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Gross Nitrogen Dynamics in the Mycorrhizosphere of an Organic Forest Soil

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Abstract

The rhizosphere is a hot-spot for biogeochemical cycles, including production of greenhouse gases, as microbial activity is stimulated by rhizodeposits released by roots and mycorrhizae. The biogeochemical cycle of nitrogen (N) in soil is complex, consisting of many simultaneously occurring processes. In situ studies investigating the effects of roots and mycorrhizae on gross N turnover rates are scarce. We conducted a ^{15}N tracer study under field conditions in a spruce forest on organic soil, zwhich was subjected to exclusion of roots and roots plus ectomycorrhizae (ECM) for 6 years by trenching. The forest soil had, over the 6-year period, an average emission of nitrous oxide (N_2O) of 5.9 ± 2.1 kg N_2O ha^{-1} $year^{-1}$. Exclusion of roots + ECM nearly tripled N_2O emissions over all years, whereas root exclusion stimulated N_2O emission only in the latest years and to a smaller extent. Gross mineralization–ammonium (NH_4^+) immobilization turnover was enhanced by the presence of roots, probably due to high inputs of labile carbon, stimulating microbial activity. We found contrasting effects of roots and ECM on N_2O emission and mineralization, as the former was decreased but the latter was stimulated by roots and ECM. The N_2O emission was positively related to the ratio of gross NH_4^+ oxidation (that is, autotrophic nitrification) to NH_4^+ immobilization. Ammonium oxidation was only stimulated by the presence of ECM, but not by the presence of roots. Overall, we conclude that plants and their mycorrhizal symbionts actively control soil N cycling, thereby also affecting N_2O emissions from forest soils. Consequently, adapted forest management with permanent tree cover avoiding clearcutting could be a means to reduce N_2O emissions and potential N leaching; despite higher mineralization in the presence of roots and ECM, N_2O emissions are decreased as the relative importance of NH_4^+ oxidation is decreased, mainly due to a stimulated microbial NH_4^+ immobilization in the mycorrhizosphere.

Key words: histosol; mineralization–immobilization turnover; nitrification; nitrous oxide emissions; Norway spruce; ^{15}N tracer.

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Authors contributions TR, LK and PB planned and designed the ^{15}N experiment; MH conducted ^{15}N experiment and data analysis under supervision by TR and YK; MA conducted N_2O measurements under supervision by ÅK and LK; MH together with TR wrote the first draft of the paper; all authors participated in writing of the final paper as well as discussion and interpretation of results.

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Introduction

Nitrogen (N) is the nutrient that most often limits productivity of terrestrial ecosystems (Vitousek and Howarth 1991; LeBauer and Treseder 2008). On the other hand, excess N has negative consequences for the environment (Sutton and others 2011). One particular concern is the emission of nitrous oxide (N_2O), which is both a powerful greenhouse gas (Badr and Probert 1993) as well as the main contributor to destruction of stratospheric ozone (Ravishankara and others 2009). Emission of N_2O from terrestrial ecosystems is directly related to the soil N cycle, which is complex in nature as it includes several simultaneously occurring transformation processes often working in opposite directions (Myrold and Tiedje 1986; Nason and Myrold 1991; Hart and others 1994). The rates of the different N cycle processes are influenced by various factors such as ecosystem type, soil type, land cultivation, microbial community and by the plant itself (Mary and others 1996; Canary and others 2000; Gödde and Conrad 2000; Chapman and others 2006). The rhizosphere has been identified as a hot-spot for many biogeochemical processes, including production of greenhouse gases (Philippot and others 2008; Frank and Groffman 2009).

Plants affect soil N cycling mainly by exudation of labile carbon (C) to the rhizosphere that stimulates microbial activity (Frank and Groffman 2009). This may cause a higher gross N mineralization in the rhizosphere compared to bulk soil, which has been found in laboratory (Herman and others 2006; Landi and others 2006) and greenhouse studies (Norton and Firestone 1996; Dijkstra and others 2009). However, these studies might not be representative of in situ conditions. Only few studies have investigated changes in gross N transformation rates by in situ root/mycorrhizae exclusion (Ross and others 2001; Holub and others 2005). However, the effect of ectomycorrhizae (ECM) on gross N transformations is unclear, but potentially even more important than those of roots (Ernfors and others 2011). In a trenching experiment on organic forest soil, Ernfors and others (2011) reported a doubling of N_2O emissions after exclusion of ECM plus roots, whereas exclusion of roots alone did not affect N_2O emissions.

To explain the findings by Ernfors and others (2011), we conducted an in situ ^{15}N tracer study with and without exclusion of roots and roots plus ECM. Our aim was to elucidate how the internal soil N cycle changed as a consequence of exclusion of roots or roots and ECM. In particular, we wanted to reveal changes of gross N transformation rates that might lead to increased N_2O emissions. A secondary aim was to investigate the persistence of root and ECM effects on the N_2O emission. As root exudates stimulate microbial activity, we hypothesized that trenching reduces gross mineralization and NH_4^+ immobilization rates. We further hypothesized that an increased relative importance of nitrification for NH_4^+ consumption, caused by decreased NH_4^+ immobilization, can explain higher N_2O emissions after exclusion of ECM.

Materials and Methods

Site Description

The experiment was conducted at the Skogaryd Research Catchment, a part of the SITES station network (www.fieldsites.se), located in southwestern Sweden (58°23′N, 12°09′E) 60 m above sea level. Long-term (1961–1990) mean annual temperature and mean annual precipitation measured at Vänersborg weather station (12 km east of the site) are 6.4°C and 709 mm, respectively. The site is a well-drained former mesotrophic peatland with peat depths greater than one metre, which was afforested by Norway spruce (*Picea abies* (L.) Karst.) in the 1950s. For a more detailed description of the site, see Ernfors and others (2011) and Meyer and others (2013). General soil properties are shown in Table 1.

