Biogeochemistry (2010) 97:21–30
DOI 10.1007/s10533-009-9294-z

N_2O emission in a Norway spruce forest due to soil frost: concentration and isotope profiles shed a new light on an old story

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Received: 25 September 2008 / Accepted: 6 February 2009 / Published online: 25 February 2009

Abstract In mountain regions of Central Europe an increase of soil frost periods is predicted for this century due to reduced snow fall. To investigate the effects of freezing and thawing on soil N_2O fluxes in a mature Norway spruce forest in the mountainous Fichtelgebirge, Germany, the natural snow cover on three experimental plots was removed to induce soil frost. Three plots with natural snow cover served as controls. Soil N_2O fluxes were recorded in biweekly to monthly intervals during the frost and subsequent thawing period of the below-average cold winter in 2005/2006 and in the above-average warm winter in 2006/2007. In addition, N_2O concentrations and isotope signatures in soil air were measured along soil profiles in six different depths (from 6 to 70 cm). The soil of the snow removal plots was frozen down to 15 cm depth from January to April 2006 while the soil of control plots remained unfrozen under snow cover. Both soil freezing and thawing resulted in almost tenfold enhanced N_2O fluxes on snow removal plots contributing 84% to annual N_2O emissions. In the subsequent winter without soil frost no effects were observed. Vertical gradients of N_2O concentrations together with isotope abundance suggest that the subsoil of all plots was a probably weak, but continuous N_2O source throughout the year. Isotope signatures and N_2O concentration gradients in the soil profile indicate that microbial N_2O production and reduction of N_2O to N_2 did not or just marginally occur in frozen soil layers of the snow removal plots. Consequently, elevated N_2O fluxes in the late winter were attributed to the release of accumulated N_2O originating from the subsoil. At unfrozen soil, however, N_2O emissions were reduced due to a shift of the N_2O production-consumption ratio towards more consumption in the topsoil of both the control and snow removal plots. These findings contradict the general assumption that N_2O production in the organic layer is responsible for bursts of N_2O due to soil frost.

Keywords N_2O · N_2O consumption · ^{15}N · ^{18}O · Soil frost · Stable isotopes · Thawing

Introduction

The trace gas N_2O considerably contributes to the greenhouse effect and is involved in the destruction of ozone in the stratosphere. Emissions from terrestrial soils are assumed to be the largest source of atmospheric N_2O (IPCC 2007). N_2O is produced in soils mainly during microbial nitrogen transformations—primarily nitrification and denitrification. The

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amount of N_2O emission during the intermediate steps in these processes is controlled by a wide range of soil parameters. Most pronounced are soil temperature, soil water content and substrate availability (Granli and Bøckman 1994; Smith et al. 2003). In general, the highest microbial activities are observed in temperate regions during the warm summer growing season (Sommerfeld et al. 1993). However, high N_2O emissions have repeatedly also been found at low soil temperatures during the winter season (Röver et al. 1998; Teepe et al. 2000; Yashiro et al. 2006; Maljanen et al. 2007; Wagner-Riddle et al. 2008), and even the largest seasonal emissions of N_2O have been observed specifically during freezing and thawing events (Papen and Butterbach-Bahl 1999; Teepe et al. 2000; Teepe and Ludwig 2004; Groffman et al. 2006; see also Matzner and Borken 2008). Nonetheless, the mechanisms involved in these N_2O emission bursts still remain a matter of debate.

With regard to forest soils it is generally assumed that N_2O production and/or consumption occurs predominantly in the organic layer (e.g. Menyailo and Huwe 1999; Pihlatie et al. 2007). Accordingly, N_2O peaks during soil frost have mostly been attributed to substrate accumulation in small water films resulting in enhanced microbiological activity in the unfrozen soil water (Papen and Butterbach-Bahl 1999; Teepe et al. 2000) and N_2O emission bursts during thawing periods have been ascribed to enhanced microbial N_2O production in the topsoil due to increased substrate and easily decomposable carbon availability (Papen and Butterbach-Bahl 1999; Neilsen et al. 2001; Teepe et al. 2000). The quantitative importance of forest soil freezing and thawing for annual stand-level N_2O budgets is highly uncertain. Nonetheless, estimates from some field investigations indicate that such events may contribute up to 70% to the annual N_2O emissions (Papen and Butterbach-Bahl 1999; Teepe et al. 2000). In contrast to these findings from field studies, in the vast majority of laboratory freeze/thaw experiments with forest soils similar high N_2O emission peaks never could be observed (Priemé and Christensen 2001; Teepe and Ludwig 2004; Goldberg et al. 2008b).

