Sources of nitrous oxide and fate of mineral nitrogen in sub-Arctic permafrost peat soils

J. Gil[1,2], M.E. Marushchak[1,3], T. Rütting[4], E.M. Baggs[5], T. Pérez[6], A. Novakovskiy[7], T. Trubnikova[1], D. Kaverin[7], P.J. Martikainen[1] and C. Biasi[1]

[1]Department of Environmental and Biological Sciences, University of Eastern Finland, Kuopio, P.O. Box 1627, FI-70211, Finland.

[2]Department of Integrative Biology, Great Lakes Bioenergy Research Center, Michigan State University, 288 Natural Science Bldg., East Lansing, MI 48824-1302, USA.

[3]Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35, FI-40014 University of Jyväskylä, Finland.

[4]Department of Earth Sciences, University of Gothenburg, Box 460, 405 30 Gothenburg, Sweden.

[5]Global Academy of Agriculture and Food Security, The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush Campus, Midlothian EH25 9RG, UK.

[6]Centro de Ciencias Atmosféricas y Biogeoquímica. IVIC. Aptdo. 20634. Caracas 1020A. Venezuela.

[7]Institute of Biology, Komi SC UB RAS, 167982 Syktyvkar, Russia.

Correspondence to: J. Gil (jenie.gillugo@uef.fi)

Abstract

Nitrous oxide (N_2O) emissions from permafrost-affected terrestrial ecosystems have received little attention, largely because they have been thought to be negligible. Recent studies, however, have shown that there are habitats in subarctic tundra emitting N_2O at high rates, such as bare peat surfaces on permafrost peatlands. The processes behind N_2O production in these high-emitting habitats are, however, poorly understood. In this study, we established an *in situ* ^{15}N-labelling experiment with the main objectives to partition the microbial sources of N_2O emitted from bare peat surfaces (BP) on permafrost peatlands and to study the fate of ammonium and nitrate in these soils and in adjacent vegetated peat surfaces (VP) showing low N_2O emissions. Our results confirm the hypothesis that denitrification is mostly responsible for the high N_2O emissions from BP. During the study period denitrification

contributed with ~79% to the total N_2O emission in BP, while the contribution of ammonia oxidation was less, about 19 %. Both gross N mineralization and gross nitrification rates were higher in BP than in VP, where the high C/N ratio together with low water content was likely limiting N transformation processes and, consequently, N_2O production. Our results show that multiple factors contribute to high N_2O production in bare peat surface on permafrost peatlands, the most important factors being absence of plants, intermediate to high water content and low C/N ratio, all factors affecting the mineral N availability for soil microbe including those producing N_2O. The process understanding produced here is important for development of process models that can be used to evaluating future permafrost–N feedbacks to the climate system.

Keywords: Permafrost soils, Arctic, sub-Arctic, soils, N_2O emissions, ^{15}N-labelling, source partitioning, denitrification, nitrification, mineralization, gross N turnover rates, permafrost-climate feedbacks

1 Introduction

The Arctic and sub-Arctic regions store more than 50% of the Earth's soil carbon (C) pool (1330–1580 Pg) (Schuur *et al.,* 2015). The possible increase in release of the greenhouse gases carbon dioxide (CO_2) and methane (CH_4) from these carbon stocks as a result of increased decomposition processes (aerobic and anaerobic) to the atmosphere under a changing climate has been intensively studied (Schuur *et al.,* 2009; Schuur *et al.,* 2015; Schädel *et al.,* 2016). However, Arctic soils store not only a huge amount of C but has also a large nitrogen (N) reservoir (conservative estimate for 0–3 m: 67 Pg N) (Harden *et al.,* 2012), but little is known of the potential of this N to be released as the strong greenhouse gas nitrous oxide (N_2O). Soils world-wide are important N_2O sources responsible for 60% of the global emissions (IPCC, 2013). Traditionally it has been suggested that N_2O emissions from Arctic soils are negligible because of the low concentrations of mineral N in soils underlain by permafrost (Ma *et al., 2007*; Takakai *et al.,* 2008; Siciliano *et al.,* 2009; Goldberg *et al.,* 2010). However, this generalization has been challenged by the identification of hotspots of N_2O on raised permafrost peatlands (Repo *et al.,* 2009; Marushchak *et al.*, 2011) and by measurements of high N_2O concentrations (Abbott & Jones, 2015) and high N_2O emissions (Marushchak *et al.,* 2021) in mineral tundra soils following permafrost thaw. A field warming experiment in a permafrost peatland further showed that soil warming (average increase of 0.95°C) promotes N_2O release not just from bare peat hotspots, but also from adjacent vegetated surfaces that do not emit N_2O under the present climate (Voigt *et al.,* 2017a). In addition, results from mesocosms