Experimental Setup

The root trenching experiment was established in August 2007. Prior to that, greenhouse gas emissions had been measured for 1 year. The experiment is a randomized block design including six replicates (see Ernfors and others 2011). Each block consists of three plots of 1 m^2, which were randomly assigned to one of the three treatments: (a) control (Ctrl), (b) roots excluded (exclR) and (c) roots and ECM excluded (exclRM). Each plot consists of a collar for GHG measurements and additionally of two spots for the injection of ^{15}N-labelled nitrate (NO_3^-) or ammonium (NH_4^+). In total, the experiment includes 18 plots (six replicated blocks x three treatments = 18). The trenching was implemented by the installation of a rectangular frame (1 × 1 m, 0.6 m high) made out of PVC. Into each side of the frame a rectangular hole was cut. Into these holes nylon mesh was inserted. For exclR, the nylon mesh had a diameter of 50 μm allowing penetration of hyphae, but not

Table 1. Gravimetric Water Content (GWC), Soil Organic Matter (SOM), Total Carbon (TC), Total Nitrogen (TN) and C/N Ratio for an Organic Forest Soil in South-Western Sweden

Treatment	GWC (%)	SOM (%)	TC (g kg^{-1})	TN (g kg^{-1})	C/N
Control	270.5 (94.1)	72.8 (15.2)	346.3 (66.4)	17.5 (2.3)	19.8 (3.2)
exclR	292.2 (130.6)	63.9 (14.5)	376.1 (76.2)	18.3 (2.7)	20.6 (3.3)
exclRM	315.7 (121.1)	72.1 (18.4)	386.4 (59.3)	19.1 (3.4)	20.5 (3.3)

Treatments were exclR = roots excluded, exclRM = roots and mycorrhizae excluded. Standard deviation is given in brackets; n = 6. None of the soil properties were significantly affected by treatments (one-way analysis of variance (ANOVA) or Kruskal–Wallis test for SOM).

of the roots, and the hole had the size of 38 × 60 cm, whereas for exclRM the nylon mesh had a diameter of 1 µm, which is smaller than the hyphae diameter, and the hole a size of 38 × 30 cm (Ernfors and others 2011).

N_2O Flux Measurements

Nitrous oxide fluxes at the soil surface were measured using static closed chambers made of stainless steel. Each chamber consisted of a permanently installed collar and a manually operated lid, which was equipped with an inserted butyl rubber septum for gas sampling. The flux measurements were carried out every 2 weeks from Aug. 2006 to July 2013 generally during the morning and early afternoon, except for the periods Dec. 2009 to April 2010 and Dec. 2010 to Feb. 2011 due to snow cover. During flux measurements, the lids of the chambers were placed on the collars. Gas samples were collected 4, 8, 16 and 32 min after closing the chambers in 22-ml glass vials sealed with butyl rubber septa by circulating the air for 30 s between the chambers and the glass vials using an electric pump. The collected samples were analysed afterwards by gas chromatography (Agilent 7890A, Agilent Technologies, Santa Clara, CA, USA) equipped with an auto-sampler (7697A). The N_2O fluxes were calculated from the slope of the linear regression of gas concentrations plotted against time. A detailed description of the chambers, gas sampling and flux calculation can be found in Ernfors and others (2011), who reported fluxes from the experiment until Dec. 2009.

^{15}N Labelling and Soil Sampling

Gross soil N dynamics were investigated in situ. ^{15}N tracing was conducted in May 2013 using the virtual soil core approach (Rütting and others 2011; Staelens and others 2012). For each trenching frame, five sampling locations (6 × 6 cm) were marked by sticks in two opposing corners, allowing sampling at five different time steps. The distance between each location was at least 5 cm. The ^{15}N labelling was conducted by injection of either $^{15}NH_4NO_3$ or $NH_4^{15}NO_3$ (99%) at eleven spots per location each 1 ml to a soil depth of 5.5 cm using spinal needles with a diameter of 0.65 mm and a template that was slipped over the marking sticks (Rütting and others 2011). In each sampling location, 19.71 µg ^{15}N was injected which resulted in a content of 0.48 µg ^{15}N g^{-1} dry soil. Soil sampling was conducted using another template and PVC tubes with a diameter of 4 cm, sampling a core from the centre of the labelling spot to a depth of 6 cm. Soil sampling was conducted 1.3, 25.8, 72.7, 144.3 and 239.3 h after labelling.

Sample Preparation and Analysis

Thirty grams of fresh soil, devoid of coarse materials, was extracted with 60 ml of 2 M KCl, which was shaken for 60 min (250 rpm) followed by filtration with Whatman G/F filter paper, after 30 min of sedimentation. Extracts were stored frozen until further analyses. Concentrations of NH_4^+ and NO_3^- were determined using an auto-analyser (AA3, Bran and Luebbe, Germany). For ^{15}N analysis, NH_4^+ and NO_3^- were separately converted to N_2O (Laughlin and others 1997; Stevens and Laughlin 1994). The ^{15}N in the N_2O was then analysed using a trace gas preparation unit (ANCA-TGII, PDZ Europa, UK) coupled to an Isotope Ratio Mass Spectrometer (IRMS) (20–20, SerCon, UK). Bulk soil ^{15}N was determined on oven-dried and ground soil using an elemental analyser (Fisons-Instruments, Rodano, Milano, Italy) connected to a Delta plus IRMS (Finnigan MAT, Bremen, Germany). Soil organic matter (SOM) content was determined by loss-on-ignition at 550°C.

