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Sources of nitrous oxide emitted from European forest soils

P. Ambus[1], **S. Zechmeister-Boltenstern**[2], **and K. Butterbach-Bahl**[3]

[1]Risø National Laboratory, Biosystems Department (BIO-309), 4000 Roskilde, Denmark
[2]Federal Research and Training Centre for Forests, Natural Hazards and Landscape (BFW), Vienna, Austria
[3]Karlsruhe Research Centre, Institute for Meteorology and Climate Research, Atmospheric Environmental Research (IMK-IFU), Garmisch-Partenkirchen, Germany

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Abstract. Forest ecosystems may provide strong sources of nitrous oxide (N_2O), which is important for atmospheric chemical and radiative properties. Nonetheless, our understanding of controls on forest N_2O emissions is insufficient to narrow current flux estimates, which still are associated with great uncertainties. In this study, we have investigated the quantitative and qualitative relationships between N-cycling and N_2O production in European forests in order to evaluate the importance of nitrification and denitrification for N_2O production. Soil samples were collected in 11 different sites characterized by variable climatic regimes and forest types. Soil N-cycling and associated production of N_2O was assessed following application of ^{15}N-labeled nitrogen. The N_2O emission varied significantly among the different forest soils, and was inversely correlated to the soil C:N ratio. The N_2O emissions were significantly higher from the deciduous soils ($13\,ng\ N_2O\text{-}N\ cm^{-3}\ d^{-1}$) than from the coniferous soils ($4\,ng\ N_2O\text{-}N\ cm^{-3}\ d^{-1}$). Nitrate ($NO_3^-$) was the dominant substrate for N_2O with an average contribution of 62% and exceeding 50% at least once for all sites. The average contribution of ammonium (NH_4^+) to N_2O averaged 34%. The N_2O emissions were correlated with gross nitrification activities, and as for N_2O, gross nitrification was also higher in deciduous soils ($3.4\,\mu g\,N\,cm^{-3}\,d^{-1}$) than in coniferous soils ($1.1\,\mu g\,N\,cm^{-3}\,d^{-1}$). The ratio between N_2O production and gross nitrification averaged 0.67% (deciduous) and 0.44% (coniferous). Our study suggests that changes in forest composition in response to land use activities and global change may have implications for regional budgets of greenhouse gases. From the study it also became clear that N_2O emissions were driven by the nitrification activity, although the N_2O was produced per se mainly from denitrification. Increased nitrification in response to accelerated N inputs predicted for forest ecosystems in Europe may thus lead to increased greenhouse gas emissions from forest ecosystems.

Correspondence to: P. Ambus
(per.ambus@risoe.dk)

1 Introduction

Nitrous oxide (N_2O) is an important trace gas with implications for atmospheric chemistry and radiative properties (IPCC, 2001). Several independent studies have demonstrated that N_2O is emitted in significant quantities from forest ecosystems (e.g. Ambus and Christensen, 1995; Bowden et al., 1990; Schmidt et al., 1989; Struwe and Kjøller, 1994; Papen and Butterbach-Bahl, 1999). In Europe, forest ecosystems cover about 1.9 Mill. km^2 or ca. 28% of the total land area and may thus have significant importance for European scale N_2O budgets. However, the importance of temperate forests for the atmospheric N_2O budget still remains highly uncertain with a 20-fold range in the emission estimates (IPCC, 2001). Such high uncertainty arise due to a combination of mere factors including lack of experimental data for upscaling, inevitable inaccuracy in flux measurements, and large spatial and temporal variability of fluxes (Kroeze et al., 2003). Moreover, insufficient process understanding for example on the importance of forest type and atmospheric N-deposition is also an impediment to narrow flux estimates (Kroeze et al., 2003; Skiba et al., 1999).

Microbial C and N turnover processes, including mineralization, nitrification, denitrification and microbial immobilization are the main reason for N_2O production and consumption in soils (Conrad, 2002). Therefore, a detailed knowledge of these processes is of fundamental importance to understand the microbially mediated biosphere-atmosphere exchange of trace gases, and to build, parameterize and further improve process oriented models. Such models constitute crucial tools in the upscaling of plot-scale measurements in view of the large spatial and temporal variability of environmental conditions in forest ecosystems across Europe, and as tools to predict future N trace gas emissions from forest soils (Butterbach-Bahl et al., 2004).

The exchange of N_2O between soils and the atmosphere depends specifically on the simultaneous, opposing processes of nitrification and denitrification (Wrage et al., 2001).

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Table 1. List of sampling sites with information on geographical location, species composition of tree stands, dominant undergrowth vegetation and forest age. The sites are grouped according to major forest type.

Site (country)	Location	Tree species	Dominant undergrowth vegetation	Age (yrs)
Coniferous sites				
Hyytiälä (SF)	61.85° N 24.28° E	Scots pine (*Pinus sylvestris*)	*Calluna vulgaris; Vaccinium sp.*	42
Glencorse (GB)	55.85° N 2.17° E	Sitka spruce (*Picea sitchensis*)	None	19
Speulderbos (NL)	52.22° N 5.65° E	Douglas fir (*Pseudotsuga menziesii*)	Scattered mosses	41
Höglwald (D)	48.50° N 11.17° E	Spruce (*Picea abies)*	Mosses	110
Achenkirch (A)	47.58° N 11.65° E	Spruce-beech (*Picea abies; Fagus sylvatica*)	*Carex alba; Sesleria albicans; Melica nutans*	127
Nyirjes (HU)	47.89° N 19.95° E	Spruce (*Picea abies*)	None	40
San Rossore (I)	43.73° N 10.28° E	Pine (*Pinus pinaster)*	*Pistacia lentiscus; Erica arborea*	40
Deciduous sites				
Sorø (DK)	55.48° N 11.63° E	Beech (*Fagus sylvatica*)	*Anemone nemorosa*	>80
Schottenwald (A)	48.23° N 15.25° E	Beech (*Fagus sylvatica*)	*Allium ursinum, Salvia glutinosa*	142
Bosco negri (I)	45.20° N 9.07° E	Oak (*Quercus robur*); Poplar (*Populus spp*); Ash (*Fraxinus spp*)	*Convallaria majalis; Anemone nemorosa*	>150
Parco Ticino (I)	45.20° N 9.07° E	Poplar (*Populus euroamericana I-214*)	None	13

Nitrification is an oxidative process that requires the availability of molecular oxygen and during which ammonium (NH_4^+) is oxidised to nitrite (NO_2^-) and nitrate (NO_3^-). In contrast, denitrification is a reductive process, which mainly occurs in oxygen depleted soil zones. Under anaerobic conditions, some microbes use NO_3^- and NO_2^- as alternative electron acceptors, thereby reducing NO_3^-/NO_2^- sequentially to NO, N_2O and finally to N_2 (Conrad, 2002). Although nitrification and denitrification are characterized by different environmental controls and have optima under different environmental conditions, it is well known that both processes may occur simultaneously in the soil, thus giving rise to duplicate sources for N_2O (Davidson et al., 2000).

