REGULAR ARTICLE

Atmospheric impact of nitrous oxide uptake by boreal forest soils can be comparable to that of methane uptake

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Abstract

Aims Environmental factors controlling nitrous oxide (N_2O) uptake in forest soils are poorly known, and the atmospheric impact of the forest N_2O sink is not well constrained compared to that of methane (CH_4).

Methods We compared nitrous oxide (N_2O) and CH_4 fluxes over two growing seasons in boreal forest soils located in Eastern Finland. Within a spruce forest, we compared plots with long-term nitrogen (N) fertilization history and non-fertilized plots, and additionally pine forest plots without a fertilization history. The flux data was complemented with measurements of climatic conditions and soil physical and chemical characteristics, in order to identify factors affecting N_2O and CH_4 fluxes.

Results Non-fertilized spruce forest soils showed the highest cumulative N_2O uptake among the sites, whereas the pine forest site displayed low cumulative N_2O emission. Nitrous oxide uptake was favored by high soil silt and water content. The low temperature seasons, spring and autumn, had the highest N_2O uptake, likely associated with high soil water content typical for these seasons. In the spruce forest the N_2O uptake was seasonally decoupled from the CH_4 uptake.

Conclusions Applying the Global Warming Potential (GWP) approach, the cooling effect of N_2O uptake in the spruce forest was on average 35% of that of CH_4 uptake showing that N_2O uptake should be considered when evaluating the atmospheric impact of boreal forests.

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Introduction

The concentration of nitrous oxide (N_2O) in the atmosphere has increased from 270 ppb in preindustrial times to 328 ppb today (Blasing 2017). This increase is mainly due to agricultural N_2O emissions, but also resulting from wastewater treatment and fossil fuel burning (Machida et al. 1995; Flückiger et al. 1999; MacFarling Meure et al. 2006; Thomson et al. 2012;

Blasing 2017). Nitrous oxide is a strong greenhouse gas which has a 298 times higher global warming potential than CO_2 (based on a 100-yr time horizon; Myhre et al. 2014). Nitrous oxide also participates in reactions destroying ozone in the stratosphere. The contribution of N_2O to global warming accounts for 6% of all greenhouse gases (Myhre et al. 2014). Due to the steady increase in the atmospheric N_2O concentration the relative increase in global warming caused by N_2O has been the second largest after CO_2 during the last two decades (Hofmann et al. 2006; Forster et al. 2007).

Soils, particularly agricultural soil but also natural soils from the Tropics, represent 56–70% of the largest global source of N_2O (Butterbach-Bahl et al. 2013; Syakila and Kroaze, 2011). In terrestrial ecosystems, N_2O is produced in several microbiological N cycling processes such as chemolithotrophic ammonium (NH_4^+) oxidation (Wrage et al. 2005), denitrification (Braker and Conrad 2011), nitrifier denitrification (Kool et al. 2011), codenitrification (Spott et al. 2011), dissimilatory nitrate reduction (DNRA) (Laughlin and Stevens 2002), heterotrophic nitrification (Zhang et al. 2015) and chemodenitrification (van Cleemput and Samater 1995). Denitrification and nitrification are considered to be the most important processes for N_2O production, with other processes contributing less than 1% to the total N_2O emissions (Chapuis-Lardy et al. 2007).

The factors that regulate denitrification and nitrification in soils have been categorized as proximal and distal controls (Groffman et al. 1988). The key proximal factors directly controlling the reduction pathway in denitrification are soil oxygen (O_2) and nitrate (NO_3^-) concentrations, availability of energy (organic compounds) and temperature (Tiedje 1987; Butterbach-Bahl et al. 2013). For chemolithotrophic nitrification availability of O_2, NH_4^+ and temperature are important proximal controls (Robertson 1989; Kowalchuk and Stephen 2001). Distal factors such as plants, management practices, soil texture and water availability indirectly regulate denitrification and nitrification activities by affecting the proximal controls (Tiedje 1987; Groffman et al. 1988; Wallenstein et al. 2006).

Soils can act as a source or sink for N_2O (Chapuis-Lardy et al. 2007; Schlesinger 2013). Though the importance of soils as N_2O sinks is considered to be small at a global scale, N_2O uptake may be important regionally, e.g. in the boreal region, since most of the uptake seems to take place at high latitudes (Syakila and Kroeze 2011, Stewart et al. 2012; Brummell et al. 2014).

Denitrification is the most important microbial process known to consume N_2O. In denitrification, NO_3^- is reduced stepwise over several intermediates to N_2O, and finally to dinitrogen (N_2), the end product of denitrification. A key question is how efficiently the intermediate N_2O is reduced to N_2 and what factors regulate N_2O reduction. Nitrous oxide is reduced to N_2 by nitrous oxide reductase (N_2OR). High availability of NO_3^-, low pH, and low soil water content (high O_2 availability), are among the factors decreasing the N_2OR activity and increasing the ratio of N_2O to N_2 ($N_2O + N_2$) in denitrification (Weier et al. 1993; Tate 1995; Thomas et al. 1994; Schneider et al. 2012). In contrast, low NO_3^- availability and high soil water content favour the N_2OR activity over the previous reductive steps, which could lead to net N_2O consumption (uptake of N_2O from the atmosphere) (Chapuis-Lardy et al. 2007). In nitrifier denitrification, N_2O can also be consumed by reduction of N_2O to N_2 (Zart and Bock 1998; Shrestha et al. 2002; Megonigal et al. 2004).

Nitrous oxide uptake has been observed both in wetland and upland soils (Ryden 1981; Arah et al. 1991; Chapuis-Lardy et al. 2007). Water saturated ecosystems such as peatlands regularly show atmospheric N_2O uptake (Martikainen et al. 1993; Marushchak et al. 2011). Upland forest soils, even when well-aerated, can also act as a sink for atmospheric N_2O. Periodic N_2O uptake has been observed during dry periods in temperate Norway spruce forests (Bowden et al. 1991; Klemedtsson et al. 1997; Butterbach-Bahl et al. 1998; Goldberg and Gebauer 2009), during spring and autumn in temperate Red pine (Bowden et al. 1990) and Scots pine forests (Butterbach-Bahl et al. 2002) and boreal pine dominated forest (Machakova et al. 2019), in deciduous forests (Dong et al. 1998; Goossens et al. 2001), as well as in Mediterranean pine (Rosenkranz et al. 2006) and oak forests (Shvaleva et al. 2015). According to the best of our knowledge, data on N_2O uptake from boreal spruce forests, an important coniferous forest type in the boreal region, is currently lacking.

Boreal forests cover approximately 11% of the total land area in the world (Archibold 1995) and play an important role in the global CO_2 and CH_4 balance (Brumme et al. 2005). Boreal forest soils have low mineral N availability (Turkington et al. 1998) and the nitrification activity in these N limited boreal forest soils is low (Martikainen 1984; Priha and Smolander 1999; Priha et al. 1999). Therefore, N_2O emissions in non-fertilized boreal coniferous forests with low atmospheric

N deposition are generally low (Ambus and Robertson 2006; Pihlatie et al. 2007; Kanakidou et al. 2016). However, the capacity of these N limited soils to consume atmospheric N_2O is currently not known. If we consider the known controls on N_2O reduction, the conditions in boreal forest soils could both support and inhibit N_2O reduction: the low soil NO_3^- availability limits the overall denitrification but supports reduction of N_2O to N_2 (Palmer et al. 2011). Low pH, however, which is typical in boreal coniferous soils (even below 4) and low soil temperature could inhibit the activity of N_2OR and thus support N_2O production.