Data Analysis and Statistics

The calculation of gross N transformation rates was conducted via the ^{15}N tracing model *Ntrace* (Müller and others 2007). The conceptual model included

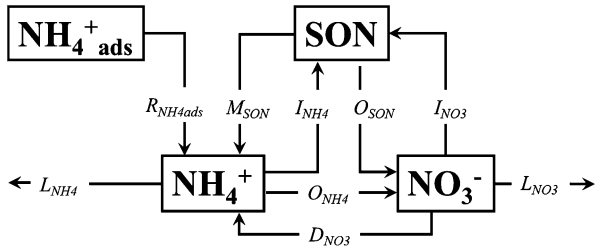

Figure 1. Conceptual model of the soil nitrogen (N) cycle for quantification of gross N transformation rates in a drained organic forest soil in south-western Sweden (modified from Müller and others 2004 and Staelens and others 2012). Considered N pools are ammonium (NH_4^+), nitrate (NO_3^-), soil organic nitrogen (SON) and NH_4^+ adsorbed to soil particles ($NH_4^+{}_{ads}$). The N transformations are described in Table 2 (R_{NH_4ads} was not considered in final data analysis, as inclusion did not improve the model fit).

four N pools and nine N transformation processes (Figure 1). The N pools are NH_4^+, NO_3^-, soil organic N pool (SON) and one pool for adsorbed NH_4^+ ($NH_4^+{}_{ads}$). The processes and kinetic settings are presented in Table 2. Initial contents of the mineral N pools and ^{15}N enrichment were estimated by linear back-extrapolation of the first two data points (Müller and others 2004), and for SON measured total soil N content (TN; Table 1) was used. Based on the ^{15}N enrichment and content of TN, the recovery of added ^{15}N was calculated. The initial ^{15}N amount in TN was linearly back-extrapolated from the first two measurements and was set to 100%, due to uncertainty in the exact amount of soil per labelled virtual soil core. In the $^{15}NH_4^+$-labelled exclR and exclRM soil, not all of the ^{15}N was initially (at t = 1.3 h) recovered in the form of NH_4^+, indicating an adsorption of $^{15}NH_4^+$. This adsorbed NH_4^+ was explicitly considered in the *Ntrace* model ($NH_4^+{}_{ads}$ pool; Figure 1), which was initialized as described in Münchmeyer (2001). Based on the total ^{15}N recovery, loss fluxes of NH_4^+ (L_{NH_4}) and NO_3^- (L_{NO_3}) were estimated, which include emission of gaseous N species, leaching, lateral diffusion and roots/ECM N uptake.

Parameter optimization was carried out using a Markov chain Monte Carlo approach, which performs a random walk in the model parameter space (Müller and others 2007). The optimization algorithm was programmed in MatLab 7.9 (The MathWorks Inc.) and calls the model set up in Simulink (Version 6, The MathWorks Inc.). Gross N transformations rates are quantified by minimizing the misfit function expressed as a quadratic weighted error between the observed data and the model data, as described in detail elsewhere (Müller and others 2007). For the fluxes that followed first-order kinetics, average rates were calculated by integrating the gross N rates over the duration of the experiment divided by the time (Rütting and Müller 2007). To find the model that best described the measured data, several model modifications regarding the kinetic settings and considered N transformation processes were tested. This procedure was guided by the Akaike's information criterion (AIC) (see Staelens and others 2012). Based on this, the release of adsorbed NH_4^+ (R_{NH_4ads}) was not considered for final data analysis, as this did not improve the model fit of the experimental data. Net mineralization and net nitrification rates were calculated as the sum of all modelled processes producing, subtracted by the sum of all modelled processes consuming, NH_4^+ or NO_3^-, respectively.

Table 2. Gross N Transformation Rates (Mean ± Standard Deviation) for an Organic Forest Soil in South-Western Sweden, for Control Soil and Soil with Exclusion of Roots (exclR) or Exclusion of Roots and Ectomycorrhiza (exclRM)

N transformation		Kinetic	Transformation rate (μg N g^{-1} d^{-1})		
			Control	exclR	exclRM
M_{SON}	Mineralization of SON	0	4.49 (0.55)[a]	4.46 (0.78)[a]	1.34 (0.32)[b]
I_{NH_4}	Immobilization of NH_4^+	1	2.21 (0.44)[a]	1.77 (0.33)[b]	0.80 (0.21)[c]
O_{SON}	Oxidation of SON to NO_3^-	0	5.67 (0.54)[a]	3.91 (0.51)[c]	4.59 (0.38)[b]
I_{NO_3}	Immobilization of NO_3^-	1	0.95 (0.29)	n.d.	n.d.
O_{NH_4}	Oxidation of NH_4^+ to NO_3^-	1	1.68 (0.36)[a]	2.75 (0.37)[b]	1.72 (0.47)[a]
D_{NO_3}	Dissimilatory NO_3^- reduction to NH_4^+	1	1.42 (0.18)[b]	2.04 (0.39)[a]	0.85 (0.23)[c]
L_{NH_4}	Losses of NH_4^+	1	1.34 (0.30)[b]	2.63 (0.30)[a]	n.d.
L_{NO_3}	Losses of NO_3^-	1	4.48 (0.39)[a]	2.63 (0.31)[c]	3.99 (0.29)[b]

Gross rates that were significantly different between treatments are followed by different letters within a row. Kinetics were either zero order or first order; n.d. = not detected.