and soil incubation studies show that arctic soils have potential for high N_2O emissions after permafrost thawing (~ 3–4 mg N_2O m^{-2} d^{-1}, Elberling *et al.,* 2010; Voigt *et al*., 2017b). In a recent review it was concluded that the emissions of N_2O from permafrost soils could be up to 1.27 Tg N_2O-N yr^{-1}, which represents 11.6 % of total N_2O emissions from natural soils (Voigt *et al.,* 2020). Thus, N_2O emissions from permafrost soils cannot be ignored anymore.

Even though there is increasing evidence of N_2O production from permafrost soils, with potential global importance (Voigt *et al.,* 2020), mechanisms underlying the release of this strong greenhouse gas remain largely unclear. A better understanding of N_2O production from permafrost soils is needed to evaluate the role the Arctic and sub-Arctic play in the global N_2O budget at present and in future. Under the present climate, N_2O emissions from bare surfaces of permafrost peatland (–0.24 to 31 mg N_2O m^{-2} d^{-1}) (Repo *et al.,* 2009; Voigt *et al.,* 2017a; Gil *et al.,* 2017)—the until now strongest known sources of N_2O from the Arctic—can achieve similar magnitudes per unit area as those from temperate and boreal agricultural soils (Maljanen *et al.,* 2010) and tropical forests soils (Werner *et al.,* 2004). It is thought that these hotspots have developed through frost action and wind erosion (Kaverin *et al.* 2013). The absence of vegetation together with low C/N ratio and intermediate soil water content (~60% water-filled pore space; WFPS) have been suggested to be the key environmental factors associated with the high N_2O emissions from these bare peat surfaces (Repo *et al*. 2009). Generally, the main processes responsible for N_2O production in soils are nitrification (ammonia oxidation) and the nitrate reducing pathway of denitrification which tend to predominate under suboxic and anaerobic conditions, respectively (Baggs, 2011). In unfertilized, natural ecosystems with low atmospheric deposition of N like the Arctic, nitrate produced during nitrification is the main N source for denitrification. Therefore, these two processes are tightly coupled in Arctic soils (Siljanen *et al*. 2019). Low C/N ratios of the bulk soil in these systems (23 ± 2; Repo et al., 2009) may favor net N mineralization and nitrification, and intermediate soil water status may allow both aerobic (including nitrification) and anaerobic (including denitrification) processes to take place simultaneously. The lack of vegetation and consequently N uptake by plants means better availability of mineral N for soil microbes. All in all, the bare peat environment can be considered conducive to microbial N_2O production both in nitrification and denitrification.

Although we have some understanding on the factors controlling N turnover and N availability for microbes in the permafrost peat soils, the role of various microbial processes in N_2O production in these soils is still limited. It is important to get more information on these processes to be able to better predict responses of N_2O emissions from Arctic ecosystems to climate induced changes. For example, increase