These contradictory findings in field and laboratory investigations have been attributed to (1) other effects of freezing being responsible for a stimulation of C and N cycling under field conditions, e.g. disruption of soil structure (Neilsen et al. 2001), (2) artifacts, i.e. experimental designs in laboratory experiments that do not reflect natural conditions (Henry 2007) or (3) apparently minor contribution of the organic layer to the total soil N_2O emission after freezing (Teepe and Ludwig 2004; Goldberg et al. 2008b).

The objective of this field study was to induce dry-cold winter climate conditions and to examine N_2O fluxes during and after soil frost in comparison to unfrozen control conditions. Specifically, we wanted to obtain more information about reasons for the phenomenon of N_2O bursts during soil freezing and thawing by using a new tool: The investigation of temporal variations in N_2O concentration and isotope signature along soil profiles.

Methods

Site description

The experiment was carried out in a mature Norway spruce forest (*Picea abies* (L.) Karst.) in the Fichtelgebirge (NE Bavaria), Germany, at the Coulissenhieb II research site (50°8′N, 11°52′E) in an elevation of 770 m a.s.l. This site belongs to the Lehstenbach catchment (size: 4.5 km^2), which is almost completely covered by Norway spruce forest. The experimental site is exposed to WNW with a slope of about 3%.

The mean annual temperature in this region is +5.3°C and the average annual precipitation is 1,160 mm of which approximately 20–30% are snow or mixed precipitation (Foken 2003). The soil is a Haplic Podsol with a 6–10 cm thick humus layer with distinct Oi, Oe and Oa horizons. The pH (H_2O) follows a rather small vertical gradient from 4.0 in the Oa horizon to 4.5 in the Bv/Cv horizon. For more detailed description of the soil see Table 1. The understorey vegetation is dominated by *Calamagrostis villosa* (Chaix.) J.F. Gmel., *Deschampsia flexuosa* L., *Vaccinium myrtillus* L. and *Oxalis acetosella* L.

Experimental design

Three control (hereafter C plots) and three snow removal plots (hereafter SR plots), each of an area of 20 m × 20 m, were established in the summer of 2005. All plots were equipped with identical basal instrumentations for measurement of soil temperature,

Table 1 Chemical properties, soil texture, and porosity of the Norway spruce forest soil after Zuber (2007) and Hentschel et al. (2008)

Horizon	Depth [cm]	C [%]	N [%]	Sand [%]	Silt [%]	Clay [%]	Porosity [%]
EA	−10	7.4	0.4	37	46	16	74.3
Bh	−12	5.5	0.3	30	47	22	68.2
Bs	−18	3.4	0.2	33	41	24	67.5
Bw	−55	1.3	0.1	31	48	20	65.7
Cv	<−55	0.4	<0.05	32	46	19	51.0

soil matric potential and soil solution chemistry. To induce soil frost snow was manually removed at the SR plots between the end of December 2005 and the beginning of February 2006. To avoid damage to the forest floor due to snow removal plastic nets (mesh width 1 cm) were used to cover the soil. During the snow removal period the snow cover on the non-manipulated C plots amounted to 20–40 cm depth. This snow cover remained at these plots until the end of March 2006. The amount of snow removed from the SR plots was equal to 147 mm of water (Fig. 1a).

Measurements of N_2O fluxes

On each of the six plots, three stainless steel collars, 8 cm in height and with an inner diameter of 19.5 cm, were installed permanently for N_2O flux measurements. During the snow cover period the chambers on the C plots were inserted 4 cm into the snowpack just before measurement. Concentrations of N_2O, CO_2 and H_2O in the headspace were determined using a closed chamber technique in conjunction with a photoacoustic infrared gas analyser (Multigas Monitor 1312, INNOVA, Denmark, see Yamulki and Jarvis 1999). To exclude influences of changing CO_2 and H_2O concentrations on N_2O concentration measurements (see Yamulki and Jarvis 1999), the air was pumped through a CO_2 trap filled with soda lime pellets (Merck KGaA, Darmstadt, Germany) and a water trap filled with Drierite (98% $CaSO_4$, 2% $CoCl_2$, 8 mesh, W.A. Hammond Drierite Co. LTD, Xenia, USA) before passing the Multigas Monitor. N_2O concentrations were measured with a precision of ±15 ppb for a single N_2O concentration measurement.