For evaluating statistical differences in the gross N fluxes between the treatments, the method proposed by Payton and others (2000) was applied, which compares the 85% confidence intervals of the N fluxes between treatments. This statistical approach was applied as the high number of iterations of the ^{15}N tracing model does not allow the application of more common statistical tests (Rütting and others 2010). Treatment effects on annual N_2O emission were evaluated by testing if the confidence intervals of the response ratios overlap, as suggested by Ernfors and others (2011). Response ratios were calculated as the natural logarithm of the ratio of annual N_2O emission from treated plots and control plot, separately calculated for the two trenching treatments and each experimental year.

Results

N_2O Fluxes

The annual fluxes of the control plots varied between 2.0 and 12.2 kg N_2O ha^{-1} y^{-1} (Figure 4). Peak emissions occurred during summer months with warm soil conditions, and these fluxes generally follow the order exclRM > exclR > ctrl (Supplemental Figure 1). In all years following trenching, exclRM had higher annual N_2O emissions compared to control, whereas for exclR an enhanced N_2O emission was only apparent from the third year after trenching onwards (Figure 4). Summarized over the entire 6-year period, exclusion of roots increased N_2O emission by 62%, whereas concurrent exclusion of ECM almost tripled N_2O emission compared to control (Figure 4).

^{15}N Recovery in Soil

The amount of ^{15}N in soil decreased over the course of the experiment in all treatments, with the $^{15}NO_3^-$-labelled soil showing a slightly steeper decline compared to the soils labelled with $^{15}NH_4^+$ (Supplemental Figure 2). For the control treatment, measured ^{15}N recovery at day 0 in the $^{15}NH_4^+$ labelling was lower compared to day 1. At the end of the incubation, exclRM had the highest ^{15}N recovery (70 ± 11% of the ^{15}N input) and exclR the lowest (56 ± 8%) for the $^{15}NH_4^+$ tracer (Supplemental Figure 2). Most of the ^{15}N recovered in control soil was immobilized into the SOM, which was lower for the exclR and exclRM treatments (Supplemental Figure 3). For the $^{15}NO_3^-$ tracer, the final ^{15}N recovery was considerably lower than that for the $^{15}NH_4^+$ tracer and was lowest for control soil (32 ± 6%) and highest for exclR soil (44 ± 5%). Only small amounts of the added $^{15}NO_3^-$ were recovered in soil organic matter (Supplemental Figure 3).

N Pool Sizes, ^{15}N Enrichment Over Time and Model Fit

For the control treatment, the content of both NH_4^+ and NO_3^- remained constant during the experiment (Figures 2 and 3), but were highly variable ranging from 8.9 to 22.2 μg N g^{-1} for NH_4^+ and from 8.6 to 21.6 μg N g^{-1} for NO_3^-. Ammonium contents for the treatment excluding roots were lower compared to the control and decreased slightly over time. A similar pattern was found for the exclRM treatment which exhibited even lower NH_4^+ contents. Nitrate contents exhibited a similar increase in the exclR and exclRM treatments, from about 20 μg N g^{-1} to approximately 40 μg N g^{-1} at the end of the experiment.

The ^{15}N enrichment of the NH_4^+ pool labelled with $^{15}NH_4^+$ decreased exponentially during the experiment for all treatments to about 0.5% (Figure 2), which was steeper for the exclR and exclRM treatments. After applying the $^{15}NO_3^-$ tracer, the ^{15}N enrichment of the NH_4^+ pool increased until the third day of the experiment and decreased afterwards (Figure 2), whereas the ^{15}N enrichment of NO_3^- decreased throughout the experiment and was lower for the treatments exclR and exclRM compared to control (Figure 3). In the $^{15}NH_4^+$-labelled soil, the ^{15}N enrichment of $^{15}NO_3$ increased until the third day after the labelling and decreased afterwards. The control showed slightly lower enrichment of $^{15}NO_3$ after applying $^{15}NH_4^+$ compared to the treatments exclR and exclRM.

In general, the final chosen models fit well to the data for ^{15}N recovery for total nitrogen (Supplemental Figure 2) as well as for NH_4^+ and NO_3^- (Figures 2 and 3). One exception is the $^{15}NH_4^+$ enrichment in the control after labelling with $^{15}NH_4^+$, where the dilution was underestimated by the model (Figure 2), which leads to an underestimation of gross mineralization. Further, the model slightly overestimated $^{15}NO_3^-$ enrichment in the exclRM treatment after adding $^{15}NO_3$ tracer.

Gross and Net N Transformation Rates

Gross NH_4^+ immobilization (I_{NH_4}) was about half as high as mineralization of soil organic N (M_{SON}) in all treatments. Exclusion of roots did not affect gross mineralization, but decreased NH_4^+ immobilization by 20% compared to control (Table 2). In contrast, excluding roots + ECM decreased gross