Our aim was to study in situ N_2O dynamics in boreal spruce and pine forests. The spruce and pine forest study sites had distinct soil characteristics, and were subjected to different management practices (fertilized and non-fertilized), allowing us to study how soil characteristics and N fertilization affect N_2O dynamics. We also quantified the isotopic signature of N_2O in soil profile gas concentrations to insights into further partition N_2O and behaviour of boreal forest soils processes within the soil profile as previous work has demonstrated reduction of N_2O to N_2 results in an ^{15}N enrichment in N_2O (Vieten et al. 2007; Gil et al. 2017). Forest soils are known to be a major global sink for atmospheric methane (Dutaur and Verchot 2007; Kirschke et al. 2013; Yu et al. 2017).Oxygen availability in well-drained upland forest soils is generally high, supporting microbial (methanotrophs) methane oxidation in these soils. Therefore, along with N_2O dynamics, we assessed CH_4 dynamics and compared the relative atmospheric impact of this trace gases over the growing seasons.

We aimed to answer three main questions with this research: 1) which soil management practices and 2) environmental characteristics support the potential for N_2O uptake, and 3) what is the atmospheric importance of N_2O uptake compared to that of CH_4 uptake. We hypothesized that there are three main controls driving the capacity of boreal forest soils to act as a sink or source for N_2O: 1) soil texture; 2) soil moisture; and 3) availability of mineral N (ammonium and nitrate).

Materials and methods

Study sites

The studied spruce forest was located in Heinävesi (62°41′ N, 28°71′E), and the pine forest in Suonenjoki (62°38.782′ N, 27°01.406′ E), Eastern Finland. The spruce site was established by the Finnish Forest Research Institute in the early 1960s to study the long-term effects of fertilization on forest growth. The tree stand consists mainly of Norway spruce (*Picea abies*) mixed with Rowan (*Sorbus sp.*), and the understory vegetation is dominated by blueberry (*Vaccinium angustifolium* Ait.). *Sphagnum sp.* mosses dominate the ground layer in wetter areas, whereas dryer areas had only *V. angustifolium*. The soil has a sandy loam texture. Two sub-sites in the spruce forest were studied, which were laid out in a split-block random design: 1) control (non-fertilized) site (CS site), and 2) nitrogen-fertilized site (NS site), where N fertilizer was applied in 4–10-year intervals. From 1958 to 1965, ammonium sulphate was used as the N fertilizer and from 1972 onwards ammonium sulphate was replaced by ammonium nitrate with lime following the general changes in forest fertilization practices in Finland. The total amount of applied of N until the year 2009 was 714 kg ha^{-1} (Saarsalmi et al. 2014).

The pine forest site (CP) has no fertilization history, consists exclusively of pine (*Pinus sylvestris*), the understory vegetation has a sparse coverage of lingonberry (*Vaccinium vitis-idaea*) and the ground layer is dominated by lichens (*Cladonia rangiferina*) with some *Sphagnum sp.* mosses. The pine forest soil has loamy sand texture (ISO 14688).

Both soils are classified as Orthic podzol according to FAO guidelines (IUSS Working Group WRB 2006). The soil horizons A, E (eluviated), B (alluvial) and C - horizons were overlain by an organic layer (O thickness 3-10 cm).

Site preparation

To measure N_2O and CH_4 flux rates, four replicate aluminum collars (60 cm × 60 cm) were installed permanently for the gas flux measurements at each of the three sites (CS, NS, CP) in June 2012. The distance between collars was 10–20 m. Additionally, we inserted perforated PVC tubes covered with gas permeable nylon fabric (Pihlatie et al. 2007) into the soil at the CS and NS sites for taking soil gas samples. The tubes were installed horizontally into the O, A, E, B and C -horizons, at depths of 5 cm, 15 cm, 25 cm, 35 cm and 50 cm, respectively (Fig. 3b and S2). To measure soil O_2 concentration, soil moisture, soil and air temperatures continuously (data were collected every 30 min from which

daily means were calculated) a Campbell Scientific CR3000 data logger equipped with multiplexer was used in 2013 and 2014 at the CS site. Data was collected with following sensors: moisture sensor (CS-616 water content reflectometer, Campbell Scientific, Inc., Shepshed, UK), temperature sensor (107-L temperature probe, Campbell Scientific, Inc., Shepshed, UK) and O_2 sensor (GS Oxygen Sensor KE-25, Figaro USA Inc., Illinois, USA). Sensors were installed and calibrated as described previously (Gil et al. 2017) and inserted by drilling them into the soil profiles in March 2013 when soil was frozen.

Measurements of nitrous oxide and methane fluxes

The gas tightness between the soil and the collars was ensured by fixing a polyethylene membrane outside of the collars extending from the collars to the surrounding forest floor. Sand was added on the membrane on the forest floor. The collars were not pushed into the soil to avoid breakdown of plant roots and disturbances in nutrient uptake by plants and release of root exudates, all important factors for the activity of soil microbes. The upper part of the collar had a groove filled with water to create an airtight seal between the opaque aluminum chamber (60 cm × 60 cm × 10 cm, volume 36 l) and the collar during gas sampling. Each chamber had a circular vent tube to prevent development of under pressure within the chamber headspace during sampling (Nykänen et al. 1995). The chambers were equipped with a fan to ensure mixing of chamber air.

Gas samples were withdrawn from the chambers at intervals of 5, 10, 20 and 40 min with polypropylene syringes (Terumo, equipped with three-way stopcocks) during the 40 min enclosure time. Gas samples were transferred into N_2-flushed and pre-evacuated glass vials closed with rubber septa (Labco Exetainer) within 24 h following sampling. The gas fluxes were measured at regular intervals during the 2012 growing season at all sites (approx. Every 3 weeks) (in total 27 flux measurements in 2012). The measurement frequency was increased in the 2013 growing season (between 9th April and 28th October 2013) at the CS and NS spruce sites so that fluxes were measured at regular 1–3 week intervals (in total 69 flux measurements in 2013).

The spatial heterogeneity of N_2O fluxes at the CS site was studied by a grid sampling approach. On 16th July 2013, the fluxes and soil NO_3^- content were measured once across the site over a 14 × 22 m plot using 25

measurement points (Fig. S1). These fluxes were measured with a round stainless steel chamber (volume ~ 15 dm^3). The open bottom part of the chambers was pushed into soil to a depth of 3–7 cm.

Gas samples were analyzed for N_2O and CH_4 with an Agilent gas chromatograph (GC) equipped with a Hayesep Q 80/100 mesh column (length 1.8 m) and an electron capture detector (ECD) for N_2O and a flame ionization detector (FID) for CH_4 (Nykänen et al. 1995). The flux rates were calculated by linear regression between the gas concentration data and sampling time. A correlation coefficient > 0.60 was used as a quality criterion to accept the flux. The minimum detectable flux was calculated based on the GC detection limit (signal variation of standards in GC caused by the method) corresponding to ±0.77 s.d. 0.41 mg CH_4 m^{-2} d^{-1} for CH_4 and ± 20.76 s.d. 9.86 μg N_2O m^{-2} d^{-1} for N_2O ($N = 35$). Fluxes under this detection limit were excluded from further analyses. In total, 20% of CH_4 and 7% of N_2O fluxes were excluded. The atmospheric impact of N_2O and CH_4 fluxes was compared in CO_2-equivalents using the Global Warming Potential approach (GWP, 100 year-time-horizon). The calculation of CO_2-equivalents used 34 and 298 times greater GWP for CH_4 and N_2O, respectively (Myhre et al. 2013). Cumulative fluxes were calculated by first calculating daily fluxes from measured hourly fluxes (assuming no diurnal variation) and then linearly interpolating between the measured daily fluxes for each sampling period (time between two sampling days) and summing the fluxes over all sampling periods.