The importance of other soil processes in the production of N_2O, including any role of dissimilatory nitrate reduction to ammonium, heterotrophic nitrification by fungi and anaerobic oxidation of NH_4^+, remains poorly known, (e.g. Dalsgaard et al., 2003; Wolf and Brumme, 2002; Wrage et al., 2001).

In this experiment, we have investigated the quantitative and qualitative relationships between N-cycling and N_2O production in European forests in order to evaluate the importance of nitrification and denitrification for N_2O production. Soil samples were collected in 11 different sites characterized by variable climatic regimes and forest types, and incubated in the laboratory under controlled environmental conditions. Soil N-cycling and associated production of N_2O was assessed following application of ^{15}N-labeled nitrogen as tracer.

2 Materials and methods

2.1 Study sites and sampling

The study included 11 different forest sites situated across Europe from Finland in the north to Italy in the south, and from Scotland in the west to Hungary in the east. Main botanical characteristics are listed in Table 1 and soil characteristics are compiled in Table 2. Undisturbed soil samples were collected at each site in response to predefined conditions with respect to soil temperature to ensure identical preincubation conditions. Subsequent incubation took place in the laboratory under uniform temperature and moisture conditions. This procedure allowed identifying to which extent differences in chemical and biological conditions among the sites would lead to different N_2O emission rates. At least two sampling occasions were considered for each site, i.e. at the onset of the growing season and at the end of the growing season, which subsequently will be mentioned as the spring time and autumn time samplings, respectively. The spring sampling took place when average soil temperature (5 cm depth) exceeded 8°C and the autumn sampling took place

Table 2. Soil characteristics of the study sites.

Site	Soil texture	pH	%C (dw)	%N (dw)	C:N	BD[§] ($g\,cm^{-3}$)	WFPS[!] (% vol vol^{-1})
Hyytiälä	Sandy loam	3.7	4.3	0.27	16.3	0.71	48±7
Glencorse	Silty loam	4.2	4.5	0.32	13.8	0.78	55±11
Speulderbos	Sand	3.7	6.9	0.26	26.4	0.85	66±14
Höglwald	Loam	3.7	12.5	0.56	22.7	0.33	62±13
Achenkirch	Loam	7.0	14.1	0.80	17.8	0.43	68±14
Nyirjes	Sandy loam	3.9	4.8	0.27	16.2	0.87	73±21
San Rossore	Sand	5.8	2.7	0.08	35.9	0.86	38±8
Sorø	Loamy sand	4.5	2.6	0.15	17.7	0.85	58±8
Schottenwald	Silty loam	4.2	3.6	0.25	15.0	0.88	58±12
Bosco negri	Loamy sand	4.2	5.6	0.42	12.5	0.58	50±12
Parco Ticino	Sandy loam	5.9	1.2	0.10	12.0	1.15	70±28

[§] Bulk density of incubated soil cores.
[!] Water filled pore space (±SE) measured after incubation.

when soil temperature had decreased to below 5°C. In the Hyytiälä and Sorø sites, two additional samplings were conducted in early summer (June) and late summer (September), respectively. In the San Rossore site a spring time sample was not achieved until June.

At each sampling, 16 intact soil cores, 5 cm diam., were collected from the top 0–10 cm soil after removal of the litter layer by pushing down 10 cm long PVC-cylinders sharpened at the bottom end. The cores were gently excavated, sealed firmly by wrapping with Parafilm® and then shipped in a cool box to the laboratory by overnight carrier.

2.2 Incubation

Upon arrival to the laboratory, the samples were stored in a cold room (5°C) until incubation was initiated, within one week. Incubation included a two-step procedure. Firstly, the soil water content was adjusted to a pF value of 2.36. The Parafilm® was removed and the cores wrapped at the bottom by a cotton cloth and then gently wetted with 40 ml of deionised water. Subsequently a 230 cm water suction was applied to the bottom of the cores achieved by placing them in an elevated sandbox for 60 h at 5°C. After equilibration of the soil water content, the cores were re-sealed at the bottom. Then, three quadruplicate sets of cores were treated with different combinations of nitrogen-15 enriched substrates prior to incubation for N_2O flux measurements and gross N-cycling assay. In treatment *A* ^{15}N-enriched NH_4^+ was added as a $^{15}(NH_4)_2SO_4$ solution with 2 atom% excess (APE) ^{15}N; in treatment*N* ^{15}N-enriched NO_3^- was added as a $K^{15}NO_3$ solution (2 APE). A third set of cores received both $^{15}NH_4^+$ and $^{15}NO_3^-$ and was subject to immediate extraction for dissolved inorganic N to assess start values. A fourth treatment constituted a zero N control by adding deionised water. The solutions were applied in six 1-ml doses providing 300 μg N $core^{-1}$ and a homogeneous distribution of the applied N was ensured by injecting slowly in synchrony with the vertical 0–8 cm travel of a side hole needle facilitated by the use of a precision liquid processor (Hamilton Microlab 500). The cores were then incubated in individuality at 15°C for 48 h in a 2-l gas tight Kilner jar fitted with a rubber septum in the lid for headspace gas sampling by syringe and needle. A small beaker with water in the jars served to prevent evaporation losses from the cores. Two gas samples were removed from the headspace at 0, 24 and 48 h, respectively, for subsequent analysis of headspace N_2O concentrations and ^{15}N enrichments of N_2O. A 5-ml sample was transferred to a pre-evacuated 3-ml Venoject® blood-collecting tube for the N_2O concentration measurements, and a 120-ml sample was transferred to a pre-evacuated crimp-sealed 100-ml serum bottle for determination of ^{15}N content in the N_2O. Prior to each sampling, the Kilner jars were pressurized with an equal amount of N_2 to maintain atmospheric headspace pressure.