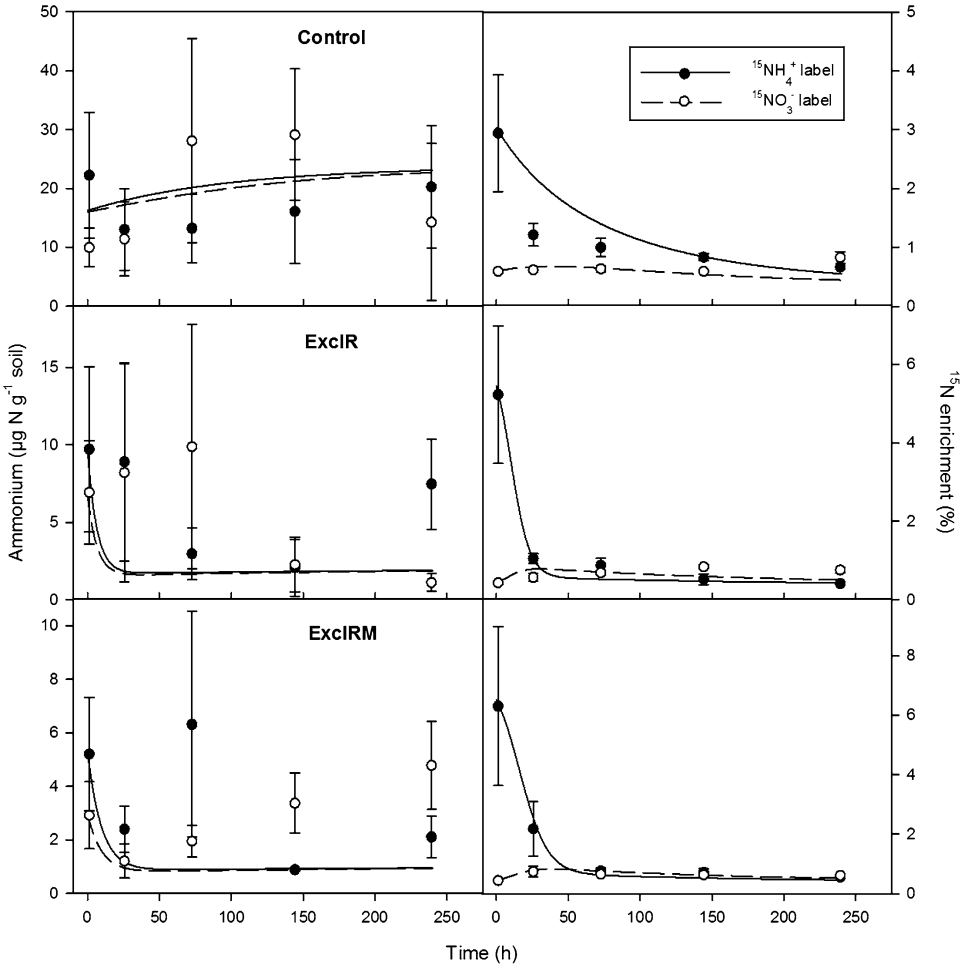

Figure 2. Measured (*symbols*; mean ± standard deviation; $n = 6$) and fitted (*lines*) ammonium concentrations (*left*) and ^{15}N enrichment of ammonium (*right*) during the 10-day soil incubations after addition of either $^{15}NH_4NO_3$ or $NH_4^{15}NO_3$ to an organic forest soil. Results are shown for control soil and soil with exclusion of roots (exclR) or exclusion of roots and ectomycorrhiza (exclRM).

rates of both processes by 64% for I_{NH_4} and 70% for M_{SON}. For NH_4^+ oxidation (O_{NH_4}), the gross rates for control and exclRM were not significantly different from each other, but exclR exhibited enhanced O_{NH_4} by 64%. Overall, a similar pattern as for O_{NH_4} was found for dissimilarity NO_3^- reduction to NH_4^+ (DNRA), but the gross rate was only half in exclRM compared to control (Table 2). Nitrification using organic N as a substrate (O_{SON}) was highest in control and decreased by 31% in exclR and 19% in exclRM. Immobilization of NO_3^- (I_{NO_3}) was only occurring in the control (Table 2).

The losses of NH_4^+ (L_{NH_4}) and NO_3^- (L_{NO_3}) were estimated based on the total ^{15}N recovery (Supplemental Figure 2). High L_{NH_4} was found for the exclR treatment, which was about twice as high as in the control (Table 2), whereas no L_{NH_4} was detected in the exclRM treatment. In contrast, highest L_{NO_3} occurred in the control soil and was lowest in exclR treatment (Table 2).

Although net mineralization in the control was 0.59 µg N g^{-1} d^{-1}, net N immobilization occurred for the trenched treatments (−0.96 for exclR and −0.47 µg N g^{-1} d^{-1} for exclRM). The opposite was true for net nitrification, which increased with trenching, and was 0.5 µg N g^{-1} d^{-1} for the control but 2.08 and 2.18 µg N g^{-1} d^{-1} for the trenched treatments.

Discussion

N_2O Emissions

Ernfors and others (2011) reported and discussed N_2O emissions during 2.5 years following trenching. We here extend the time series, covering now 6 years after trenching. Although during the initial period N_2O emissions were only affected in the exclRM treatment (Ernfors and others 2011), a novel pattern emerges in the new data set. From the third year after trenching, N_2O emissions were also consistently enhanced in the exclR treatment, though to a smaller extent than in the exclRM treatment. Moreover, the stimulation of N_2O emission from exclRM was stronger during years 3–6 compared to the first two years, even though we