Environmental variables and soil characteristics

Air temperature and precipitation data was obtained from a permanent meteorological station of the Finnish Meteorological Institute in Heinävesi (2 km away from the study site). Soil temperatures at 0, 5, 10, and 20 cm soil depth were measured next to each collar during gas sampling. Photosynthetically Active Radiation (PAR) above the chambers was measured with a PAR meter (LI-250A, Li-Cor, Lincoln, USA) during the gas flux measurements.

Three soil cores (0–30 cm depth) were collected once per month near each collar after gas flux measurements using a corer (35 mm in diameter). The collected profile consisted of an O, A, and E-horizon which were separated by solid color differences. Soil moisture and chemical characteristics were determined from the two top

soil layers (organic O and A-horizon). After sampling, soil layers were put in plastic bags sealed tightly with plastic liners and stored at +4 °C in Styrofoam box with ice packages until soil analyses were done. Before analyses, soils were homogenized and then sieved to remove roots and stones (mesh size 3 mm).

Soil water content of the O and A-horizons was measured by drying samples over night at +60 °C and + 105 °C, respectively. Soil particle density was measured by boiling a soil-water suspension of homogenized soil in pycnometers of known volumes for 30 min. Water-filled pore space was calculated based on volumetric soil water content, particle density and bulk density. Organic matter (OM) content was estimated by loss-of-ignition at 550 °C for 2 h (Marushchak et al. 2011).

Two grams of field-moist soil were extracted with 15 ml of 1.0 M KCl in sterile 50 ml polypropylene tubes for determining NO_3^- and NH_4^+ concentrations. The soil-KCl suspension was shaken for 60 min at 150 rpm and subsequently centrifuged at 5000 rpm for 10 min. Extracts were filtered through 0.2 μm sterile filters. Filtrates were then collected and frozen and refrigerated at −80 °C until analysis. Soil pH and electrical conductivity were measured from soil-water suspension (1:2).

Nitrate concentrations were analyzed spectrophotometrically after reduction to nitrite. In brief, NO_3^- was reduced to nitrite with vanadium reagent (VCl_3) (at +37 °C, 90 min) and nitrite was then measured spectrophotometrically at 544 nm using a mixture of N- (1-napthyl) ethylenedianminedihydrochloride (NED) and sulfanilamide (SA) (Miranda et al. 2001). Ammonium was determined spectrophotometrically (630 nm) with the sodiumphenate, −prusside and -hypochlorite method (Fawcett and Scott 1960; Voigt et al. 2017).

To measure soil water retention characteristics, intact soil cores (diameter 50 mm) were sampled next to the study plots from A-horizons. Particle size distribution was measured from air-dried mineral soil using a series of sieves (20 to 0.002 mm; $n = 3$). Dry bulk density was determined as the ratio of dry mass (dried at 105 °C) to saturated volume (n = 3). Particle density was estimated using the water pycnometer method (Heiskanen 1993). Volumetric water content (VWC) at decreasing matric potentials (i.e. desorption water retention characteristics) was measured with intact soil cores using a pressure plate apparatus (Soil moisture Equipment Corp., Santa Barbara, California, USA) and standard methods (Klute 1986; Heiskanen 1993): metal cylinders were

filled with each soil sample, saturated, allowed to drain freely (to about −0.3 kPa), and then exposed to successive matric potential down to −1500 kPa ($n = 3$). Water content was reassessed gravimetrically at each matric potential.

Total porosity (TP) was estimated using:

TP = AFP + WC,

where AFP is air-filled porosity and WC is water content in vol.% (porespace including air and water in pores, vol.%).

Air-filled porosity (AFP) was estimated using:

AFP = TP-VWC.

where VWC is the volumetric water content at −10 kPa.

Nitrous oxide concentration in soil, $^{14}N/^{15}N\text{-}N_2O$ isotopic ratios and soil abiotic characteristics

Nitrous oxide concentration in soil profiles of the CS and NS sites were measured from gas samples taken from soil horizons, as described above (Fig. S4). Soil gases were collected with syringes (sample volume 25 ml) biweekly during the growing season after soil gas flux measurements and the N_2O concentration was analyzed as described above.

The $\delta^{15}N\text{-}N_2O$ values in N_2O in the sites having frequent N_2O uptake (CS and NS) from soil gas, chamber headspace air, and ambient air were determined for the CS and NS sites on 28th August 2013 and 3rd of October, respectively (Fig. S4). Gas samples of 120 ml were taken with a 150 ml polyethylene syringe from the air and from soil gas collectors, respectively, and immediately injected into pre-evacuated 120 ml Wheaton vials ensuring pressure equilibration. The isotopic ratio of N_2O were measured with Thermo Finnigan Delta V Plus isotope ratio mass spectrometer (IRMS) operated in continuous-flow mode, coupled with an online Finnigan pre-concentrator unit (Precon; Thermo Finnigan, Germany) and gas chromatograph (Trace GC; Thermo Finnigan, Bremen, Germany) with PoraPlot Q capillary column (0.32×25 m). Due to high aldehyde concentration in the soil gases especially in the uppermost soil horizons, the mass spectrometer was equipped with a selective aldehyde trap (Waters Inc.) which was attached in front of the ascartite column. The elemental analyzer (EA; Flash EA 112, Thermo Finnigan, Bremen, Germany) connected to the IRMS was used to measure the C and N content (%) and the C:N ratio of the soils.

The $\delta^{15}N$ values of N_2O (reported in parts per thousand; in ‰) were computed from $^{14}N/^{15}N$ ratios using the following equation:

$$\delta^{15}N-N_2O = (Rsample/Rstandard-1) \times 1000.$$

Where R is the $^{15}N/^{14}N$ ratio of N_2O and the standard is atmospheric N_2. The precision of the isotopic composition measurements in N_2O was better than 0.3‰.

Statistical analyses

All statistical tests were made with R statistical program version 3.2.2 (R Core Team 2015). The data was separated into two subsets for statistical analyses to cover the three main hypotheses of the study: 1) to identify the overall controls on N_2O fluxes in the CS, NS and CP sites sampled evenly in year 2012 (data used for testing hypotheses 1, 2, 3); and 2) to study the effect of past fertilization and availability of mineral N on N_2O fluxes in the intensively sampled spruce sites (CS and NS) over multiple years (2012–2014) (data used for testing hypothesis 2).

Prior to statistical analyses, data were tested for normal distribution using histograms and density and qq-plots coupled with the Shapiro-Wilk normality test. The differences in the cumulative fluxes between forest types were tested with Two-Way ANOVA and TukeyHSD post hoc comparison. The spatial interpolation over the sampling plots at the CS site was calculated with R package *geoR* (Paulo et al. 2016).

To examine the strength of the factors regulating N_2O flux, correlations between environmental variables and N_2O fluxes were done with the non-parametric Spearman's correlation test. We applied principle component analysis (PCA) to reduce data dimensionality and to visualize the variance structure of dataset 1, in order to identify differences between sites and treatments in terms of N_2O fluxes, soil and environmental properties. The variables included in the PCA were scaled (standardized to unit variance). Six components were included in the PCA, and PCA-biplots were created using R-packages FactoMineR (Lê et al. 2008) and factoextra (Kassambara and Mundt 2016).