2.3 Analysis

The soil cores were analyzed for inorganic N contents and ^{15}N enrichments two hours and 48 h after the label was applied. The soil was removed from the PVC cylinder, mixed thoroughly by hand and sieved to pass a 2 mm mesh. Subsamples of 20 g were suspended in 100 ml of 1 $\underline{M}$ KCl and shaken for 1 h prior to filtration and analysis of inorganic N and ^{15}N.

Gross N turnover rates were calculated based on the ^{15}N-isotope pool dilution principle using the analytical equations of Kirkham and Bartholomew (1954). Gross rates of N mineralization were obtained from the isotopic dilution of the NH_4^+ pool in treatment *A*. Gross rates of nitrification were obtained from the isotopic dilution of the NO_3^- pool in treatment *N*. Net nitrification was calculated from the change

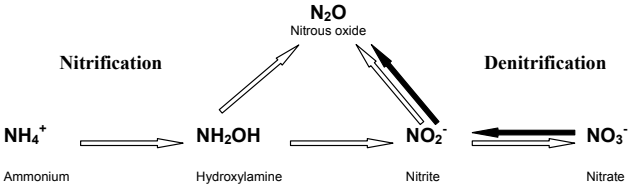

Fig. 1. Conceptual model indicating the major pathways for N_2O formation regarded in this study. Nitrification is indicated with open arrows and denitrification is indicated with bold arrows. Adapted from Wrage et al. (2001).

in soil NO_3^- concentrations in treatment *N* over the two-day incubation.

The contribution of NH_4^+ to N_2O production (Fa) was achieved in treatment *A* by the use of the linear mixing model Fa=(NOe−NIe)/(AMe−NIe), where NOe, NIe and AMe are the atom% ^{15}N enrichments of N_2O, NO_3^- and NH_4^+, respectively. NOe was calculated from the paired measurements of N_2O concentration and ^{15}N enrichments in the incubation jar. NIe and AMe were calculated as the average between the NO_3^- and NH_4^+ enrichments, respectively, observed at the onset and at the end of the incubations. The Fa-value was calculated under the assumptions that labeled NH_4^+ and labeled NO_3^- were the only sources for N_2O as illustrated in the simple, conceptual model pictured in Fig. 1. The contribution of NO_3^- to the N_2O production (Fn) was evaluated from the results of experiment *N* by the approach Fn=NOe/NIe.

Concentrations of N_2O were measured by gas chromatography (Shimadzu GC 14B, Kyoto, Japan) with electron capture detection. The ^{15}N content of N_2O was analysed using a Finnigan MAT PreCon unit (ThermoFinnigan, Bremen, Germany) interfaced with a GC coupled in continuous flow-mode to a Finnigan MAT Delta PLUS isotope ratio mass spectrometer (IRMS). Briefly, using a double hole needle and He carrier the sample in the 100-ml serum bottle was purged through a chemical trap of $Mg(ClO_4)_2$ and Ascarite (KOH) for removal of water and CO_2, respectively, and the sample N_2O was then concentrated cryogenically (liq. N_2) before injection into the GC.

Soil extracts were analysed for concentrations of NH_4^+ and NO_3^- on a Bran+Luebbe AutoAnalyzer 3 system (Bran+Luebbe, Norderstedt, Germany). To determine the ^{15}N enrichments of NH_4^+ and NO_3^-, the content of each component in an 80-ml portion of the KCl extract was concentrated on acidified filter paper prior to analysis (Sørensen and Jensen, 1991).

Soil total N and ^{15}N was determined in finely ground 40-mg portions of air-dried soil samples. Dried filter papers and soil samples were wrapped in tin cups followed by analysis on a CE 1110 elemental analyser (ThermoFinnigan, Milan, Italy) coupled in continuous flow mode to the IRMS.

Soil moisture was determined gravimetrically (105°C, 24 h) and pH was measured in water suspension (1:2.5 w:vol) of air-dried soil samples.

The sites differed considerably in bulk densities (Table 2) and to facilitate comparisons between sites data are given on basis of soil volume. Statistical evaluation of the data was undertaken using S-PLUS® (Insightful Corporation, Seattle, USA). Two-sample population means were compared using t-tests. Main effects of site and time were evaluated by analysis of variance (ANOVA). Where the main effects were significant, multiple-means comparisons were done by the Tukey critical point calculation to determine differences in response factors. Single linear regression and stepwise multiple linear regression analysis was used to describe the effect of single or multiple variables upon response variables. Data were assumed normally distributed except for N_2O fluxes, which were log-transformed before evaluation. All tests were performed at the 5% probability level.

3 Results

3.1 Site characteristics

The 11 sites included in this study represented seven different major tree species, comprising both deciduous and coniferous forest types (Table 1), which were distributed on five different soil textures ranging from sand to loam (Table 2). Except for the Achenkirch site, which was located on limestone (Schindlbacher et al., 2004), all sites were characterized by acidic pH-values (Table 2). The Höglwald and Achenkirch spruce soils revealed notably high organic C contents of 12.5% and 14.1%, respectively. For the remaining soils organic C ranged between 1.2% and 6.9% (Table 2). The soil moisture adjustments prior to the incubations combined with the subsequent substrate additions resulted in soil water filled pore spaces (WFPS) ranging from 38% in the sandy San Rossore soil to 73% in the sandy loam soil of Nyirjes (Table 2).

