 12, 45.

Jussy, J.H., Ranger, J., Bienaimé, S., Dambrine, E., 2004. 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. Annual of Forest Science 61, 397–408. https://doi.org/10.1051/forest:2004033.

Kozlowski, T.T., Kramer, P.J., Pallardy, S.G., 2012. The Physiological Ecology of Woody Plants. Academic press.

Krieger, D.J., 2001. Economic Value of Forest Ecosystem Services: A Review.

Kuzyakov, Y., Xu, X., 2013. Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. New Phytol. 198, 656–669. https://doi.org/10.1111/nph.12235.

Laffite, A., Florio, A., Andrianarisoa, K.S., Creuze des Chatelliers, C., Schloter-Hai, B., Ndaw, S.M., Periot, C., Schloter, M., Zeller, B., Poly, F., Le Roux, X., 2020. Biological inhibition of soil nitrification by forest tree species affects *Nitrobacter* populations. Environ. Microbiol. 22, 1141–1153. https://doi.org/10.1111/1462-2920.14905.

Lata, J.C., Guillaume, K., Degrange, V., Abbadie, L., Lensi, R., 2000. Relationships between root density of the African grass Hyparrhenia diplandra and nitrification at the decimetric scale: an inhibition–stimulation balance hypothesis. Proceedings of the Royal Society of London. Series B: biological sciences 267, 595–600.

Le Roux, X., Bouskill, N.J., Niboyet, A., Barthes, L., Dijkstra, P., Field, C.B., 2016. Predicting the responses of soil nitrite-oxidizers to multi-factorial global change: a trait-based approach. Front. Microbiol. 7, 628.

Legout, A., van der Heijden, G., Jaffrain, J., Boudot, J.-P., Ranger, J., 2016. Tree species effects on solution chemistry and major element fluxes: a case study in the Morvan (Breuil, France). For. Ecol. Manag. 378, 244–258. https://doi.org/10.1016/j.foreco.2016.07.003.

Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G.W., Prosser, J.I., Schuster, S.C., Schleper, C., 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442, 806–809. https://doi.org/10.1038/nature04983.

Madsen, E.L., 1995. Impacts of agricultural practices on subsurface microbial ecology. Adv. Agron. 54, 1–67. https://doi.org/10.1016/S0065-2113(08)60897-4.

Mareschal, L., Turpault, M.-P., Bonnaud, P., Ranger, J., 2013. Relationship between the weathering of clay minerals and the nitrification rate: a rapid tree species effect. Biogeochemistry 112, 293–309. https://doi.org/10.1007/s10533-012-9725-0.

Moukoumi, J., Munier-Lamy, C., Berthelin, J., Ranger, J., 2006. Effect of tree species substitution on organic matter biodegradability and mineral nutrient availability in a temperate topsoil. Ann. For. Sci. 63, 763–771. https://doi.org/10.1051/forest:2006057.

Myrold, D.D., 1999. Fate of nitrate in soil environment. In Principles and Applications of Soil Microbiology. In: Sylvia, D.M., Fuhrmann, J.J., Hartel, P.G., Zuberer, D.A. (Eds.). Prentice Hall, Inc., Upper Saddle River, N.J., pp. 278–281

Onodera, Y., Nakagawa, T., Takahashi, R., Tokuyama, T., 2010. Seasonal change in vertical distribution of Ammonia-oxidizing Archaea and Bacteria and their nitrification in temperate Forest soil. Soil Biol. Biochem. 25, 28–35. https://doi.org/10.1264/jsme2.ME09179.

Osmond, B., Ananyev, G., Berry, J., Langdon, C., Kolber, Z., Lin, G., 2004. Changing the way we think about global change research: scaling up in experimental ecosystem science. Glob. Change Biol. 10, 393–407. https://doi.org/10.1111/j.1529-8817.2003.00747.x.

Paul, A., Hissler, C., Florio, A., Didier, S., Pollier, B., van der Heijden, G., Dambrine, E., Ranger, J., Zeller, B., Legout, A., 2023. Douglas-fir plantations impact stream and groundwater chemistry in western Europe: insights from three case studies in France and Luxembourg. Environ. Pollut. 336, 122477. https://doi.org/10.1016/j.envpol.2023.122477.

Paul, A., Legout, A., Zeller, B., van der Heijden, G., Bonnaud, P., Reichard, A., Nourrisson, G., Ranger, J., 2022. Soil solution chemistry in 11 monitoring plots of Douglas-fir plantations in France: implications for soil fertility. Plant Soil 479, 207–231. https://doi.org/10.1007/s11104-022-05510-5.

Persson, H., 1983. The distribution and productivity of fine roots in boreal forests. Plant Soil 71, 87–101.

Prosser, J.I., Nicol, G.W., 2012. Archaeal and bacterial ammonia-oxidisers in soil: the quest for niche specialisation and differentiation. Trends Microbiol. 20, 523–531. https://doi.org/10.1016/j.tim.2012.08.001.