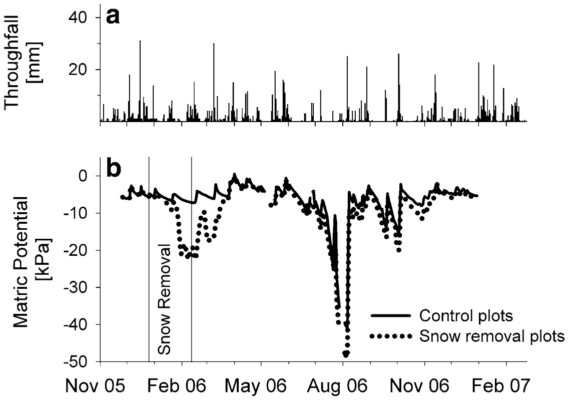

Fig. 1 Throughfall measured at the control plots (**a**) and time course of the daily mean soil matric potential at 20 cm soil depth at the control and snow removal plots (**b**)

N_2O fluxes on each of the six plots were monitored in biweekly to monthly intervals from November 2005 until February 2007. At each measurement date on two plots, one SR and one C plot, N_2O fluxes were simultaneously monitored in three replicates each as described by Goldberg and Gebauer (2009). For each chamber N_2O concentrations in the headspace were analysed five times over the total period of 1 h. Gas flux rates were calculated from the linear increase or decrease in the gas concentrations in the chamber headspace with headspace volume and time. Sum curves were created by multiplying mean emission rates of two consecutive gas flux rates with the corresponding time period and summarizing these time weighted means.

It must be noted that the chamber system used for flux measurements on the snow surface of the C plots may have resulted in a slight underestimation of the N_2O fluxes between soil and atmosphere due to the high porosity of snow.

Gas sampling along soil profiles

N_2O gas samples along soil profiles from each plot were taken by use of six sub-surface soil air tubes. The 50 cm long plastic tubes with 1.6 cm inner diameter were installed horizontally between 5 and 70 cm soil depth at the transition to the soil horizons EA, Bh, Bs, Bw, Cv. In the middle of the Bw horizon an additional soil air tube was installed, because of the thickness of this horizon. Mean installation depths of the gas tubes were 4–6, 10–15, 19–22, 24–30, 40–45 and 65–70 cm, varying from plot to plot because of differences in the thickness of soil horizons. Stainless steel tubes (ID: 1.5 mm) were connected at right angles with the soil air sampling tubes and led to the soil surface. For a more detailed description of the soil air sampling devices see Goldberg et al. (2008a).

For the manual soil air gas sampling glass bottles (100 ml, with an inlet, an outlet, and a septum) were used. The glass bottles were first flushed with N_2 and than evacuated using a membrane vacuum pump. The vacuum in the glass bottles was measured using a pressure gauge (TensioCheck TC 03S, Tensio-Technik, Geisenheim, Germany) and then gas samples from the various soil depths were taken. Soil air was sampled during induced soil frost (7 March 2006) and 3 months after the end of the soil frost period (10 July 2006) as well as in the subsequent mild winter without any soil frost periods (16 January 2007). Samples of ambient air ($n = 3$) were also taken on the respective sampling dates at 50 cm above the soil surface.

Measurement of N_2O isotope ratios and N_2O concentrations

$^{15}N/^{14}N$ and $^{18}O/^{16}O$ ratios of N_2O gas were measured using a pre-concentration device coupled with a gas chromatograph-isotope ratio mass spectrometer (PreCon-GC-C-IRMS) (Hewlett-Packard GC 5890 series II, Wilmington, USA; Combustion Interface II and gas-IRMS delta S, both Finnigan MAT, Bremen, Germany) as described by Brand (1995). The internal reproducibility of the measurement system is typically ±0.15‰ for N and ±0.30‰ for O. Isotope ratios are presented as δ-values, which are defined as:

$$\delta x = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \cdot 1000 \quad [‰] \tag{1}$$

where δx is the δ-value of the heavy isotope x (^{15}N or ^{18}O, respectively) and R is the ratio of heavy isotope (atom percent, at%) to light isotope (at%).

N_2O concentrations were calculated from the sampled gas volume and the peak area on mass 44 with the help of a calibration curve. The reproducibility of N_2O quantification based on this method is ±4 ppb. For further details on this method see Goldberg et al. (2008a).

Data analysis

Each treatment was replicated three times and on each plot, gas flux measurements were carried out with three chambers to consider spatial heterogeneity. For each plot, mean fluxes were calculated by treating the chambers on the same plot as pseudo-replicates and the different plots as true replicates. Thus, for both treatments (SR and C plots) means of $n = 3 \pm$ standard errors were calculated including error propagation. For the calculation of cumulative N_2O emissions, N_2O fluxes between two consecutive measuring dates were linearly interpolated.