To account for the differences among sites (spruce vs. pine (dataset 1)) as well as treatment (fertilized vs. non-fertilized spruce (dataset 2)), and to account for repeated measurements on the same plot in multiple years, we applied linear mixed-effects models (R-package *lme4*, Bates et al. 2015), following the procedure described by Zuur et al. (2009) and Voigt et al. (2017).

We created two distinct statistical models: model 1 (dataset 1) included both forest types (sites CS and CP) for the evenly sampled 2012 dataset to evaluate which of the environmental factors were explaining variation in N_2O fluxes, and model 2 (dataset 2) included data obtained from the spruce forest sites (CS and NS) (years 2012–2014), with fertilized and non-fertilized treatments (in total, 11 measured timepoints). The final output for model 1 was constructed using forest type (pine vs. spruce), treatment (fertilized vs. non-fertilized), soil layer (O or A-horizon), air temperature and pH in the fixed effects structure, and the interaction of sampling time and treatment as random effects. Model 2 included treatment, air temperature, pH, WFPS, as well as the interactions of NH_4^+ with air temperature and pH as fixed effects, and plot nested in sampling time as random effects.

Results

Soil physical and chemical properties

Spruce soil had sandy loam soil texture, whereas the pine soil had loamy sand texture (Table 1). Content of OM was highest in the O-horizon of the fertilized spruce forest soil. However, the effect of fertilization was not seen in the C:N ratio of OM. In general, NO_3^- concentration in the pine soil was lower than in the spruce soils, and the O-horizon of the fertilized spruce soil had the highest NO_3^- concentration. The same trend was noticeable for NH_4^+ concentrations in O-horizons, but generally NH_4^+ concentrations were an order of magnitude lower than NO_3^- concentrations. Soil pH was lowest in the non-fertilized spruce soil (CS). Soil electrical conductivity was lowest in the pine soil (CP). We observed a trend towards higher water content and water-filled-pore-space (WFPS) in the spruce sites compared (CS and NS) to the pine site. Field capacity of the A horizon of the spruce forest (CS and NS) was higher than that of the pine forest A horizon.

Table 1 Soil chemical and physical properties of the spruce and pine forests on July 2012 ($N = 4$). Significant differences ($P < 0.05$) to spruce control (CS) soil are marked with asterisk

O-horizon	Tair	NO_3^- [µg NO_3^--N g^{-1} soil (DW)]	NH_4^+ [µg NH_4^+-N g^{-1} soil (DW)]	EC	pH	Soil water content %	WFPS %	Soil type
Spruce control (CS)	18,6 ± 1,14	610,53 ± 239,74	32,60 ± 1,53	86,55 ± 35,3	3,44 ± 1,74	61,06 ± 1,77	80,93 ± 2,34	Sandy loam
Spruce N fertilized (NS)	20,17 ± 1,43	1802,59 ± 1167,14	50,19 ± 18,58	68,0 ± 1,89	4,38 ± 0,13	59,54 ± 0,68	97,44 ± 1,12 *	
Pine (CP)	17,5 ± 0	518,11 ± 271,25	17,41 ± 1,30 *	37,67 ± 2,18 *	4,31 ± 0,04 *	58,19 ± 1,79	71,36 ± 2,19 *	
A-horizon	**Coordinates**							
Spruce control (CS)	62°24.542′ N, 28°36.571′ E	225,58 ± 202,79	11,01 ± 0,21	22,0 ± 6,60	3,22 ± 2,25	22,60 ± 1,18	51,69 ± 2,71	Sandy loam
Spruce N fertilized (NS)	62°24.556′ N, 28°42.494′ E	208,81 ± 89,41	10,51 ± 0,30	19,33 ± 0,54	4,55 ± 0,04	17,66 ± 0,84 *	33,34 ± 1,58 *	Sandy loam
Pine (CP)	62°38.782′ N, 27°01.406′ E	39,63 ± 28,44 *	9,62 ± 1,28	11,67 ± 9,25	5,51 ± 0,02	8,78 ± 0,45 *	18,63 ± 0,95 *	Loamy sand

O-horizon	Field capacity	Total porosity	SOM	C%	N%	C/N	Tree stand
Spruce control (CS)		0,75 ± 0,004	50,45 ± 0,41	27,59 ± 0,1	1,07 ± 0,02	25,79 ± 0,51	Picea abies (>95%), Sorbus sp. (<5%)
Spruce N fertilized (NS)		0,61 ± 0,07 *	63,59 ± 4,77 *	43,81 ± 0,50 *	1,31 ± 0,01 *	33,55 ± 0,56 *	Picea abies (>95%), Sorbus sp. (<5%)
Pine (CP)		0,82 ± 0,004 *	48,72 ± 11,44	24,94 ± 6,31	0,73 ± 0,18 *	34,34 ± 0,13 *	Pinus sylvestris (100%)
A-horizon							**Understory vegetation**
Spruce control (CS)	37,04 ± 0.98	0,44 ± 0,07	12,37 ± 0,56	6,32 ± 0,45	0,31 ± 0,01	20,27 ± 0,90	Vaccinium myrtillus (25%), Sphagnum sp. (75%)
Spruce N fertilized (NS)	N/A	0,53 ± 0,031 *	9,12 ± 0,11 *	5,04 ± 0,13 *	0,25 ± 0,00 *	19,88 ± 0,75	Vaccinium myrtillus (25%), Sphagnum sp. (75%)
Pine (CP)	18,20 ± 1.31 *	0,47 ± 0,031 *	4,24 ± 0,98 *	4,88 ± 0,87 *	0,18 ± 0,00 *	27,33 ± 0,00 *	Vaccinium vitis-idaea (20%), Cladonia rangiferina (60%), Sphagnum sp. (10%)

Nitrous oxide fluxes

Nitrous oxide fluxes in the spruce and pine sites

Nitrous oxide fluxes varied between uptake rates of -631.7 µg N_2O m^{-2} d^{-1} to emissions of 591.3 µg N_2O m^{-2} d^{-1}, and CH_4 fluxes ranged between -10.8 and 4.2 mg CH_4 m^{-2} d^{-1} across all study sites during the 2012 growing season. The cumulative N_2O fluxes differed significantly between sites (Fig. 1a. $F^{sites} = 7.325$; $P < 0.05$). In June–July 2012, the pine forest showed cumulative N_2O emissions, whereas the spruce control site displayed cumulative N_2O uptake (Fig. 1.), and the fluxes differed significantly from each other and from zero ($P < 0.05$). All sites showed cumulative CH_4 uptake rates, which were highest at the CS site, and slightly (not significant) lower at the NS and P sites.

Comparison of nitrous oxide uptake with methane uptake

Methane and nitrous oxide showed a pronounced decoupling at the CS site in 2013 (Fig. 2a), i.e. N_2O fluxes were larger during wet periods when CH_4 fluxes were close to zero. On the other hand, during times of pronounced CH_4 uptake the N_2O fluxes were fluctuating around zero. The global warming potential (GWP) approach was used to compare the atmospheric impacts of the cumulative N_2O and CH_4 uptake (Fig. 1b, calculated with data shown in Fig. 2a) in the CS site during growing season 2013. Expressed as CO_2 - equivalents, the GWP of the N_2O uptake was on average 35% of the CH_4 uptake (Fig. 1b).