Soil inorganic nitrogen contents varied between sampling times in three of the sites (Table 3). Between the sites, NH_4^+ was uniform in the spring, whereas in the autumn the Hyytiälä site contained most NH_4^+. Soil NO_3^- in the spring reached peak values in Glencorse whereas in the autumn soil NO_3^- in Bosco negri exceeded most other sites ($P<0.001$ for all effects). The early summer and late summer samplings in Hyytiälä and Sorø showed that the two sites differed in NH_4^+ in the late summer, but otherwise remained similar.

3.2 Emissions of N_2O from soil columns

During the 48 h incubation time, the majority of the forest soil columns emitted N_2O at median values ranging from <0.5 ng N_2O-N $cm^{-3}\,d^{-1}$ in the Hyytiälä soils to about 15 ng N_2O-N $cm^{-3}\,d^{-1}$ in soil from the Schottenwald beech forest (Fig. 2). At three occasions a slight decrease in N_2O headspace concentrations was observed suggesting that

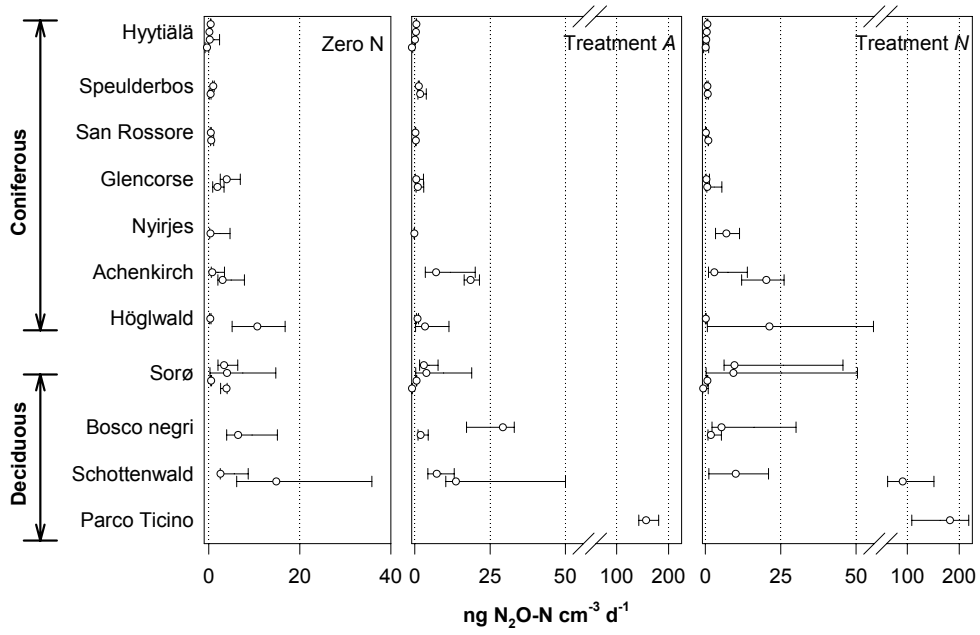

Fig. 2. Emissions of N_2O from soil cores incubated after soil moisture adjustment only (zero N), after $^{15}NH_4^+$ additions (treatment *A*) and after $^{15}NO_3^-$ additions (treatment *N*), respectively. Data points indicate the median value for each sampling occasion and horizontal bars indicate the 25 percentile and 75 percentile boundaries. For each forest type the sites are grouped along the vertical axis according to N_2O emission magnitude. Sampling times for the sites were Hyytiälä (May, June, August, November); Speulderbos (April, December); San Rossore (June, Janauary); Glencorse (April, December); Nyirjes (December); Achenkirch (April, November); Höglwald (April, December); Sorø (May, June, September, November); Bosco negri (May, January); Schottenwald (April, November); Parco Ticino (May).

N_2O uptake occurred, but always at low rates <1 ng N_2O-N $cm^{-3}\,d^{-1}$ (Hyytiälä autumn with NH_4^+, Nyirjes with NH_4^+, and Sorø autumn with NH_4^+, respectively). Sampling time had no influence on the N_2O activity except for the Höglwald site, where N_2O emission was greater in the autumn than in the spring ($P<0.05$). Addition of the NH_4^+ and NO_3^- substrates roughly doubled the N_2O activity, independent of substrate form, when all sites and sampling times are combined (Fig. 2). However, due to the prevalent scatter in data the N effect was only significant in the Nyirjes soil in the autumn where activity peaked at 7 ng N_2O-N $cm^{-3}\,d^{-1}$ after NO_3^- additions and in the Höglwald soil in the spring where activity peaked at 0.9 ng N_2O-N $cm^{-3}\,d^{-1}$ upon NH_4^+-addition.

The N_2O emission varied significantly among the different forest soils both under zero N conditions as well as for the nitrogen amended soil columns ($P<0.001$). This site related effect on N_2O emissions remained significant also when the asymptotic high values for the Parco Ticino (Fig. 2) was removed in the ANOVA. The average N_2O emission in the zero N samples was inversely correlated ($P<0.05$) to the soil C:N ratio (Table 1), but did not correlate with any other of the soil variables listed in Tables 2 and 3. The N_2O emissions from the zero N samples were significantly ($P<0.01$) higher from the deciduous soils (13 ng N_2O-N $cm^{-3}\,d^{-1}$) than from the coniferous soils (4 ng N_2O-N $cm^{-3}\,d^{-1}$). A similar effect of forest type was also significant ($P<0.05$) with the NO_3^- treated soil columns, and a trend ($P<0.06$) was evident for the NH_4^+ treated soils, also when the asymptotic values for the Parco Ticino poplar site were excluded from the analysis.

3.3 Sources for N_2O

The data in Fig. 3 shows the average ^{15}N enrichments of the inorganic N pools during the course of the incubation, combined with the ^{15}N enrichment of the cumulated N_2O. Two days after the $^{15}NH_4^+$-labelling (treatment *A*) an increase in $^{15}NO_3^-$ could be observed for most of the soils indicating that nitrification occurred (Fig. 3). The ^{15}N enrichments of the N_2O emitted from $^{15}NH_4^+$-labeled soil columns varied considerably among the different sites. For some sites, e.g. Hyytiälä, Speulderbos (spring), and San Rossore the emitted N_2O contained almost no excess ^{15}N despite significant enrichments of both the NH_4^+ and NO_3^- pools. In the remaining sites, the N_2O enrichments were intermediate to the enrichments of the NH_4^+ and NO_3^- pools, apart for three observations (Hyytiälä late summer, Achenkirch autumn and Höglwald autumn) where the N_2O ^{15}N enrichments exceeded the enrichments of both inorganic N pools (Fig. 3). With the $^{15}NO_3^-$-labelled soil (treatment *N*) no increase in $^{15}NH_4^+$ was observed. The ^{15}N isotopic signature of emitted N_2O was less than that of the NO_3^--pool to varying degrees indicating variable source strengths of NO_3^- for N_2O

Table 3. Soil inorganic N contents at each sampling occasion.