The significance of correlations between isotope signatures and N_2O concentrations in the soil profiles from the same treatment and sampling date were tested by using the correlation test after Pearson. N_2O concentrations were log-transformed before executing the analysis to attain a linear relationship between the two according variables.

Results

Soil matric potential, air and soil temperature

Before beginning of the snow removal period in the winter of 2005/2006 no major differences in matric potentials of both C and SR plots were to be found in a depth of 20 cm beneath soil surface (Fig. 1b). During soil freezing and thawing (mid January to May 2006), the soil matric potentials of C and SR plots in 20 cm soil depth differed significantly due to decreased matric potentials on the SR plots. Almost constant matric potentials between −7 and 0 kPa were measured on the C plots, whereas the soil matric potential of the SR plots rapidly decreased to −20 kPa at the end of January 2006 and stepwise increased to the level of the controls again from March to May. In contrast, the soil matric potential in 40 and 90 cm soil depth did not show any significant differences between both treatments throughout the whole year (data not shown). Soil matric potentials in these greater depths varied between −10 to 0 kPa (40 cm) and −5 to 10 kPa (90 cm), respectively, during soil freezing and thawing.

Compared with a 10-year average of −1.5°C the mean winter temperatures (December–March) in 2005/2006 were below-average cold (−3.8°C), whereas the following, snow-free winter (2006/2007) was above-average mild (1.2°C) (Fig. 2a). Thus, climate conditions in the winter period 2005/2006 were almost ideal for the experiment. Due to snow removal below-zero temperatures were attained down to 15 cm soil depth. In January and February the daily mean soil temperature at 5 cm depth dropped to −5°C and remained <0°C until 26 March (Fig. 2a). At 15 cm soil depth, temperatures ranged between −1°C and −0.1°C from 24 January to 29 March. At 25 cm soil depth, temperatures did not differ between C and SR plots at any time (data not shown). The soil of the C plots remained unfrozen at all depths throughout the entire measurement period.

N_2O fluxes

Prior to the snow removal treatment and at the beginning of the soil freezing period, N_2O fluxes between soil and atmosphere of both C and SR plots were similar and ranged between 0.0 and 0.1 µmol m^{-2} h^{-1} (Fig. 2b). From end of February until end of April 2006 soil N_2O emissions on SR plots were considerably enhanced compared with the C plots. This period of enhanced soil N_2O emissions included a major part of the soil frost period as well as the soil thawing period. During this time mean N_2O fluxes of the SR plots ranged between 1.2 ± 0.5 µmol m^{-2} h^{-1} and 3.5 ± 0.9 µmol m^{-2} h^{-1}. Maximum N_2O fluxes occurred on 6 March 2006, after an extreme cold week with daily mean temperatures <−5°C and on 30 March 2006, 3 days after the air temperature showed a first maximum of 8°C. Thereafter, N_2O emission rates decreased with increasing air and soil temperature, but were still five times higher than that of the C plots 3 weeks after the beginning of soil thawing. N_2O emissions on the SR plots reached the level of the C plots in mid of May 2006. Mean N_2O fluxes of the SR plots were 10 to 20-fold higher compared to those of the C plots, but C plots also showed highest emission rates during the late winter and spring period. From June 2006 to the end of the investigation period in January 2007, N_2O fluxes between SR and C plots did not differ significantly and were always <0.1 µmol m^{-2} h^{-1}.