Seasonal and spatial variation of nitrous oxide fluxes

Nitrous oxide fluxes in the CS and NS sites varied from low emission to uptake in 2013 (Supplementary Fig. S2). At the CS site, N_2O emissions were highest during the summer, whereas there was uptake in early spring, late autumn and winter. In contrast, N_2O fluxes at the NS site were less variable over the seasons. However, despite the different seasonal patterns in N_2O fluxes at the CS and NS sites, the mean fluxes at these sites did not differ significantly (Linear mixed effect model: Fig. S2, $P = 0.334$ in Table S1). The interaction effect of NH_4^+ concentration in A-horizon and soil pH on N_2O fluxes in the spruce sites was the strongest and only significant factor explaining the variation ($P = 0.045$ in Table S1., results of model 2 retrieved from dataset 2). The highest uptake occurred in April before snow melt (Fig. 2a), when soil was still frozen (Fig. 2c). During this peak N_2O uptake in spring temperature was low, soil water content was high and there was a decrease in O_2 concentrations in the soil profile (minimum O_2 concentration about 10%) (Fig. 2 b, c, d).
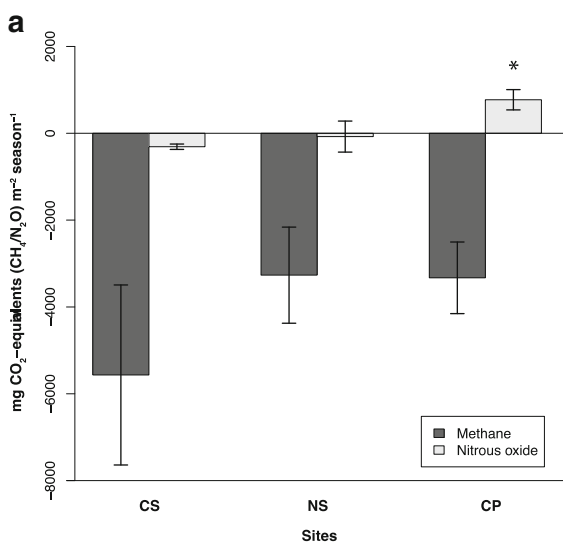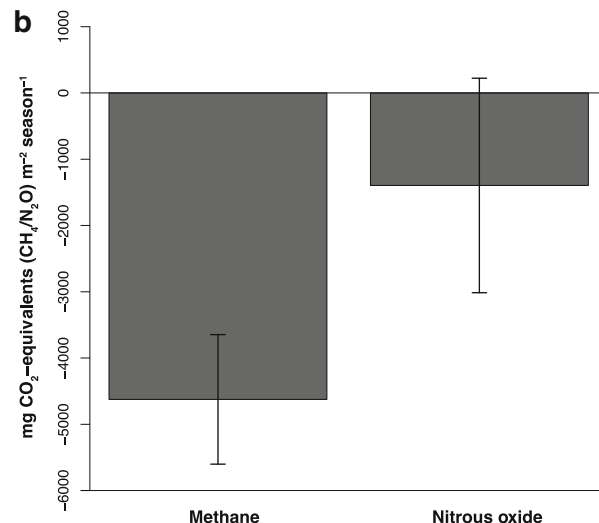

Fig. 1 **a** Cumulative CH_4 and N_2O fluxes shown as CO_2-equivalents from boreal forests. Measurements were conducted during 12th June – 27th July in 2012 (n = 4). Significant differences as compared to CS site are shown with asterisk (P < 0.05). **b** Comparison of cumulative N_2O and CH_4 fluxes as CO_2 equivalents for the growing season in the CS sub-site. Measurements were done between 9th April and 3rd October in 2013
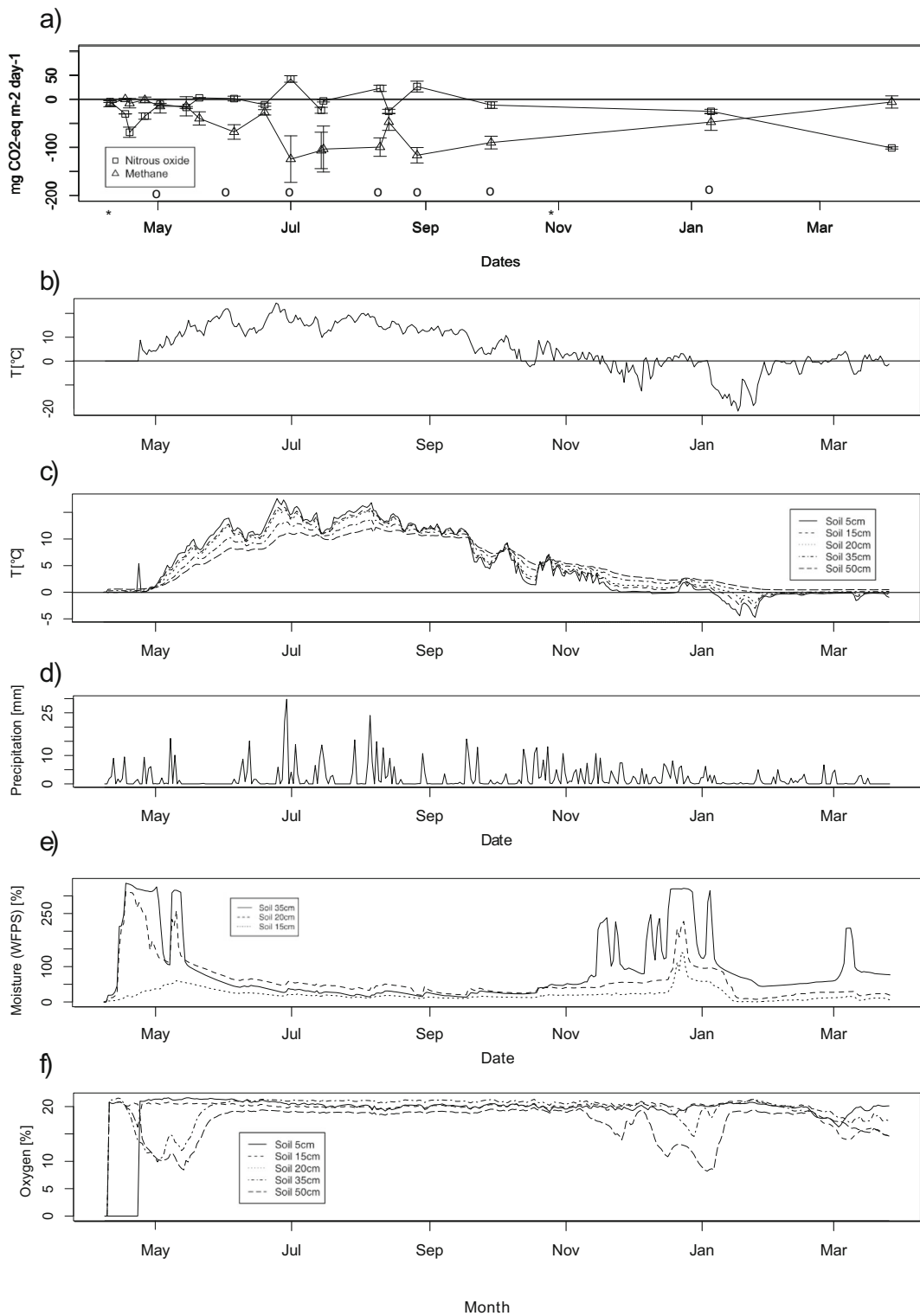
Fig. 2 N_2O and CH_4 fluxes in 2013–2014 expressed as CO_2-equivalents at the CS site (**a**) soil sampling points shown with °-mark, air temperature (**b**), soil profile temperatures (**c**), precipitation (**d**), soil moisture (**e**) and O_2 concentration in soil profile (**f**). The beginning and the end of the growing season in 2013 (9th April and 28th October) is marked with asterisk in (**a**)

Spatial variation of N_2O fluxes was measured at the CS site with a grid sampling approach. While N_2O fluxes at the CS site displayed spatial variation, the mean fluxes measured on the same day from 25 measurement points of a 5 m × 15 m spatial sampling plot (Fig. 3a) and permanent collars both showed N_2O uptake (-84.6 s.d. 33.1 µg N_2O m^{-2} d^{-1} and -181.64 s.d. 132.2 µg N_2O m^{-2} d^{-1}, respectively).