in soil water content, as predicted e.g., for Alaska (Douglas *et al.,* 2020), will affect the dominant microbial pathways. Nitrification and denitrification are differently controlled by environmental factors, most importantly soil moisture. Compared to nitrification, denitrification releases usually more N_2O under wetter, more anaerobic conditions and has been suggested as the key process for N_2O production in bare peat surfaces (Repo *et al.,* 2009). This is supported by results from laboratory incubations where nitrate addition stimulated N_2O production under anoxic conditions (Palmer *et al.,* 2011). On the other hand, isotope analysis of N_2O (^{15}N natural abundance, site preference values) from these hotspots in tundra in a dry year with low net emissions suggested that ammonia oxidizing nitrifiers could play a major role in dry conditions (Gil *et al.,* 2017). However, the limitations of such natural abundance approaches are well documented (Decock and Six, 2013; Toyoda *et al.,* 2015; Gil *et al.,* 2017), and include overlapping source signals and changing isotope fingerprints under variable environmental conditions. To overcome them, ^{15}N-enrichment approaches provide the ability to quantify and distinguish microbial sources of N_2O *in situ*, particularly ammonia oxidation and denitrification (Stevens *et al.*, 1997; Baggs, 2011). This approach also enables tracing of ^{15}N through the plant-soil system, providing valuable information on N processes including gross turnover rates and N uptake into plants (Gardner *et al.,* 2009; Harrison *et al.,* 2012; Wild *et al.,* 2015). Particularly data on gross N turnover rates including gross ammonification and nitrification are still rare from the Arctic (Ramm *et al*. 2022).

In this study, we conducted an *in situ* ^{15}N-enrichment experiment using a single and double ^{15}N- labeled ammonium nitrate method (Baggs et al., 2003) with a virtual core injection technique (Rütting *et al.,* 2011). Our objectives were, first, to partition between denitrification and nitrification as sources of N_2O emitted from the N_2O hotspots (bare peat; BP) located on permafrost peatlands, and second, to trace the fate of applied ^{15}N in BP and adjacent vegetated peat (VP). VP has shown low N_2O emissions in previous studies. We hypothesized that (1) denitrification is the predominant pathway of N_2O production in the BP, when emissions are high under typical climatic conditions, (2) a major proportion of the added ^{15}N is released as nitrogenous gases from BP but in VP microbial immobilization is the most important sink of N, indicating that competition for N is a key regulator of N_2O in these peatlands. Further, we hypothesized that (3) in addition to the absence of vegetation, lower C/N ratios and higher water content support higher N turnover rates in BP (as compared to VP) and are important factors leading to higher N_2O fluxes there.

2 Materials and methods

2.1 Study site and soil characteristics

The experiment was carried out at the Seida study site which is located in sub-Arctic northwestern Russia (67º03'N, 62º57'E) in the discontinuous permafrost zone. Some common geographical features occurring in discontinuous and sporadic permafrost zone are the so-called palsas and peat plateaus (Seppälä, 2011, Sannel and Kuhry, 2011, *Borge et al.,* 2017). They are formed by permafrost aggradation, which lifts the peat surface, leading to drier conditions than the surrounding unfrozen peatland surface (Seppälä, 2003). As a result of wind abrasion, parts of the palsas and peat plateaus lack vegetation (Seppälä, 2003). The unvegetated bare peat surfaces (BP) which were studies here are located on a large peat plateau characteristic for the Seida area and are round in shape with an average diameter of 20 m and have only sporadic bryophytes and lichens (Kaverin *et al.* 2016). The growing season in the study region lasts approximately 3 months, from mid-late June to early-mid September. The mean annual precipitation is 505 mm and the mean annual air temperature is –5.8 °C. The warmest month is usually July with a mean air temperature of 12.5 °C followed by August with 9.4 °C (30 years averages, data from weather station at Vorkuta (67º48'N, 64º01'E); Komi Republican Center for Hydrometeorological and Environmental Monitoring). The mean precipitation sums for the period July – September is 121 mm. Additional information on the site characteristics and climatic conditions can be found in Repo *et al.,* (2009); Marushchak et *al.* (2013) and Biasi *et al.,* (2014). In 2010, when our study was undertaken, the warmest month was July with a mean air temperature of 12.9 °C, similar to the long-term mean, while August was warmer than the long-term mean. The maximum daily air temperature (22 °C) was registered in August. The cumulative precipitation for the period July-September was close to the long-term average, 113 mm. Most of the rainfall took place during mid-August, which resulted in high soil water content at this time.