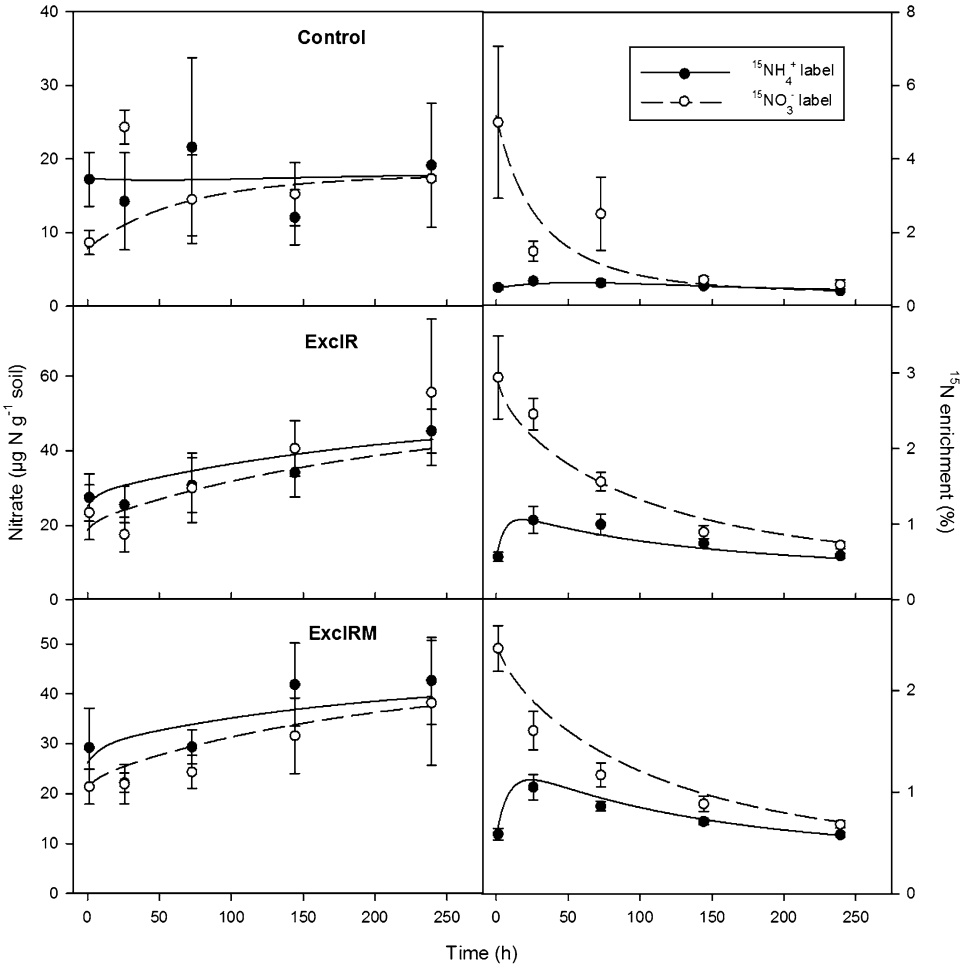

Figure 3. Measured (*symbols*; mean ± standard deviation; $n = 6$) and fitted (*lines*) nitrate concentrations (*left*) and ^{15}N enrichment of nitrate (*right*) during the 10-day soil incubations after addition of either $^{15}NH_4NO_3$ or $NH_4^{15}NO_3$ to an organic forest soil. Results are shown for control soil and soil with exclusion of roots (exclR) or exclusion of roots and ectomycorrhiza (exclRM).

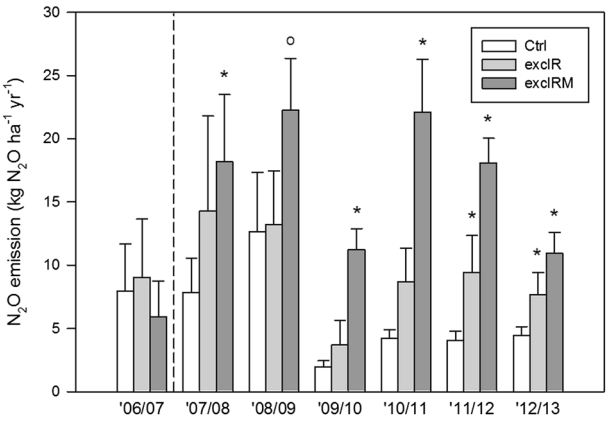

Figure 4. Annual nitrous oxide (N_2O) emission for an organic forest soil in south-western Sweden for three treatments: Ctrl = control, exclR = root exclusion, exclRM = exclusion of roots and mycorrhizae (2006/2007 = pre-treatment). Variation is given as standard error of mean (*$P < 0.05$; °$P < 0.1$; $n = 6$).

see a declining trend in year 6 (Figure 4). Ernfors and others (2011) suggested decreased N uptake by trees, mainly via the ectomycorrhizal symbiont, as the most likely explanation for a stimulated N_2O emissions after exclusion of roots plus ECM. In the later years, we also observed a stimulation of N_2O emission in exclR, which suggests that roots also play a role in N uptake.

The increase in N_2O emission in the order control < exclR < exclRM reverses the pattern that we observed for gross mineralization, which contrasts other studies reporting a positive correlation between these two processes (Matson and Vitousek 1987; Ambus 2005). Our findings suggest that factors other than mineral N supply are important for regulating N_2O emissions. For forests and agricultural ecosystems, the ratio between the competing processes of autotrophic nitrification (NH_4^+ oxidation) and NH_4^+ immobilization (*N*/*I*) has been suggested as a proxy for ecosystem N losses via NO_3^- leaching (Tietema and Wessel 1992; Stockdale and others 2002). As NO_3^- is the precursor for denitrification, supposedly the main pathway of N_2O production in the investigated site (Björk and others 2010), we propose that the *N*/*I* ratio should also be a useful proxy for predicting N_2O emissions.