Location	Sampling time (*mo*)	Inorganic nitrogen content (μg N cm^{-3})	
		NH_4^+	NO_3^-
Hyytiälä	Spring (May)	7.7§	0.3§
	Early summer (June)	0	0.7
	Late summer (Aug)	6.4	2.0
	Autumn (Nov)	30.3	0.2
Glencorse	Spring (April)	7.1	11.1
	Autumn (Dec)	1.8	5.8
Speulderbos	Spring (April)	3.7	0.5§
	Autumn (Dec)	6.1	5.4
Höglwald	Spring (April)	0.6	4.2
	Autumn (Dec)	1.1	2.2
Achenkirch	Spring (April)	2.6	7.1
	Autumn (Nov)	2.9	1.6
Nyirjes	Spring (n.a.)	n.a.	n.a.
	Autumn (Dec)	1.5	0.5
San Rossore	Spring/Summer (June)	0	0.3
	Autumn (Jan)	1.3	0.4
Sorø	Spring (May)	0§	1.4§
	Early summer (June)	8.3	0.2
	Late summer (Sep)	0.6	0.8
	Autumn (Nov)	0	1.2
Schottenwald	Spring (April)	3.5	6.8
	Autumn (Nov)	0.4	5.5
Bosco negri	Spring (May)	2.8	11.3
	Autumn (Jan)	5.3	13.3
Parco Ticino	Spring (May)	19.7	11.0
	Autumn (n.a.)	n.a.	n.a.

§: Indicates significant differences between sampling times.
n.a.: Data not available.

production between the sites. Unexpected high ^{15}N enrichments of N_2O were observed at two occasions, i.e. Nyirjes autumn and Achenkirch autumn (Fig. 3).

The contribution of NH_4^+ to N_2O in experiment *A* and the contribution of NO_3^- to N_2O in experiment *N*, calculated from the ^{15}N values in Fig. 3, suggests that NO_3^- was the dominant source for N_2O on most occasions across sampling time and location (Table 4). The contribution of NH_4^+ to N_2O averaged 34%. Data for the Achenkirch site showed that the contribution from NH_4^+ consistently exceeded 50%, which was significantly ($P<0.05$) higher than for the other sites except Hyytiälä, Bosco negri and Parco Ticino. In contrast, we found that the contribution of NO_3^- to N_2O averaged 62% and exceeded 50% in at least one observation in each site, but independent of sampling time. The Höglwald site revealed a significantly higher NO_3^- source strength ($P<0.01$) than Hyytiälä, San Rossore, Achenkirch and Sorø (Table 3). The source strengths of NH_4^+ and NO_3^- to N_2O were not influenced by major forest type, i.e. deciduous versus coniferous.

3.4 Gross N cycling rates

Gross N mineralization varied significantly between the sampling sites for both springtime samples and autumn-time samples, and four sites, i.e. Hyytiälä, Glencorse, Achenkirch, and Sorø, also varied between sampling times (Fig. 4). The lowest gross mineralization, 0.6 μg N cm^{-3} d^{-1}, was observed in a coniferous site (Hyytiälä in autumn) and the highest, 13.3 μg N cm^{-3} d^{-1}, in a deciduous site (Bosco negri in spring). The average coniferous activity of 3.3 μg N cm^{-3} d^{-1}, however, did not differ from the average deciduous activity of 4.2 μg N cm^{-3} d^{-1}. A step-wise multiple regression analysis with mean gross mineralization rates as response variable and soil physical and chemical characteristics (Tables 2 and 3) as main effects indicated a negative correlation with soil total C ($P<0.05$) and a positive correlation with soil total N ($P<0.05$).

Gross nitrification rates ranged from <0.1 μg N cm^{-3} d^{-1} to 5.7 μg N cm^{-3} d^{-1}, and fluctuated significantly among sites in the spring, but not autumn (Fig. 4). Sampling time fluctuations were evident only at two sites, i.e. Hyytiälä and

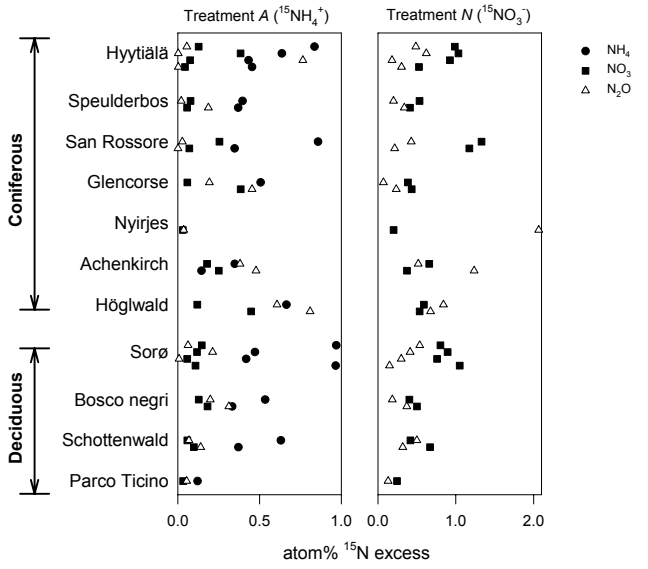

Fig. 3. Nitrogen-15 enrichments of soil NH_4^+, soil NO_3^- and emitted N_2O, respectively, after $^{15}NH_4^+$ additions (treatment *A*) and $^{15}NO_3^-$ additions (treatment *N*). Data points indicate the atom% ^{15}N excess for each component. Data for NH_4^+ and NO_3^- are averages between the initial and final samplings during the incubation. The sites are grouped along the vertical axis according to Fig. 2.