BP surfaces consist mainly of decomposed fen peat. VP surfaces have typical bog vegetation including vascular plants such as *Ledum decumbes, rubus chamaeomorus, Vaccinium uliginosum*, and mosses (e.g., *Sphagnum, Dicranum sp.)* and lichens (e.g., *Cladina sp.*) (Table 1).

Table 1. Soil characteristics of the topsoil (0-10cm) of the bare peat (BP) and vegetated peat (VP) soil

Soil type	pH	BD ($g\ cm^{-3}$)	SOM (%)	%C	%N	C/N	$[NO_3^-]$ (mg N kg^{-1} DW)	$[NH_4^+]$ (mg N kg^{-1} DW)	WFPS (%)	Max. seasonal thaw depth (cm)
BP	3.2±0.1	0.27±0.02[a]	96±2[a]	54±6[a]	2.2± 03[a]	23±2[a]	60±11[a]	116±39[a]	67±5[a]	70±5[a]
VP	3.4±0.1	0.05 ±0.02[b]	98±1[b]	47±2[b]	0.8±0.2[b]	62±16[b]	11±4[b]	35±6[b]	30±7[b]	60±12[b]

Values are mean ± 1 SE, for the sampling period during the growing season 2007,2008 (Marushchak *et al*., 2011) and 2010 (this study).
Different letters indicate statistically significant differences between surface types ($p < 0.005$).
n=3 for each soil type.

2.2 ^{15}N-enrichment experiment

2.2.1 Experimental design

The study took place during the growing season 2010, between July 21 and August 13 (24 days). The ^{15}N labelling experiment was conducted *in situ* on BP and adjacent VP in three replicates per treatment type (n=3). The soil surfaces were selected based on their contrasting N_2O emission rates reported in previous field campaigns at the site (2007–2008; Repo *et al.,* 2009; Marushchak et *al.,* 2011). Bare peat surfaces are known to act as N_2O hotspots in contrast to VP where the N_2O fluxes are low.

Following the approach previously applied by Baggs *et al., (*2003), the experiment comprised three different ^{15}N-labelling treatments with either single or double ^{15}N-labelling: $^{14}NH_4{}^{15}NO_3$ (Treatment 1; T1), $^{15}NH_4{}^{14}NO_3$ (Treatment 2; T2) and $^{15}NH_4{}^{15}NO_3$ (Treatment 3; T3) with each of applied at 98 at% ^{15}N. Briefly, we used T1 with $^{15}N\text{-}NO_3^-$ label to calculate gross nitrification and to quantify N_2O emissions produced by nitrate reduction in denitrification. Nitrous oxide emissions from nitrification were estimated using the difference in $^{15}N\text{-}N_2O$ flux between the treatments T3 ($^{15}NH_4{}^{15}NO_3$) and T1 ($^{14}NH_4{}^{15}NO_3$). Treatment 2 ($^{15}NH_4{}^{14}NO_3$) was used to calculate gross mineralization and to account for $^{15}N\text{-}N_2O$ fluxes from $^{15}N\text{-}NH_4^+$ after it had been first nitrified to $^{15}N\text{-}NO_3^-$. The approach is based on the assumptions of negligible nitrate ammonification (DNRA) and negligible re-mineralization of $^{15}NH_4$ within the first 72 hours (Braun *et al.*, 2018). The application rate of the label solutions was adjusted to soil inorganic N concentrations in 2007–2008 using previously determined bulk densities and corresponded approximately to 50% of the native extractable N pools in the soils during the growing season (Table 1). For VP, the label solutions were applied at a rate of 5 mg NO_3^--N kg^{-1} dry soil (1 µg N cm^{-2}) and 17 mg NH_4^+-N kg^{-1} dry soil (2 µg N cm^{-2}) while for BP, the application rates were 30 mg