The N_2O fluxes were highly variable across the study site. Even though this approach was used only once, it highlights the spatial variability of N_2O fluxes. The average fluxes obtained from the grid sampling measurements reflect the areal estimate of the overall N_2O flux of the study site (mean -84.62 ± 33.13 µg N_2O m^{-2} d^{-1}). The mean flux rate from the grid sampling approach was rather similar to the mean flux rate measured from permanent plots (-132 µg N_2O m^{-2} d^{-1}) on the same day, located inside the grid sampling plot.

During the growing season, the N_2O concentrations in CS soil profile increased with depth, being highest at a depth of 35 cm. This was reflected by high N_2O emissions (Fig. 3b). When N_2O uptake was measured, N_2O concentrations were below the ambient concentration in the uppermost soil horizons (O- and A-horizons) (Fig. 3b). $\delta^{15}N$ enrichment of N_2O was present at 15 cm depth of the spruce forest (Fig.

S2C), corresponding to a decrease in N_2O concentration at this depth (Fig. S2B). Additionally, N-N_2O in the chambers was enriched compared to the ambient N-N_2O at the end of the 60 min measurement period (Fig. S2C).

Controls on nitrous oxide and methane fluxes

In the 2012 growing season, the N_2O fluxes from the pine and spruce sites correlated positively with soil pH and negatively with SOM content, soil water content, WFPS, water retention capacity (with 5–33 kPa pressure) and with fine sand/silt particles (Table 2). There was no correlation between N_2O fluxes and precipitation. However, time lagged (three days) precipitation correlated negatively with the N_2O flux (Table 2) and NH_4^+ and NO_3^- content in soil (Table 2). The negative correlations were stronger in the A- than in the O-horizon. Methane fluxes correlated positively with SOM content and water retention at 0.3 kPa pressure in O-horizon, and negatively with electrical conductivity, NH_4^+ concentrations, total porosity and water retention (with 0.3–1 kPa).

Principal component analysis revealed differences in the physical-chemical properties, such as particle size distribution, in soils of the pine and spruce sites (Fig. 4). Fine sand, silt and clay abundance in the spruce soil,
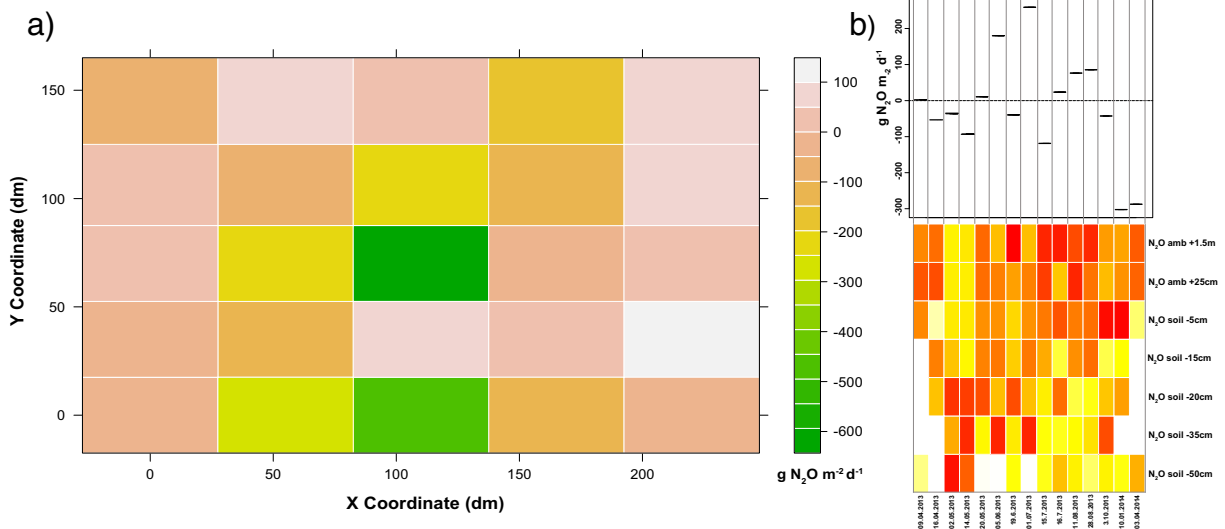

Fig. 3 Spatial grid sampling of N_2O fluxes (**a**) in site CS on 16th of June 2013. Sampling grid was 15 × 22 m, and 25 flux measurements were done across the area. The collars used to measure seasonal variation in the fluxes were located inside this area. Soil and ambient air N_2O concentrations in site CS (**b**). Mean N_2O flux of same sampling points are plotted above. The color code is scaled to N_2O concentrations of each sampling date, highest value of the day is presented with red and smallest with pale yellow (in printed with greyscale, highest value is black and smallest with pale gray). White cell presents "not analyzed"

Table 2 Correlations between the background soil/environmental variables and the N_2O and CH_4 fluxes from pine and spruce sites in 2012. The correlations with soil variables were done either with the data from O- or A-horizons. EC = Electrical conductivity, LOI = Loss of ignition, SWC = Soil water content, WFPS = Water filled pore space, TP = Total porosity, VWC = Volumetric water content at matrix potential e.g. 0.3 kPa. Significant correlations are bolded and shown with symbols (& significant at $P < 0.15$, # significant at $P < 0.10$, * significant at $P < 0.05$)

| Spearman's correlation coefficiency Data from 2012 three forest types together	Spearman's *rho* O-horizon N_2O flux vs.	Spearman's *rho* A-horizon N_2O flux vs.	Spearman's *rho* O-horizon CH_4 flux vs.	Spearman's *rho* A-horizon CH_4 flux vs.
pH	**0.42 ***	**0.303 &**	0.130	0.186
EC	0.061	−0.005	**−0.456 ***	−0.294
LOI	0.236	**−0.307 &**	**0.369 #**	−0.19
NH_4^+ concentration	−0.181	−0.003	**−0.384 #**	**−0.408 ***
NO_3^- concentration	0.218	0.010	0.211	0.096
Soil water content	−0.186	**−0.433 ***	−0.182	−0.064
WFPS	−0.154	**−0.424 ***	−0.256	−0.073
TP	0.223	0.019	0.211	**−0.452 ***
WC 0.3 kPa	−0.019	−0.135	**0.512 ***	**−0.383 #**
WC 1 kPa	−0.186	−0.236	0.115	**−0.336 &**
WC 5 kPa	**−0.394 #**	**−0.303 &**	0.0	0.062
WC 10 kPa	**−0.355 #**	**−0.425 ***	0.088	−0.274
WC 33 kPa	−0.297	**−0.425 ***	0.114	−0.274
WC 100 kPa	−0.297	−0.316	0.114	−0.034
WC 1500 kPa	−0.10	0.127	0.228	−0.003
Coarse sand	NA	0.306	NA	0.020
Medium sand	NA	0.339	NA	0.060
Fine sand	NA	**−0.379 #**	NA	−0.180
Clay	NA	−0.200	NA	−0.269
Silt sum	NA	**−0.314 &**	NA	−0.048
Sand sum	NA	0.283	NA	0.110
Gravel sum	NA	−0.224	NA	−0.208
Precipitation sum 24 h		−0.11		0.154
Precipitation sum 48 h		−0.11		−0.002
Precipitation sum 72 h		0.160		0.204
Time lagged precipitation 24 h after		0.105		0.154
Time lagged precipitation 3 days after		−0.239		−0.251