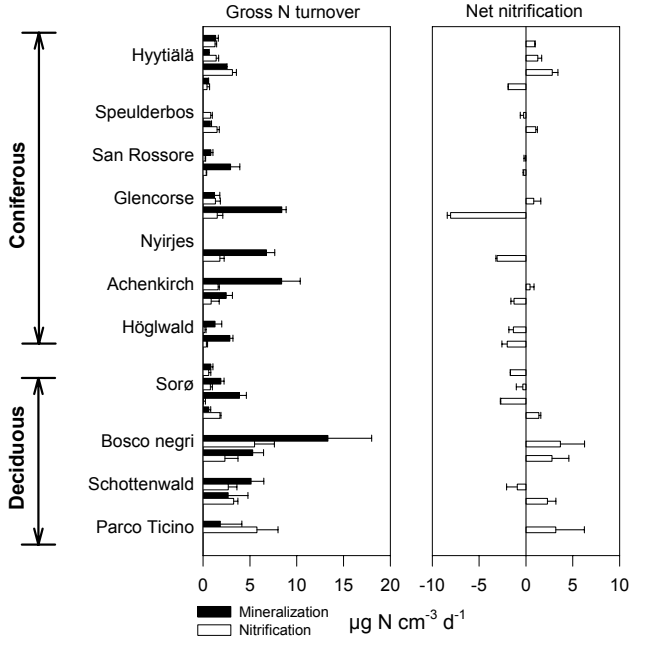

Fig. 4. Left hand plot shows rates of gross N mineralization and gross nitrification obtained from treatment *A* and treatment *N*, respectively. Right hand plot shows net nitrification rates obtained from treatment *N*. The bars indicate mean rates (n=4+1SE) from each sampling occasion. The vertical grouping is according to Fig. 2.

Table 4. The contribution of NH_4^+ and NO_3^- to N_2O formation. The numbers are percent of produced N_2O derived from ^{15}N-labeled NH_4^+ (treatment *A*) and NO_3^- (treatment *N*), respectively.

Location	Sampling time (*mo*)	% contribution to $N_2O^{\S}$	
		From $^{15}NH_4^+$	From $^{15}NO_3^-$
Hyytiälä	Spring (May)	0±9	50±4
	Early summer (June)	0±9	60±29
	Late summer (Aug)	∼100±24	19±5
	Autumn (Nov)	0±1	88±14
Glencorse	Spring (April)	32±11	19±4
	Autumn (Dec)	n.a.	53±9
Speulderbos	Spring (April)	0±5	37±6
	Autumn (Dec)	40±8	84±21
Höglwald	Spring (April)	92±11	100±16
	Autumn (Dec)	n.a.	100±20
Achenkirch	Spring (April)	100±15	80±11
	Autumn (Nov)	∼100±12	∼100±21
Nyirjes	Spring (n.a.)	n.a.	n.a.
	Autumn (Dec)	n.a.	∼100±8
San Rossore	Spring/Summer (June)	0±8	33±15
	Autumn (Jan)	0±2	19±8
Sorø	Spring (May)	0±8	62±34
	Early summer (June)	27±23	47±7
	Late summer (Sep)	0±1	40±6
	Autumn (Nov)	n.a.	18±19
Schottenwald	Spring (April)	2±3	100±20
	Autumn (Nov)	12±7	47±16
Bosco negri	Spring (May)	21±11	45±3
	Autumn (Jan)	53±17	69±23
Parco Ticino	Spring (May)	22±2	53±3
	Autumn (n.a.)	n.a.	n.a.

§: The contribution of $^{15}NH_4^+$ and $^{15}NO_3^-$, respectively to $^{15}N_2O$ production in experiment *A* and experiment *N*. Mean of n=2–4 samples ±SE.
n.a.: data not available.

Sorø, where sampling took place four times during the season. The average coniferous gross nitrification of 1.1 μg N cm^{-3} d^{-1} was significantly ($P<0.01$) lower than the deciduous activity of 3.4 μg N cm^{-3} d^{-1}. Across all sites, the average gross nitrification was inversely correlated to the soil C:N ratio ($P<0.05$).

Gross nitrification was generally lower than the gross mineralization. The ratio between gross nitrification and gross mineralization tended to be higher for the deciduous soils (0.83±0.14; n=4±SE) than for the coniferous soils (0.50±0.14; n=7±SE). Variations in the ratio occurred independently of the soil parameters listed in Tables 2 and 3.

As for gross nitrification, net nitrification fluctuated also significantly among the different sites (Fig. 4) indicating that some sites each time provided a net NO_3^- source (e.g. Bosco negri) and others a net NO_3^- sink (e.g. Höglwald). Net nitrification also differed significantly ($P<0.05$) between the coniferous sites (-1.1 μg N cm^{-3} d^{-1}) and deciduous sites (1.6 μg N cm^{-3} d^{-1}).

Nitrous oxide emissions were not correlated to the gross mineralization rates, neither among all sites, nor within the