NO_3^--N kg^{-1} dry soil (10 µg N cm^{-2}) and 58 NH_4^+-N kg^{-1} dry soil (20 µg N cm^{-2}). The total quantity of mineral N added never exceeded maximum NO_3^- or NH_4^+ content found in the native, unamended soils. The ^{15}N-solutions were added *in situ* to the depth of 0–6 cm adopting the virtual core injection technique described by Rütting *et al.,* (2011). For the ^{15}N-labelling and samplings a 20 cm × 20 cm sub-plot was demarcated within each plot. Inside these sub-plots, a smaller area (16 cm × 16 cm) was marked with sticks, and this template was used for N addition and soil sampling. For the injection of ^{15}N-solutions, 49 syringes (1 mL) were attached to a plastic frame within the template in a regular 7×7 grid lay-out to release the ^{15}N-solutions from the syringes into the soil as uniformly as possible (both horizontally and vertically). Since the ^{15}N-labelled areas were to be sampled destructively for each of the 7 sampling occasions, the label injection was repeated 7 times in each replicate plot at randomly selected locations. The total number of injections amounted to 126 (2 surface types × 3 replicates × 3 ^{15}N-treatments 7 sampling occasions). For logistical reasons, it took two days (21–22 July 2010) to complete all the ^{15}N-applications, but both soil surface types (VP and BP) were always labelled at the same time for each treatment to ensure comparable results for the two soil types.

After the ^{15}N-addition, the following samples were taken at 0 h, 1 h, 24 h and 3, 5, 9, 15, 24 days: Surface gas flux samples for N_2O concentration and ^{15}N-N_2O isotopic analyses; soil samples for mineral N (NH_4^+ and NO_3^-), total N (TN) and ^{15}N-enrichment in these three N pools. In addition, we collected all above-ground plants as well as roots from VP surfaces on the same days. All samples were analyzed for N concentrations and ^{15}N-enrichments as described below.

2.2.2 Gas sampling and analysis

Emissions of ^{15}N-N_2O were determined using the static chamber technique (Heikkinen *et al*, 2002). A circular plastic collar was inserted to the soil one hour before the gas sampling and, for the measurement, a small PVC chamber (diameter 10 cm, volume 920 cm^3) was attached to the collar. The chamber had an inlet (polyamide nylon tube) equipped with a three-way stopcock (Steritex ®3W, CODAN Limited, UK) for gas sampling. Gas samples were taken for analyses of N_2O concentrations and ^{15}N-N_2O content twice: once before closing the chamber (ambient, t = 0) just above the soil surface and second time 40 min after closure from chamber headspace. The N_2O fluxes were calculated from the concentration difference between the two sampling points. The 2-point measurement method was chosen because of the small chamber volume which prevented taking several samples during the measurement. This methodology was compared against the static chamber technique with 4–5 sampling points within 40

min used at the site during this experiment and previous sampling campaigns (Repo *et al.,* 2009; Marushchak et *al.,* 2011, Gil *et al.,* 2017). The test showed that the concentration increase during the 40 min measurement time was linear and the two methods give essentially similar results. Samples were taken using a polypropylene syringe with a Luer lock tip (Terumo®, Tokyo, Japan) fitted with a three-way stopcock (as above). Temperature inside the chamber was recorded at the beginning and at the end of each closure period.

Gas samples of 20 mL for analysis of N_2O concentrations were transferred into 12 ml pre-evacuated exetainers equipped with butyl rubber septa (Labco Ltd, UK) the same day of sampling. Concentrations of N_2O were analyzed 1-2 months later at the University of Eastern Finland. A leakage test with a standard gas showed that leakage for N_2O was negligible (≤ 3% over the storage period). The concentration of N_2O was measured with a gas chromatograph as described in Gil *et al.* (2017). (Repo *et al.,* 2009; Marushchak et *al.,* 2011, Gil *et al.,* 2017).

Samples for ^{15}N-N_2O determination were stored in 60 ml gas-tight glass flasks (Supelco, UK) and their $^{14}N/^{15}N$ ratios determined at the Stable Isotope Facility at the University of California, Davis, using a Delta V Plus isotope ratio mass spectrometer (IRMS) operated in continuous flow mode (Thermo Scientific, Bremen, Germany) coupled with an online pre-concentrator and a GasBench (Thermo Finnigan, Bremen, Germany). The ^{15}N-N_2O flux rates were calculated from linear regression slope fitted to the at % excess ^{15}N of the samples against time.