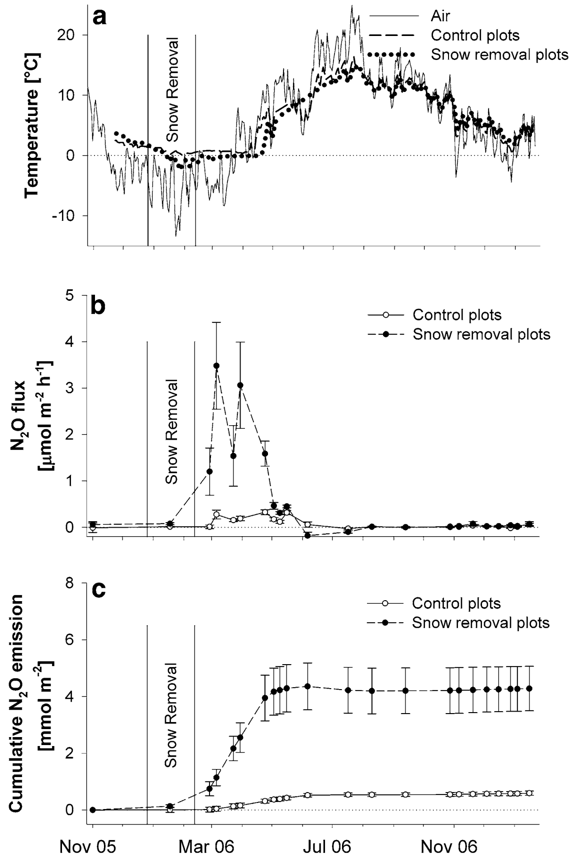

Fig. 2 Time course of daily mean soil temperature at 5 cm depth and air temperature (**a**), mean N_2O fluxes (**b**), and mean cumulative N_2O flux rates (**c**). *Error bars* indicate the standard error of the mean ($n = 3$)

On an annual scale the frost and thaw period of 78 days (from 27 February to 16 May 2006) contributed 84% to the N_2O emissions (3.5 mmol m^{-2}). Even on the snow covered C plots—that were not subjected to soil freezing and thawing—enhanced N_2O emissions during this period contributed 75% (0.4 mmol m^{-2}) to the total annual N_2O emissions of these plots. In total, 4.3 mmol N_2O m^{-2} were emitted from the SR plots throughout the whole course of the experiment (14 months), which significantly exceeded the emissions from the C plots (0.6 mmol N_2O m^{-2}; Fig. 2c).

N_2O concentration and isotope profiles

Throughout the experiment N_2O concentrations exponentially increased with increasing soil depth (Fig. 3). In the uppermost mineral soil horizon (Ea) of both C and SR plots N_2O concentrations were mostly close to ambient air N_2O concentrations (310–380 ppbv). In the lowermost horizon of the investigated soil profiles N_2O concentrations ranged between 850 and 3,400 ppbv. The only exceptions from this general trend were the N_2O concentration profiles of the SR plots during soil frost on 7 March 2006: On this date N_2O concentrations were drastically enhanced in all