favoured low N_2O fluxes (N_2O uptake) (Fig. 4). On the other hand, coarse and medium soil particles together with low soil pH were associated with higher N_2O fluxes (emissions). Fine sand and silt/clay particles were linked to high soil water retention capacity (e.g. WC33 kPa = water retention at 33 kPa pressure) both associated with low N_2O fluxes (Fig. 4).

Based on the physical and chemical variables in the A horizon, linear mixed-effects models identified the forest type (pine vs. spruce) as a factor affecting the N_2O fluxes (Table 3.).

Discussion

In our study, the spruce forest showed more frequent N_2O uptake than the pine forest. The cumulative N_2O fluxes over the growing season in control spruce forest sub-sites were negative, suggesting that the soil conditions strongly support microbial processes responsible for N_2O uptake. High water retention capacity in fine structured soil, high soil water content and WFPS were identified as the most important factors promoting N_2O uptake in this system.

Fig. 4 The association between the N$_2$O fluxes and environmental factors/physical and chemical properties of A-horizon (year 2012, all sites) studied by principal component analysis (PCA). EC = Electrical conductivity, LOI = Loss of ignition, SWC = Soil water content, WFPS = Water filled pore space, WC33kPa = Soil water retention as volumetric water content at matrix potential at 33 kPa pressure, NH$_4^+$ = soil ammonium concentration, NO$_3^-$ = soil nitrate concentration
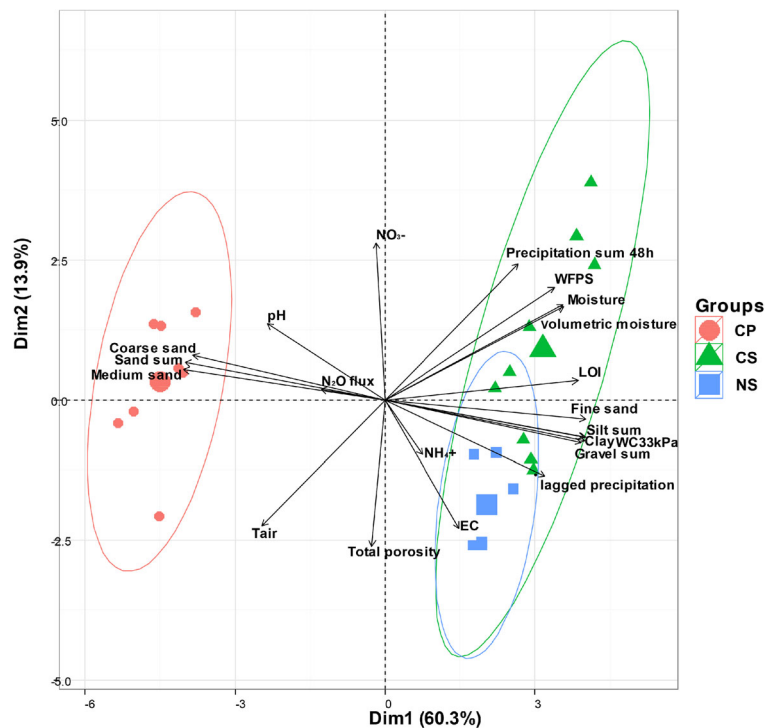
High WFPS reduces the O$_2$ diffusion rates and can create O$_2$ deficiency in soils. Our data demonstrates that N$_2$O uptake took place primarily during winter and early spring when soil water content was high, there was the negative correlation between the N$_2$O flux and the soil water content/water retention, suggesting that higher soil water content supports lower N$_2$O fluxes. On the other hand, highest CH$_4$ uptake rates were measured in summer when soil moisture was low and temperature high, promoting CH$_4$ oxidation. High soil water content and the associated shortage of O$_2$ is known to favor the reduction of N$_2$O to N$_2$ via denitrification (Davidson 1991; Miller et al. 2008). Our isotope results support the

frequent occurrence of N$_2$O-to-N$_2$ reduction in the spruce soils and thus N$_2$O consumption. On the other hand, the highest CH$_4$ uptake rates were measured in summer when soil moisture was low and temperature high. Nitrous oxide uptake peak periods have been reported in springtime in Australian savannahs, temperate pine and deciduous forest (Peichle et al. 2010; Livesley et al. 2011; Ullah and Moore 2011); periodic N$_2$O uptake can thus be connected frequently to pulsing ground water tables (Mander et al. 2015).The maximum N$_2$O uptake of −631 μg N$_2$O-N m^{-2} d^{-1} in our study occurred with high soil water content and is in the median range of reported N$_2$O uptake fluxes for

Table 3 Linear mixed effect model results for N$_2$O fluxes in pine and spruce forests on the growing season 2012 (equal sampling of P, CS and NS sites, dataset 1). Level of significance: ** significant at $P \leq 0.01$, * significant at $P \leq 0.05$, × marginally significant at $P \leq 0.1$

	Estimate	Std. Error	t-value	2.5% CI	97.5% CI	*P* value	Significance
Fixed effects:							
(Intercept)	−96.83	345.92	−0.28	−760.97	571.19	0.789	
Forest type	−132.39	47.55	−2.78	−230.45	−37.66	0.011	*
Soil layer	34.03	38.31	0.89	−42.90	107.41	0.383	
Treatment	82.50	93.77	0.88	−118.63	266.09	0.550	
Tair	−12.65	6.29	−2.01	−25.46	0.29	0.074	×
pH	81.68	60.16	1.36	−32.11	198.16	0.189	

terrestrial ecosystems in general (Chapuis-Lardy et al. 2007). We can assume that denitrification was the key process for the N_2O uptake since maximum uptake occurred when soil water content was high. Nitrous oxide concentration and the isotopic composition of N_2O in soil profiles supported the conclusion that there was N_2O reduction in the uppermost soil horizons. It is noteworthy that although low pH is known to limit N_2O reductase (N_2OR activity) (Simek and Cooper 2002; Richardson et al. 2009; Liu et al. 2010; Bakken et al. 2012), the only known biological sink for N_2O, N_2O reduction was high enough to allow N_2O uptake in these acidic soils. These soils could therefore harbor acid-tolerant denitrifiers as previously found in temperate and arctic wetlands (Palmer et al. 2010; Palmer et al. 2011).