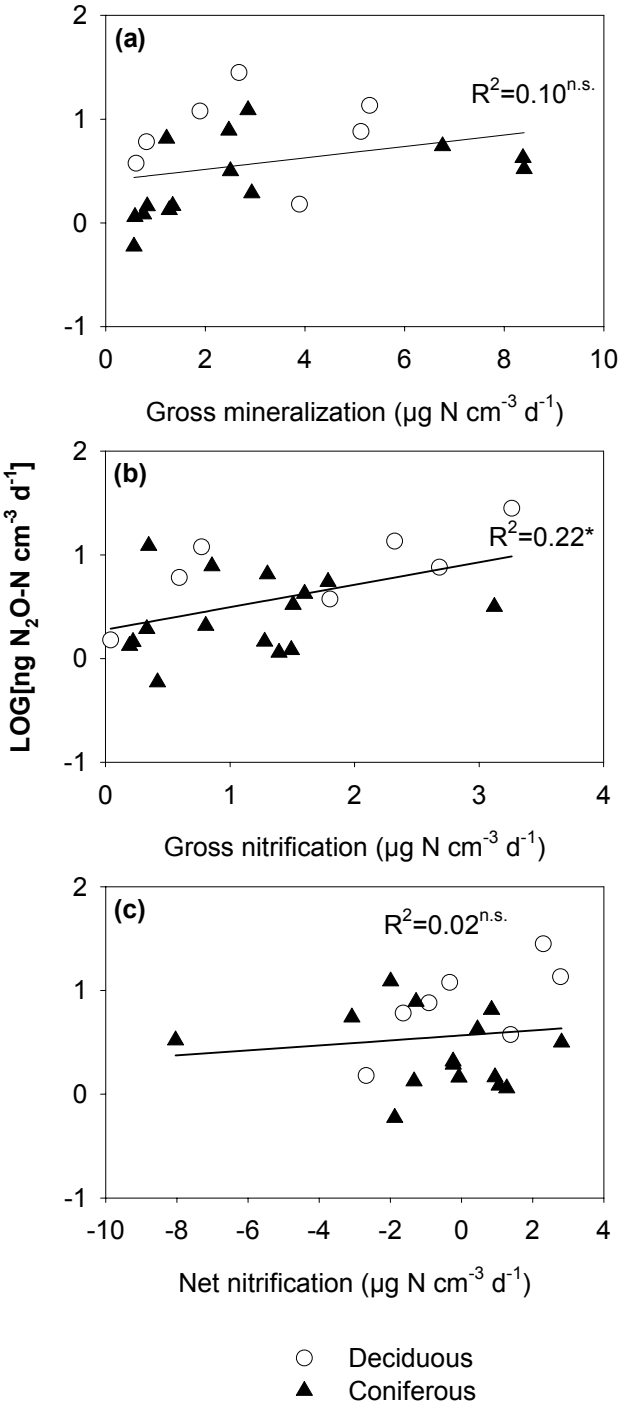

Fig. 5. X-Y scatter plots indicating the relationships between LOG[N_2O emission] and gross N mineralization activity **(a)**, gross nitrification activity **(b)**, and net nitrification activity **(c)**, respectively. The positions of values for coniferous and deciduous soils are indicated by different legends. The trend lines indicate the positions of linear regressions lines when all data are included in the analysis. n.s. not significant; * $P<0.05$.

two forest types (Fig. 5a). However, a trend in the data ($P<0.09$) indicated a positive relationship between N_2O and gross mineralization for the coniferous soils. The N_2O emissions correlated significantly ($P<0.03$) with gross nitrification activities when all sites were included in the analysis (Fig. 5b). For the individual forest types, however, the N_2O emissions were not correlated to gross nitrification although a positive trend ($P<0.08$) was observed for the deciduous soils. In contrast to the relationship with gross nitrification, the N_2O emissions were not related to net nitrification activities, but a positive trend ($P<0.08$) could be observed for the deciduous soils (Fig. 5c).

4 Discussion

4.1 Impact of forest type and C- and N-pools

Nitrogen cycling processes in forest soils is widely believed to be regulated by litter quality parameters such as C:N ratio, contents of N, lignin and phenolic compounds (Venterea et al., 2004). It is also believed that deciduous tree species generally increase nutrient cycling and microbial activities compared to coniferous tree species (Menyailo et al., 2002; Smolander et al., 2005). In accordance with this general perception, incubation of intact samples from a range of European coniferous and deciduous forest soils indicated that soils under deciduous forest types emit more N_2O than soils under coniferous forest types. A similar difference between these two main forest types was also observed in a field study by Butterbach-Bahl et al. (1997) who found that a German beech forest emitted approximately twice as much N_2O as a spruce forest. Field measurements within the sites encountered in the current study also indicated that deciduous forests are stronger sources of N_2O than coniferous forests (Butterbach-Bahl, 2005). Other soil incubation studies have also confirmed that soils under deciduous forests may emit more N_2O compared with soils under coniferous forests (Menyailo and Huwe, 1999; Menyailo et al., 2002).

We also observed that the N_2O production was inversely correlated to the C:N ratio of soil carbon and nitrogen pools. This observation suggests that not only the chemical quality of the litter input, but also external factors, such as nitrogen deposition, which may have a long-term cumulated effect on the soil C:N ratio constitute important proxy controllers for the N_2O production across European forest sites. Lovett et al. (2004) found that standard measures of litter quality (e.g. N, lignin, and phenolic contents) could not explain mechanisms of control on forest N cycling and suggested that external factors may play an important role. In line with this, the soil characteristics encountered in this study as indices for quality of litter input (Table 2) did not differ between the two major forest types despite the different N_2O emission strengths.

The supply of inorganic N, as expressed by gross N mineralization, was also similar in magnitude for the two forest types and did not explain the difference in N_2O emissions. Gross mineralization in hardwood and Pine stands of Harvard Forest (Venterea et al., 2004) were similar in magnitude to those reported herein and, in agreement with our conclusion, not different between the two forest types. Not surprisingly, however, current gross mineralization increased with increasing total N contents among all sites and decreased with increasing C content. The combined effect of these two parameters thus suggests that gross mineralization would increase with a decreasing C:N ratio of the soil organic matter, but in our analysis, this relationship was just about significant ($P<0.06$).

The extent of nitrification in relation to substrate input, as expressed by the ratio between gross nitrification and gross mineralization, is basically regulated by the competition between heterotrophic and autotrophic NH_4^+ assimilation (Venterea et al., 2004). The current data suggests that the competitive ability of nitrifying communities in deciduous soils is stronger than in the coniferous soils, which may explain why both gross and net nitrification in the deciduous sites exceeded nitrification in the coniferous sites. The magnitude of nitrification was also mediated through the soil C:N ratio, in agreement with observations in other studies suggesting that nitrification increases with decreasing C:N ratio below a threshold value of 22–25 (Lovett et al., 2004). The current C:N ratios were almost all below this threshold.