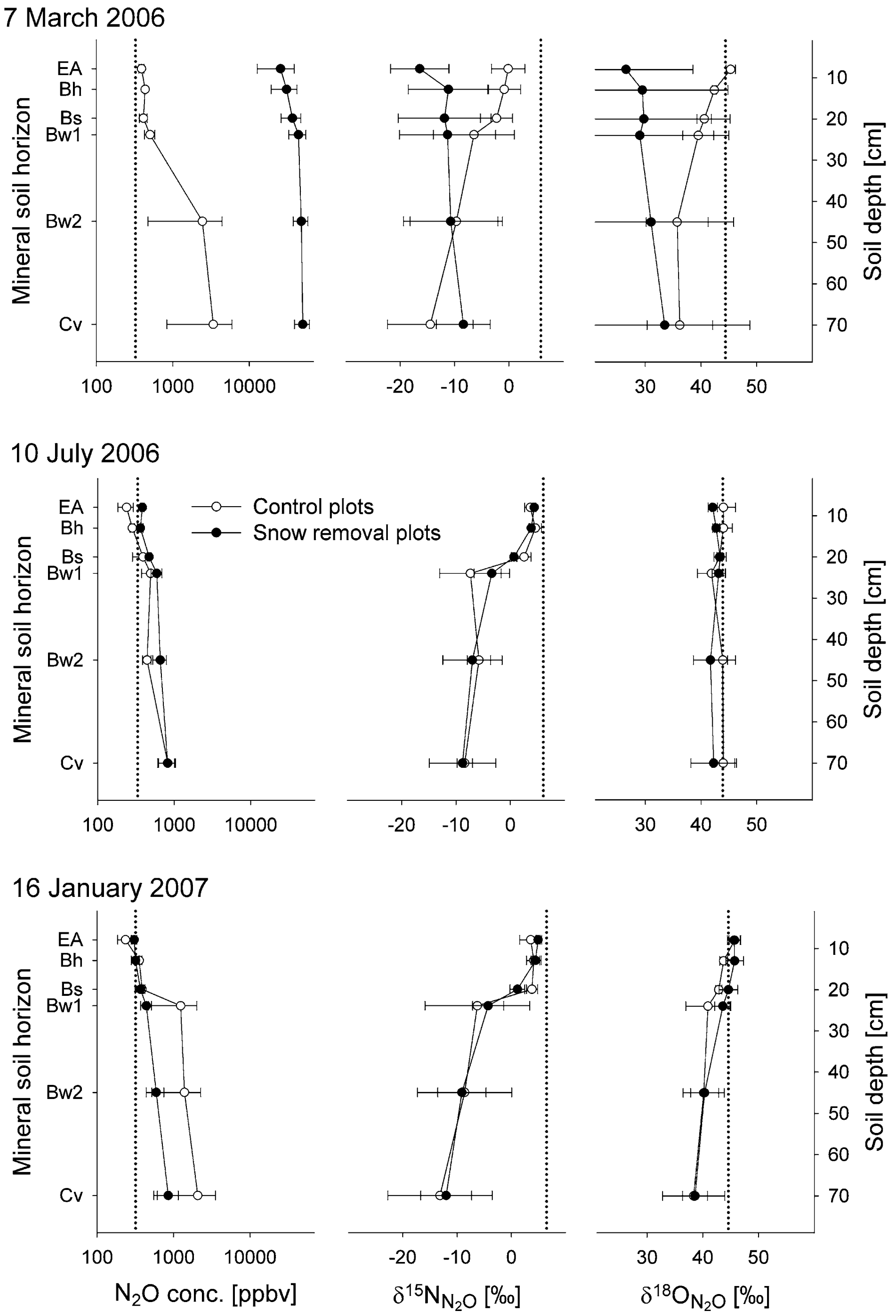

Fig. 3 N_2O concentrations, $\delta^{15}N$ and $\delta^{18}O$ values of N_2O in soil air along soil profiles during soil frost (7 March) and after the experiment (10 July) in 2006, and in the mild winter (16 January) in 2007. *Error bars* indicate the standard error of the mean ($n = 3$). The *dotted line* reflects the respective values of N_2O in the ambient atmosphere. The space between single mineral soil horizons reflects their mean distance in the field (see for comparison soil depth scaling)

soil horizons with values ranging from 26 ± 13 to 50 ± 11 ppmv along the general concentration gradient from the EA to the Cv horizon. The N_2O concentrations on the SR plots increased in a logarithmic manner with soil depth, however, the relative differences between two consecutive soil horizons were significantly lower than on the C plots. Quotients of N_2O concentrations in two consecutive soil horizons ranged between 1.0 and 4.8 in the C plots and between 1.0 and 1.2 in the SR plots. In the summer of 2006 and the winter of 2006/2007, with soil temperatures at any depth remaining above 0°C, N_2O concentrations as well as concentration gradients of the C and SR plots did not differ significantly and were similar to those found for the C plots in the winter of 2005/2006.

In general, the highest soil air N_2O concentrations in the profile coincided with lowest $\delta^{15}N$ signatures and vice versa (Fig. 3) revealing a negative logarithmic correlation. Thus, subsoil N_2O was more depleted in $\delta^{15}N$ than that of the topsoil. The gradient in $\delta^{15}N$ was similar for both C and SR plots, with a mean difference in $\delta^{15}N$ between EA and Cv horizon ranging between 12 and 17‰. In contrast to this general trend, during soil frost on 7 March 2006 $\delta^{15}N$ values of soil air N_2O on the SR plots slightly increased from the unfrozen subsoil to the frozen topsoil and N_2O concentrations were in a positive logarithmic manner correlated with the $\delta^{15}N$ signatures. On this date, mean $\delta^{15}N$ values of -8.4 ± 9‰ were observed in the Cv horizon, stepwise decreasing to -16.4 ± 9‰ in the EA horizon, with the largest mean depletion of 5.3‰ occurring from the Bh to the EA horizon.

$\delta^{18}O$ values mostly followed the same pattern as the $\delta^{15}N$ signatures (Fig. 3) and were in most cases significant in a negative logarithmic manner correlated with N_2O concentrations. This trend holds true for the N_2O depth profiles investigated on C and SR plots on 10 July 2006 and 16 January 2007 as well as for the C plots on 7 March 2006. However, as for $\delta^{15}N$ signatures this relationship to N_2O concentrations over soil depth was positive logarithmic on the SR plots on 7 March 2006. Again, the largest mean depletion in $\delta^{18}O$ (2.9‰) was found in N_2O diffusing from the Bh to the EA horizon on the SR plots on this date. $\delta^{18}O$ and $\delta^{15}N$ values were always strongly correlated, with only one exception in summer. On 10 July 2006 the $\delta^{18}O$ gradient of soil air N_2O along the soil profile was extremely slight. Gradients in $\delta^{18}O$ were always smaller than for $\delta^{15}N$, with differences between the lower- and upper-most soil horizon ranging between 0 and 9‰.