2.2.3 Soil sampling and analyses

Immediately after gas sampling, soil samples were taken by pushing a PVC tube (length: 15 cm; diameter: 5 cm; volume: 70 cm^3) into the soil (0–10 cm) at the center of the labeling subplot area. Soil samples were sieved, homogenized, and extracted with KCl (2M) on the day of collection and extracts were preserved frozen for later analysis of concentrations of NH_4^+-N and NO_3^--N and their ^{15}N-enrichments. A subsample of the soil was dried at 60 °C and preserved for later analysis of total N and its ^{15}N-enrichment. The concentrations of NH_4^+ and NO_3^- in the extracts were measured by spectrophotometry (Wallac-Data Analyzer) using a microtiter plate format, following the protocol of Fawcett and Scott (1960) for NH_4^+ (630 nm) and Griess method for NO_3^- (544 nm) (Miranda *et al.,* 2001). The ^{15}N-enrichment in mineral N was determined by the micro-diffusion method (Herman *et al.,* 1995) and analyzed on an elemental analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS), which included a Thermo Finnigan DELTA XP Plus IRMS, Flash EA 1112 Series Elemental Analyzer,

and a Conflow III open split interface (Thermo Finnigan, Bremen, Germany) at the University of Eastern Finland. The ^{15}N data were expressed as at % ^{15}N excess relative to the natural abundance ^{15}N of NO_3^- and NH_4^+ in the soils from the unlabeled plots. Dried bulk soil samples were also analyzed for total N and ^{15}N concentrations using the same EA-IRMS, and at % ^{15}N excess values were calculated). The reproducibility of 10 standard runs (EA-IRMS) was typically better than 0.5‰ (1σ, n=10).

2.2.4 Plant sampling and analyses

Aboveground parts of plant and roots were quantitatively sampled from the labelled VP plots. Aboveground parts of plants were cut at the soil surface level and classified into higher plants (e.g., *Betula nana, Ledum decumbens, Rubus chamaemorus, Vaccinium uliginosum*) and lower plants (e.g., *Sphagnum, Dicranum sp.*). Roots were removed by hand and rinsed with water to wash off any soil. Then, the aboveground parts of plants and roots were oven dried in the field laboratory, weighed and stored until further processing at the University of Eastern Finland. There, the aboveground biomass and roots were milled to fine powder (RetschMM301, Haan, Germany) and the total N and ^{15}N contents in shoot and root material were determined by the EA-IRMS system described above.

2.3 Calculations

2.3.1 Mass balance approach for estimating ^{15}N label recovery

To assess ^{15}N partitioning and losses in the evaluated pools during the sampling period, we determined a mass balance which consisted of calculating the recovery of applied ^{15}N into the different ecosystem components (plants, soil and N_2O) for each sampling point. We used area-based N pool size estimates and changes in ^{15}N content of the individual components following the ^{15}N addition. All calculations were done with at % excess values which were obtained by subtracting the natural abundance of each component (plants, including higher and lower plants, soil and gas flux), measured before the labelling started (1σ, n=12; approx. 0.3663 at-% ^{15}N for all) from the at %^{15}N values measured after labelling. The mass of ^{15}N recovered in each ecosystem component was determined as follows:

(i) We calculated the ^{15}N mass recovered per soil area (μg ^{15}N cm^{-2}) for each sampling time (e.g., at 0, 1 h, 24 h and 3, 5, 9, 15, 24 days) in each component (plants, soil or N_2O - cumulative fluxes of ^{15}N-N_2O) by multiplying the total pool size with the at % excess.

(ii) Total ^{15}N recovery at a given time was calculated as a sum of the total mass of ^{15}N recovered in all the components. The calculation was somewhat different for BP (Eq. 1) and VP (Eq. 2). Since VP had negligible N_2O emissions, this component was ignored in the final mass balance calculations, and as there were no plants on BP, this component was excluded in calculation of the total ^{15}N recovery there.