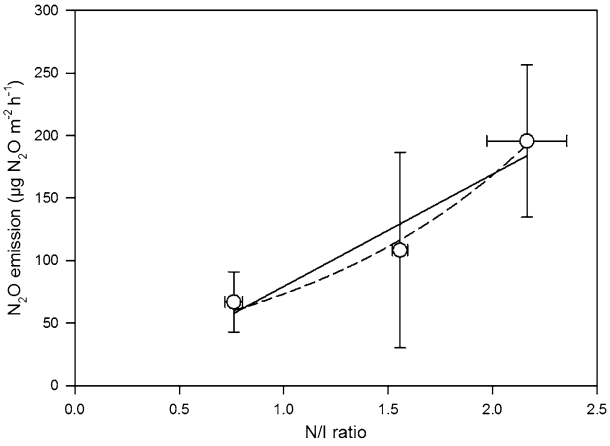

Figure 5. Correlation between average nitrous oxide (N_2O) emissions over a 6-year period for three different treatments in an organic forest soil in south-western Sweden and the ratio between gross NH_4^+ oxidation (O_{NH_4}; nitrification) and gross NH_4^+ immobilization (I_{NH_4}). Data are means and standard error; regression lines are presented for linear (*solid*; $R^2_{adj} = 0.85$) and exponential (*dashed*; $R^2_{adj} = 0.97$) fit.

Indeed, using the treatment averages of N_2O fluxes over all 6 years, a strong correlation between N_2O emissions and *N*/*I* ratio (calculated as O_{NH_4}/I_{NH_4}) was found (Figure 5). This finding points to the importance of NH_4^+ immobilization for N retention, which generally is enhanced in planted soil due to a rhizosphere priming effect (Pausch and others 2013).

Mineralization and NH_4^+ Immobilization

Rates of gross N mineralization are within the range of 0–10 µg N g^{-1} d^{-1} for temperate forests reviewed by Booth and others (2005), which only included mineral forest soils or organic forest layers. Studies investigating N transformation rates in forested organic soils are scarce. For organic soils forested with birch and poplar, gross mineralization rates ranged from 3.5 to 5.9 µg N g^{-1} d^{-1} (Willison and others 1998; Münchmeyer 2001), which is very similar to the rate of 4.4 µg N g^{-1} d^{-1} for the control plots in the present study. However, Westbrook and Devito (2004) found for a black spruce forest on organic soil a four-time higher gross mineralization (18.0 µg N g^{-1} d^{-1}), which might be related to tree species effect. Indeed, it has been shown that decomposition is higher for black spruce compared to Norway spruce needle litter (Lorenz and others 2000).

The similar pattern in response to root and ECM exclusion for gross mineralization and NH_4^+ immobilization is in agreement with Booth and others (2005) who found a positive correlation between the two processes. Reduced mineralization has previously been found in forest soils after trenching (Ross and others 2001) and tree girdling (Zeller and others 2008; Kaiser and others 2011), even though one study found increased gross mineralization after trenching (Holub and others 2005). The generally higher gross mineralization in soils with active roots is most likely a consequence of a rhizosphere priming effect (Kuzyakov 2010; Pausch and others 2013). Increased input of labile C via (mycor-)rhizodeposition stimulated microbial activity and therefore N mineralization. We hypothesized a decreased gross mineralization after trenching and expected a continuous decline towards the exclRM treatment caused by a decline in C availability. However, gross mineralization was only reduced in the exclRM treatment, but not in exclR compared to control. There could be two reasons for this: (i) mycorrhizal hyphae accelerated the rates of decomposition of N-containing organic material by stimulating soil bacteria (Hodge and others 2001; Herman and others 2006); or (ii) mycorrhizal hyphae directly mineralized organic matter (Chapman and others 2006; Jackson and others 2008; Frank and Groffman 2009). As the latter process is restricted to N-limited ecosystems (Schimel and Bennett 2004; Wu 2011), this is not a likely explanation for the investigated site. However, gross mineralization for the control treatment might have been underestimated, as the tracing model could not accurately simulate the dilution of $^{15}NH_4^+$ (Figure 2). We therefore expect that N mineralization most likely continuously declines from control over exclR towards exclRM, caused by a decrease in availability of labile C.

In contrast to gross mineralization, we found a decrease of net N mineralization after trenching. This is in agreement with other studies that found that N availability was increased in the rhizosphere compared to bulk soil (Herman and others 2006; Phillips and Fahey 2006) and that root exudation promotes N availability (Hamilton and Frank 2001). Increased net mineralization in the rhizosphere can be caused by several mechanisms, including faunal grazing, spatial heterogeneity of labile C and a fast turnover of the microbial biomass (Jackson and others 2008; Frank and Groffman 2009). However, contrasting results of increased net mineralization after trenching have been found by Fisher and Gosz (1986) and Ross and others (2001), which might be caused by the short period between trenching and sampling in

these experiments and the possibility that decaying roots in trenched plots increased net N mineralization (Ehrenfeld and others 1997).

Nitrification and NO_3^- Immobilization

The total gross nitrification ($O_{SON} + O_{NH_4}$) of 6.3–7.4 μg N g^{-1} day^{-1} is within the range of 0–10 μg N g^{-1} day^{-1} for temperate forests reported by Booth and others (2005) and comparable to those found in organic forest soils (Willison and others 1998; Münchmeyer 2001). The investigated organic forest soil is characterized by a large contribution of the organic pathway of heterotrophic nitrification (O_{SON}) to total nitrification, accounting for 77% in control. Such a dominance of O_{SON} has also been reported for other forest soils (Hart and others 1997; Rütting and others 2008; Zhu and others 2011; Staelens and others 2012). When roots were excluded (exclR), the relative importance of NH_4^+ oxidation for nitrification increased to 41%, due to a combination of decreased O_{SON} and increased O_{NH_4}.