Over the complete experimental period $\delta^{15}N$ and $\delta^{18}O$ values of N_2O in ambient air varied in narrow ranges between 4.9 and 6.2‰ or between 43.1 and 45.9‰, respectively (Fig. 3).

Discussion

N_2O emissions from our mountainous Central European Norway spruce forest soil were significantly enhanced on plots with soil frost induced by snow removal. This observation holds true for both the soil frost and the subsequent thaw period. The magnitude of these bursts in soil N_2O emission with maximum values of 3.5 µmol m^{-2} h^{-1} contributes considerably to annual N_2O emission budgets and is within the range of those found by Papen and Butterbach-Bahl (1999), Teepe et al. (2000) and Groffman et al. (2006) who observed maximum N_2O emissions of approximately 14, 4 and 2 µmol m^{-2} h^{-1}, respectively, for other temperate forest stands.

Explanations for N_2O emission peaks occurring during soil frost are often contradictory in the literature. With regard to agricultural soils, explanations range from N_2O production in unfrozen subsoil and the escape of N_2O (e.g. Burton and Beauchamp 1994; Kaiser et al. 1998) to microbial activity together with N_2O production in unfrozen compartments at the soil surface (e.g. Goodroad and Keeney 1984; Teepe et al. 2001). For temperate forest soils, however, it is assumed that a large portion of N_2O is produced in the organic layer during frost. Papen and Butterbach-Bahl (1999) and Teepe et al. (2000) suggested that huge N_2O releases resulted from high microbial N turnover rates in unfrozen water films at high concentrations of easily degradable substrates. However, results from laboratory experiments investigating specifically topsoils of similar forest soil types, in some cases even from the same study site, showed much smaller N_2O bursts as observed in field studies during soil frost events (Neilsen et al. 2001; Teepe and Ludwig 2004; Goldberg et al. 2008b). Hence, the assumption that the humus layer is the major source of frost-related N_2O bursts from temperate forest soils is rather unlikely.

The N_2O concentration profiles of both the C and SR plots indicate that N_2O was continuously produced in the subsoil, at 70 cm soil depth or deeper, throughout the year with generally low net production rates of 10–200 pmol cm^{-3} day^{-1}, as derived from turnover estimates (see Goldberg et al. 2008a). DOC and NO_3^- leaching to the subsoil is a prerequisite for potentially occurring microbial denitrification in the subsoil of this site. NO_3^- leaching from nitrification in the topsoil as well as from atmospheric deposition is known to contribute in this area considerably to the nitrate found in the groundwater (Durka et al. 1994). At any time—except for the soil frost period—largest N_2O concentrations were accompanied by most depleted $\delta^{15}N$ and $\delta^{18}O$ signatures in the subsoil. The depleted $\delta^{15}N$ and $\delta^{18}O$ signatures confirm N_2O

production in the subsoil (Goldberg and Gebauer 2009). The concentration gradient implies upward diffusion of N_2O, a transport process linked with isotopic discrimination against ^{15}N and ^{18}O ($\varepsilon_{^{15}N} = -4.4‰$, $\varepsilon_{^{18}O} = -8.6‰$, with ε being the enrichment factor, i.e. isotope enrichment of a reaction product relative to that of the substrate; detected by Pérez et al. (2000) for a tropical forest soil). Thus, decreasing $\delta^{15}N$ and $\delta^{18}O$ values during upward transported N_2O would have been expected, if diffusion was the dominating process for the soil N_2O pool. The fact, that N_2O became isotopically enriched in unfrozen topsoil throughout the year, denotes the occurrence of dominating N_2O consumption (reduction of N_2O to N_2) over new N_2O production. N_2O consumption takes place via denitrification and fractionates against ^{15}N and ^{18}O, leading to a stepwise enrichment in ^{15}N and ^{18}O of the remaining N_2O pool. Accompanying fractionation against ^{15}N and ^{18}O depends on the proportion of N_2O reduced to N_2 (Barford et al. 1999), but in general exceeds the fractionation by diffusion (Pérez et al. 2000). Similar N_2O profiles with N_2O production in subsoil and upward diffusion together with stepwise consumption of N_2O have already been observed in different soils (Pérez et al. 2000; van Groenigen et al. 2005; Rock et al. 2007; Goldberg et al. 2008a; Goldberg and Gebauer 2009).