In this experiment the litter layer was removed prior to sampling for methodological reasons. Presence of litter, however, may influence the N_2O gas exchange and it can not be ruled out that absence of litter will lead to biased results. First of all, as demonstrated by Brüggemann et al. (2005), litter from various tree species constitute a substrate for extensive mineralization and nitrification activity, suggesting that also N_2O production can take place in the litter. Moreover, different shapes of tree litters can have an influence on the gas diffusivity through the litter layer (Brumme and Borken, 1999). Litters from broad leaved trees may, in particular when they are wet, restrict oxygen diffusion into the soil to a greater extent than litters from spruce or pine needles with variable impacts on soil aeration and thus N_2O emissions. Contrasting these observations, however, Vasconcelos et al. (2004) observed no changes in N_2O and CH_4 fluxes in a tropical forest after litter had been removed.

4.2 Sources of N_2O

Although N_2O production was correlated with gross nitrification activity, the current data do imply that nitrification activity per se was not responsible for the N_2O formation in most of the sites. Exceptions to this were the Achenkirch and Höglwald sites, where all the N_2O was derived directly from NH_4^+ under nitrifying conditions. However, both of these sites were also capable for producing N_2O entirely from NO_3^- under similar soil moisture conditions. There is no obvious reason to explain why these two spruce sites in particular exhibited the capability to switch between N_2O production entirely from nitrification or from denitrification. Common characteristics to the two sites making them distinct from the other sites were a loamy texture with relatively high contents of organic C and N. This environment could sustain not only a high microbial biomass but also probably a greater microbial diversity with the capability of a rapid switch between different pathways for N_2O production, i.e. nitrification, denitrification and perhaps also less well described pathways such as heterotrophic production from NH_4^+ (Wolf and Brumme, 2002). Data for the Hyytiälä pine soil also suggested a transient production of N_2O from NH_4^+ in August (late summer) that, in combination with a reduced N_2O production from NO_3^- at the same time, do imply a shift in N_2O production pathway during the season, in accordance with the observations by Wolf and Brumme (2002).

One of the most important environmental controls in the partitioning of N_2O from nitrification and denitrification is soil aeration, which mainly is regulated by the soil moisture content. In the current experiment the pre-incubation soil moisture was regulated to pF 2.3, slightly below field capacities, with the aim to promote conditions abundant following rainfall events when peak N_2O emissions are anticipated to occur. Soil moisture conditions in the current range (Table 2) also favor simultaneous production of N_2O from both nitrification as well as denitrification (Davidson et al., 2000). Apart for the Achenkirch and Höglwald spruce sites, however, NH_4^+ constituted a less significant source for N_2O than NO_3^-, indicating that denitrification is the most important process for N_2O production across these European forests. This observation is very much in agreement with previous works by Wolf and Brumme (2002, 2003) who found that denitrification was responsible for N_2O production in German beech forests, and Ambus (1998) finding that denitrification was the only source for N_2O in Danish spruce and beech forest stands. MacDonald et al. (1997) also found evidence that denitrification was the source for N_2O in Scottish Sitka spruce plantations.

It must be emphasized that the current experiment can not distinguish if the $^{15}N_2O$ generated from $^{15}NH_4^+$ in treatment *A* was produced as an intermediate in NH_4^+ oxidation or via nitrification denitrification reduction of nitrite (Fig. 1). In some sites, e.g. Hyytiälä, Speulderbos, and Sorø, labeled N_2O was not produced in the presence of labeled NH_4^+ in spite of a significant nitrification activity, which does imply that nitrification denitrification did not contribute to the N_2O production in these soils. For soils with modest N_2O production from $^{15}NH_4^+$ it can be argued that all N_2O was produced by nitrification denitrification and that the isotope signal was diluted by unlabeled NO_2^- from denitrification reduction of NO_3^-. However, it is still debatable whether the NO_2^- products from nitrification NH_4^+ oxidation and denitrification

NO_3^- reduction, respectively, do mix in the soil due to diffusional constraints (Russow et al., 2000). In the San Rossore pine stand the lack of $^{15}NH_4^+$ derived N_2O was very likely due to the limited nitrification capacity (Fig. 4).

The key role of nitrification in the control of N_2O production was thus mostly due to the supply of substrate for denitrification, except for the Höglwald and Achenkirch sites where N_2O was produced by either pathway. The ratio between N_2O production and gross nitrification averaged 0.67% and 0.44%, respectively, for the deciduous soils and coniferous soils, which is comparable to the ratios observed in Massachusetts hardwood and pine stands (Venterea et al., 2004). Increased N inputs to forest ecosystems causes in many cases symptoms of N saturation with increased exports of various N forms such as NO_3^- leaching and gaseous losses (Venterea et al., 2004; Vervaet et al., 2004). The study by Venterea et al. (2004) suggests that these responses to increased N deposition may be explained by increased nitrification, although in sites with excessive NO_3^- consumption the N saturation symptoms will be less apparent (Vervaet et al., 2004). Our study suggests that increased nitrification in response to accelerated N inputs generally may lead to increased N_2O emissions from a wide range of European forest ecosystems. The positive relationship between N_2O emissions and gross- and net nitrification observed among the different forest systems is generally also in support of the "hole-in-the-pipe" model proposed by Firestone and Davidson (1989). This conceptual model predicts that nitrogen oxide emissions from ecosystems is related to nitrogen cycling within the system with soil water as the most robust controller in the ratio between produced nitric oxide (NO) and N_2O (Davidson et al., 2000). In the current study we attempted to maintain comparable soil moisture conditions across the sites and thereby narrow the NO:N_2O ratio and found the relationship between N_2O emission and nitrification to be true across multiple forest ecosystems with variable physical and chemical properties.

5 Conclusions and perspective

Nitrous oxide emissions were measured from soil samples collected in 11 different European forests. The data indicate that N_2O emission rates are greater from deciduous forest types compared with coniferous forest types. Changes in forest composition in response to land use activities and global change may thus have implications for regional budgets of greenhouse gases. The result emphasizes the need to include various forest types in field-based experiments on forest N_2O fluxes. From the study it also became clear that N_2O emissions were driven by the nitrification activity across all sites, although the N_2O was produced per se mainly from denitrification. Increased nitrification in response to accelerated N inputs predicted for forest ecosystems in Europe may thus lead to increased greenhouse gas emissions from forest ecosystems.

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