Total gross nitrification decreased in the order control > exclR > exclRM, following the trend in gross mineralization (Table 2), which is consistent with the generally observed positive correlation between these two processes (Booth and others 2005). This decrease in gross nitrification after trenching is, though, in contrast to earlier studies, which found increased gross nitrification after trenching (Ross and others 2001) or tree girdling (Kaiser and others 2011; Koranda and others 2011), despite decreased gross mineralization. However, by separating gross nitrification into NH_4^+ oxidation (O_{NH_4}) and oxidation of organic N (O_{SON}), we observed different root and ECM effects on these two pathways (Table 2), which were not investigated in earlier trenching/girdling studies.

NH_4^+ oxidation (O_{NH_4}), assumed to be mainly by autotrophic nitrifiers, was increased in the exclR treatment, but unaffected in the exclRM treatment compared to the control (Table 2). One possible explanation is that root exclusion reduced root-derived (or released) nitrification inhibitors (White 1986; Paavolainen and others 1998), thereby promoting autotrophic nitrification in exclRM. However, when ECMs were also excluded (exclRM), NH_4^+ supply was limiting for autotrophic nitrification, due to reduced gross mineralization. Indeed, in this treatment, O_{NH_4} actually exceeds M_{SON} (Table 2). These findings point to the importance to separate effects of root and mycorrhizae exclusion in future studies, as well as separating heterotrophic and autotrophic NH_4^+ oxidation.

Nitrate immobilization only occurred in the control but not in the trenched treatments (Table 2). This is in line with the observation that I_{NO_3} increases with increasing C availability (Hart and others 1994; Qian and others 1997; Booth and others 2005) due to increased microbial activity and, hence, N demand. Further, the absence of I_{NO_3} in the trenched plots indicates that N supply to the microbes was saturated and that NH_4^+ was the preferred N source compared to NO_3^- (Gessler and others 1998). Also, I_{NH_4} declined after trenching, reflecting a generally decreased microbial N demand probably due to absence of C input by roots and ECM.

We found significant rates of DNRA ($D^-_{NO_3}$; Table 2), which interestingly followed closely the patterns of O_{NH_4}. This is quite unexpected, as DNRA occurs under anoxic conditions (Tiedje 1988), whereas O_{NH_4} is an oxygen-demanding process. To date, most ^{15}N labelling studies have not included DNRA (Rütting and others 2011), which is inferred from the ^{15}N enrichment of NH_4^+ after labelling with $^{15}NO_3^-$ (Silver and others 2001). However, it has been shown that three processes can explain the enrichment of the NH_4^+ pool, namely DNRA, efflux of plant N and re-mineralization by microorganisms (Burger and Jackson 2004). As in the present study DNRA also occurred in the trenched plots, plant efflux can be excluded as a possibility for the observed ^{15}N pattern. Moreover, NO_3^- immobilization (I_{NO_3}), a prerequisite for the re-mineralization pathway, did not occur in trenching plots, but significant ^{15}N transfer from NO_3^- to NH_4^+ pool was observed (Figure 3). Therefore, we conclude that DNRA did occur in the investigated soil. We suggest a spatial separation of DNRA in anoxic microsites and NH_4^+ oxidation in the oxic bulk soil. The presence of ECM promoted DNRA in the present study (compare exclR with exclRM), likely due to enhanced labile C inflow (Burger and Jackson 2004). In contrast, the presence of roots decreased the DNRA rate, which we suggest to be the consequence of significant microbial NO_3^- immobilization.

N Loss Fluxes

During the 10-day incubation, we observed a loss of added ^{15}N from the labelled soil (Supplemental Figure 2), which is a common feature in in situ incubations (Davidson and others 1991; Templer and others 2008; Staelens and others 2012). This loss is explicitly included in the tracing model (Figure 1) to achieve mass balance. In the present study, L_{NH_4} was not detected in the exclRM

treatment (Table 2). Therefore, this pathway can be assigned to N uptake by plant roots and ECM. Excluding roots increased L_{NH_4} compared to control soil. This might be a consequence of the decreased competition between roots/ECM uptake and microbial NH_4^+ immobilization (Kuzyakov and Xu 2013), as the latter was decreased in the exclR treatment. Notably, the sum of the L_{NH_4} and L_{NO_3} remained about constant when roots were excluded, due to decreased L_{NO_3}. A likely explanation is a switch from NO_3^- to NH_4^+ as a source for N uptake by ECM when roots were excluded, as NH_4^+ uptake is energetically favourable (Jackson and others 2008; Gavrichkova and Kuzyakov 2010). Even when both roots and ECM were excluded, we observed considerable L_{NO_3}, which is likely due to denitrification, leaching and lateral diffusion losses (Rütting and others 2011; Staelens and others 2012).

Conclusions

Overall, this study showed that plant roots and ECM significantly affect the N cycling in forest soils. Gross rates of the internal soil N cycle were generally higher in the presence of active roots and ECM. In contrast, the presence of roots and ECM decreased annual N_2O emissions. Enhanced N turnover is probably caused by input of labile C to soil by roots and ECM resulting in an increased microbial activity. This induced an increase in microbial N immobilization, which in turn limited N_2O production. Our results suggest that plants actively control N cycling in soil and N_2O emissions. Specifically, microbial N immobilization is stimulated by root ECM, leading to a decreased importance of nitrification for NH_4^+ consumption (N/I ratio), which in turn limits N_2O production (Figure 5).

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