In contrast to this general trend, the N_2O profile affected by soil frost followed a completely different pattern. Under conditions of frozen topsoil, N_2O concentrations in the soil atmosphere were by one to two orders of magnitude higher compared to unfrozen control plots. We assert the higher N_2O concentrations in the soil atmosphere under conditions of a frozen topsoil to be due to the following reasons: (1) Continuing N_2O production in the (unfrozen) subsoil as indicated by 15-fold higher subsoil N_2O concentrations compared to those of the C plots on 7 March 2006 combined with (2) a decreased N_2O consumption during upward movement of subsoil-derived N_2O in the topsoil. Reduced N_2O consumption was most pronounced in the EA horizon as indicated by largest ^{15}N and ^{18}O depletion in N_2O diffusing from the Bh to the EA horizon. This drop of N_2O consumption in the topsoil is likely due to lowered denitrifyer activity and especially lower activity of the enzyme N_2O reductase, as observed for soil temperatures around and below 0°C (Holtan-Hartwig et al. 2002). The reduced denitrifyer and thereby N_2O reductase activity may have also been lowered because of the low content of unfrozen water during freezing. And (3) hindered N_2O exchange between topsoil and atmosphere due to frozen soil water as diffusion barrier in the topsoil as pointed out by smaller detected N_2O fluxes between soil and atmosphere as to be expected from N_2O concentrations in the top mineral soil.

Our results give evidence for the importance of microbial processes in the subsoil on N_2O production, and thus, topsoil fluxes in this forest. Furthermore, the results contradict previous assumptions that an increase in source availability and therewith in microbial activity in the organic layer are chiefly responsible for bursts of N_2O during soil frost periods.

On the C plots without soil frost highest annual N_2O emissions were found throughout the period of snow cover as observed by others, too (Sommerfeld et al. 1993; Maljanen et al. 2003; Yashiro et al. 2006). This may also result from a lowered activity of N_2O reductase in the topsoil as indicated by a shift of $\delta^{15}N$ towards more negative values there in March 2006 compared to the other sampling dates. However, N_2O emission maxima found during periods of snow cover were at least one order of magnitude lower compared to those observed on SR plots during both, soil freezing and thawing.

The observed N_2O emissions on the SR plots during soil frost were of similar importance for the annual N_2O budget as N_2O emissions in the subsequent thawing period. Peak emissions during forest soil thawing are up to now explained by stimulated microbial activity due to an enhanced supply of nutrients, which is caused by die back of microbial biomass and/or disruption of aggregates during soil frost (Papen and Butterbach-Bahl 1999; Neilsen et al. 2001; Teepe et al. 2000).

We conclude from our results that the largest amount of N_2O released from our spruce forest during soil thawing is due to a slow release of subsoil N_2O along the concentration gradient and most probably a delayed activation of N_2O reductase in the topsoil after soil frost due to low soil temperatures. Enhanced nutrient supply due to soil freezing can be excluded as reason for the enhanced N_2O emissions, as reflected by concentration measurements of dissolved organic carbon and N solutes carried out throughout the experiment (Hentschel et al. 2009). This is also confirmed by Muhr et al. (2009), who did not find enhanced CO_2 fluxes during soil thawing.

Conclusions

This study supports the until now scarce findings of a huge relevance of soil frost and thaw periods on N_2O losses from temperate forest soils to the atmosphere and emphasizes that such winter fluxes have to be taken into account in global N_2O models. Our findings of subsoil production of N_2O together with reduced N_2O consumption in the topsoil during soil frost contradict previous mechanistic explanations of soil frost effects on N_2O emission. This emphasizes the necessity to investigate N_2O dynamics along soil profiles in various temperate forest ecosystem types and to identify parameters inducing a subsoil N_2O production in order to improve our mechanistic understanding of N_2O freeze/thaw fluxes from temperate forest soils. Combining N_2O concentration with isotope abundance analysis appears to be a powerful tool in this context. Furthermore, our results give a hint for the reason of different findings in laboratory mesocosm experiments and field investigations on N_2O emissions associated with frost/thaw events in forest soils: Laboratory investigations focussed mostly on topsoil processes, and therefore, excluded potential subsoil N_2O production.

Acknowledgments This investigation was supported by the Deutsche Forschungsgemeinschaft (GE 565/6-2) and contributes to the research group FOR 562 “Soil processes under extreme meteorological boundary conditions”. We wish to thank explicitly all colleagues and students from the University of Bayreuth that contributed to the huge effort of snow removal during the winter of 2005/2006.

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