Ranger, J., Bonnaud, P., Gelhaye, D., 2003. Base de données 'Sols' du site atelier de Breuil (Morvan). Unité Biogéochimie des Ecosystèmes Forestiers. INRA Centre de Nancy, Champenoux. F54280.

Reuss, J.O., Johnson, D.W., 2012. Acid Deposition and the Acidification of Soils and Waters. Springer Science & Business Media.

Robertson, S.M.C., Hornung, M., Kennedy, V.H., 2000. Water chemistry of throughfall and soil water under four tree species at Gisburn, Northwest England, before and after felling. For. Ecol. Manag. 129, 101–117.

Rotthauwe, J., Witzel, K., 1997. The ammonia monooxygenase structural gene *amoA* as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. Appl. Environ. Microbiol. 63, 4704–4712.

Speir, T.W., Ross, D.J., Orchard, V.A., 1984. Spatial variability of biochemical properties in a taxonomically-uniform soil under grazed pasture. Soil Biol. Biochem. 16, 153–160.

Stewart, R.I.A., Dossena, M., Bohan, D.A., Jeppesen, E., Kordas, R.L., Ledger, M.E., 2013. Mesocosm experiments as a tool for ecological climate change research. Adv. Ecol. Res. 48, 71–181. https://doi.org/10.1016/B978-0-12-417199-2.00002-1.

Stopnišek, N., Gubry-Rangin, C., Pela Höfferle, S., Nicol, G.W., Mandic-Mulec, I., Prosser, J.I., 2010. Thaumarchaeal ammonia oxidation in an acidic forest peat soil is not influenced by ammonium amendment. Appl. Environ. Microbiol. 76, 7626–7634.

Subbarao, G.V., Sahrawat, K.L., Nakahara, K., Rao, I.M., Ishitani, M., Hash, C.T., Lata, J. C., 2013. A paradigm shift towards low-nitrifying production systems: the role of biological nitrification inhibition (BNI). Ann. Bot. 112, 297–316.

Subbarao, G.V., Yoshihashi, T., Worthington, M., Nakahara, K., Ando, Y., Sahrawat, K.L., Rao, I.M., Lata, J.C., Kishii, M., Braun, H.J., 2015. Suppression of soil nitrification by plants. Plant Sci. 233, 155–164. https://doi.org/10.1016/j.plantsci.2015.01.012.

Sudduth, E.B., Perakis, S.S., Bernhardt, E.S., 2013. Nitrate in watersheds: straight from soils to streams? Journal of geophysical resources. Biogeosciences 118, 291–302. https://doi.org/10.1002/jgrg.20030.

Tourna, M., Stieglmeier, M., Spang, A., Könneke, M., Schintlmeister, A., Urich, T., Engel, M., Schloter, M., Wagner, M., Richter, A., Schleper, C., 2011. *Nitrososphaera viennensis*, an ammonia oxidizing archaeon from soil. Proc. Natl. Acad. Sci. 108, 8420–8425.

van der Heijden, G., Hinz, A., Didier, S., Nys, C., Dambrine, E., Legout, A., 2019. Quantifying the uncertainty in modeled water drainage and nutrient leaching fluxes in forest ecosystems. Ecosystems 22, 677–698. https://doi.org/10.1007/s10021-018-0295-4.

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

Verhamme, D.T., Prosser, J.I., Nicol, G.W., 2011. Ammonia concentration determines differential growth of ammonia-oxidising archaea and bacteria in soil microcosms. ISME J. 5, 1067–1071.

Wertz, S., Poly, F., Le Roux, X., Degrange, V., 2008. Development and application of a PCR-DGGE tool to study the diversity of *Nitrobacter*-like *nxrA* sequences in soil. FEMS Microbiol. Ecol. 63, 261–271.

Zabowski, D., Ugolini, F.C., 1990. Lysimeter and centrifuge soil solutions: seasonal differences between methods. Soil Sci. Soc. Am. J. 54, 1130–1135.

Zak, D.R., Host, G.E., Pregitzer, K.S., 1989. Regional variability in nitrogen mineralization, nitrification, and overstory biomass in northern lower Michigan. Can. J. For. Res. 19, 1521–1526.

Zeller, B., Legout, A., Bienaimé, S., Gratia, B., Santenoise, P., Bonnaud, P., Ranger, J., 2019. Douglas fir stimulates nitrification in French forest soils. Sci. Rep. 9, 10687. https://doi.org/10.1038/s41598-019-47042-6.

Zeller, B., Recous, S., Kunze, M., Moukoumi, J., Colin-Belgrand, M., Bienaimé, S., Ranger, J., Dambrine, E., 2007. Influence of tree species on gross and net N transformations in forest soils. Ann. For. Sci. 64, 151–158. https://doi.org/10.1051/forest:2006099.

Zhang, L.M., Hu, H.W., Shen, J.P., He, J.Z., 2012. Ammonia-oxidizing archaea have more important role than ammonia-oxidizing bacteria in ammonia oxidation of strongly acidic soils. ISME J. 6, 1032–1045. https://doi.org/10.1038/ismej.2011.168.