The highest N_2O uptake was measured during the low temperature periods, at the beginning and end of the growing season. However, the N_2O uptake at low temperatures was likely a result of simultaneously high soil water content. It can be assumed that in summer when temperature is high but soil moisture is low, N_2O emissions dominate over N_2O uptake. In summer, the N_2O producing processes are enhanced by elevated temperature (Butterbach-Bahl et al. 2013) so that reduction of N_2O to N_2 through denitrification cannot compensate for the overall N_2O production. It has been reported that N_2O emissions increase with temperature both in the field and laboratory studies (Dobbie and Smith 2001; Pärn et al. 2018). Here, the highest N_2O emission in the spruce forests took place during July–August 2013 with high soil temperature and intermediate soil water content. The N_2O concentration in the soil profile increased during summer in deeper soil layers, at times of the high N_2O emissions. Enhanced soil organic matter decomposition and leaching of dissolved carbon to the deeper soil layers towards the end of the growing season are factors likely supporting N_2O production and emissions (Voigt et al. 2017).

Although maximum N_2O uptake occurred at high soil moisture, the CS site also showed periodic N_2O uptake under dry summer conditions (Fig. 2). The ability of soils to consume N_2O has been previously documented under drought conditions (Goldberg and Gebauer 2009) but poorly understood. The CS soil is characterized by a fine-grained soil structure (silty soil) which can, hypothetically, support the formation of anaerobic microsites even at low soil water content. A recent meta-analysis shows that intermediate soil water

content can trigger highest N_2O emissions, but very high soil moisture reduces N_2O emissions and even allows N_2O uptake (Pärn et al. 2018). Our results further support this finding and our linear models suggest that water retention is the strongest factor explaining the variation of N_2O fluxes over the measurement period. Moreover, there was a significant negative correlation between soil water content (WFPS) and water retention values at higher pressure and fine soil texture. Although classical denitrification is an anaerobic process, some denitrifying bacteria can reduce N_2O in aerobic conditions (Robertson et al. 1989, 1995; Chapuis-Lardy et al. 2007; Desloover et al. 2014; Qu et al. 2016).

We can expect that an increase in the availability of mineral N in soil resulting from N fertilization supports nitrification and denitrification activities, and finally N_2O emissions. It is noteworthy that although the fertilized spruce site historically received a great amount of N in the past (in total 714 kg ha^{-1} over 50 years), the content of NO_3^- and NH_4^+ in the fertilized soil was higher but not significantly different during the study period from that in the non-fertilized soil (likely due to the fact that the last fertilization occurred already 3–4 years ago), showing that the effect of fertilization is short-lived (Saarsalmi and Mälkönen 2001). This indicates that this productive forest has capacity to store added N in the tree stand and within the soil. The additional N at the NS site increased organic matter content, %C and %N in the organic horizons (Table 1). It has been shown that N fertilization increases the amount of organic matter in boreal coniferous forest soils (Martikainen 1996). However, even though there was no great difference in the mineral N content in the soils because the last fertilization occurred 3 years prior to this study, soil pH and NH_4^+ content had a significant summary effect on the N_2O fluxes in spruce sites (Table S1). Non-fertilized sub-site showed slightly higher capacity for N_2O uptake (-1.03 mg N_2O m^{-2} $season^{-1}$) than the fertilized sub-site (-0.33 mg N_2O m^{-2} $season^{-1}$). Higher pH in the fertilized spruce soil could explain this difference because soil pH correlated positively with the N_2O fluxes in the studied acidic forest soils (Table 2).

The results from the pine forest stress the importance of soil structure on N_2O fluxes. We could expect lower N_2O emissions from pine forest soil than from spruce forest soil due to the lower NO_3^- content in pine forest soil. However, the pine site had higher N_2O emissions indicating that the N_2O reduction via denitrification in

this coarse texture soil, in contrast to the fine texture spruce soil, was not strong enough to allow soil to act as a N_2O sink. Winter can contribute significantly to the annual N_2O emissions even though soil temperature is low (Maljanen et al. 2009). There are factors which favour the N_2O production in frozen soil (Koponen et al. 2004, 2006). However, the spruce soil here demonstrated N_2O uptake during winter likely resulted from low soil NO_3^- content (low N mineralization at cold temperatures) during the dormant seasons. Furthermore, low O_2 availability in the soil resulting from high soil moisture during the dormant seasons favour the reduction of N_2O.

In this study, the correlation between soil N_2O concentrations and flux rates was weak but present. Nevertheless, the N_2O concentrations in the soil profiles provided information about the fate of N_2O in soil. In general, the O and A soil horizons (-5 and -15 cm soil layer) of the CS site had lower N_2O concentrations than the deeper layers and displayed below atmospheric concentrations when the site showed N_2O uptake, suggesting that the uppermost soil layers were potential sinks for atmospheric N_2O. The ^{15}N enrichment in N_2O in the uppermost soil horizon and in the emitted N_2O in the spruce forest indicates that discrimination against the heavier isotope of N_2O-N has occurred during the microbial processes, likely during N_2O reduction. ^{15}N enrichment in the N_2O towards uppermost soil horizons has been reported previously (Goldberg and Gebauer 2009; Gil et al. 2017).

Our results show that in addition to CH_4 uptake N_2O uptake must also be considered when evaluating the atmospheric impact of the trace greenhouse gases (CH_4 and N_2O) in boreal coniferous forests. Here, the GWP of N_2O uptake in the growing season 2013 (April 9th – October 3rd) of the control spruce site (CS) (mean -1396, s.e. 1618 mg CO_2-eq season^{-1} m^{-2}), was about 35% of the GWP of the CH_4 uptake (mean -4629, s.e. 1955 mg CO_2-eq season^{-1} m^{-2}). In a Mediterranean pine forest even higher periodic contribution of N_2O uptake has been reported, 60% of the GWP of CH_4, however these measurements were done in a shorter time period, only over springtime (Rosenkranz et al. 2006). We provide here a first approximation of the seasonal N_2O budget for boreal spruce forest soils, possibly be biased by relatively low sampling frequency. However, a good spatial coverage of fluxes were included and simultaneous CH_4 budget was as expected (Saari 2003; Dutaur and Verchot 2007; Kirschke et al.

2013; Yu et al. 2017)., The measurements covered the peak emission/uptake periods and the data allow us to compare N_2O flux to CH_4 flux, which was a main aim of this study.

Methane oxidation and CH_4 uptake can be lowered by NH_4^+ addition in boreal forest soil (Saari et al. 2004) but there are also contrasting results about the effects of NH_4^+ in forest soils (Börjersson and Nohrsted 1998). According to the results reported here even the addition of a large amount of N in N-limited coniferous boreal forest ecosystem does not obviously cause drastic long-term changes in the CH_4 (no statistical difference between the CS and NS sites) (Fig. 1) and N_2O dynamics after the ecosystem has immobilized the added N.

Based on our observations, there are basic differences in the N_2O dynamics in various boreal forests, some having even negative annual N_2O balance, i.e. uptake. Therefore, the evaluation of the total N_2O balance and atmospheric impact of boreal forests requires more N_2O data from forests with different soil characteristics. Only after that, the accurate upscaling of the N_2O fluxes over the whole boreal region, and evaluation of the relevance of N_2O uptake by these ecosystems, will be possible.

Conclusions

This study shows that the capacity of boreal coniferous forests to act as sinks for atmospheric N_2O largely depends on their soil structure and soil water content. Spruce forests with finer soil structure showed higher N_2O uptake than pine forest soils with coarser soil structure. The N_2O uptake by boreal coniferous forest soils can contribute substantially to the total atmospheric impact of the trace greenhouse gas ($CH_4 + N_2O$) fluxes in these ecosystems.

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