For BP:

$$^{15}N_{total}\ (\mu g\ ^{15}N\ cm^{-2}) = {}^{15}N_{soil}\ (\mu g\ ^{15}N\ cm^{-2}) + {}^{15}N_{N2O}\ (\mu g^{15}\ N\ cm^{-2}) \quad (1)$$

For VP:

$$^{15}N_{total} = {}^{15}N_{soil}\ (\mu g\ ^{15}N\ cm^{-2}) + {}^{15}N_{plants}\ (\mu g\ ^{15}N\ cm^{-2}) \quad (2)$$

$$\text{where } {}^{15}N_{plants} = {}^{15}N_{\ higher\ plants} + {}^{15}N_{\ lower\ plants} + {}^{15}N_{roots} \quad (3)$$

The relative ^{15}N recovery in each component (Eq. 4) was calculated dividing the ^{15}N mass recovered in each component by the total label applied:

$$^{15}N\ \text{recovery}\ (\%) = ({}^{15}N_{comp}\ (\mu g\ ^{15}N\ cm^{-2}) / \text{total label applied}\ (\mu g\ ^{15}N\ cm^{-2})) \times 100 \quad (4)$$

Here we report total ^{15}N recovery for each surface type (BP and VP), as well as the relative ^{15}N recovery for each ecosystem component (in BP:soil and N_2O flux; in VP: plants and soil. Only data from T1 ($^{14}NH_4{}^{15}NO_3$) and T2 ($^{15}NH_4{}^{14}NO_3$) were used for the mass balance calculation (T3 = sum of T1 and T2, data not shown).

2.3.2 Source partitioning of N_2O emitted from the bare peat surfaces (BP)

To quantify the relative contribution of nitrification and denitrification to the overall N_2O fluxes from BP we used the single and double ^{15}N-labeled ammonium nitrate method, previously introduced by Baggs *et al.* (2003). The calculation was made individually for each plot (n=3) and sampling point (n=7). We report the averages by day after the labeling and for the entire sampling period of 24 days. The contribution of different microbial sources to the total N_2O flux was calculated as follows:

(i) The ^{15}N-N_2O emitted from T1 plots (labeled with $^{14}NH_4{}^{15}NO_3$) was assumed to represent the N_2O emission derived from denitrification (D) (Eq. 5):

$$N_2O_{\ D} = {}^{15}N_2O_{\ T1} \quad (5)$$

(ii) To the N_2O flux derived from denitrification from $^{15}NH_4^+$ after is first nitrified to $^{15}NO_3^-$ ($N_2O_{\ D\text{-}T2}$) we used data from T1 (labeled with $^{14}NH_4{}^{15}NO_3$) and T2 plots (labeled with $^{15}NH_4{}^{14}NO_3$). We assumed

that the ratio of $^{15}N\text{-}N_2O$ to the enrichment of the substrate pool ($^{15}NO_3^-$) was similar in T1 and T2, and calculated $N_2O_{D\text{-}T2}$ based on a direct relationship (Eq. 6):

$$N_2O_{D\text{-}T2} = (^{15}N_2O_{T1} / {}^{15}NO_3^-{}_{T1}) \times {}^{15}NO_3^-{}_{T2} \quad (6)$$

(iii) The N_2O flux derived from nitrification was then calculated as the difference between the $^{15}N\text{-}N_2O$ emitted from the double-labeled T3 plots (labeled with $^{15}NH_4{}^{15}NO_3$; denitrification+nitrification) and T1 plots (labeled with $^{14}NH_4{}^{15}NO_3$ (only denitrification), and subtracting the N_2O flux derived from $^{15}NH_4^+$ after it was nitrified to $^{15}NO_3^-$ (Eq. 7):

$$N_2O_N = {}^{15}N_2O_{T3} - N_2O_D - N_2O_{D\text{-}T2} \quad (7)$$

The total $^{15}N\text{-}N_2O$ emission was calculated as the sum of N_2O derived from denitrification (N_2O_D and $N_2OD_{D\text{-}T2}$) and N_2O derived from nitrification (N_2O_N), which was used to calculate the percent contribution of each process. The assumptions behind this methodology were that: (1) there was no significant dissimilatory NO_3^- reduction to NH_4^+ (DNRA; or nitrate ammonification) or re-mineralization as $^{15}N\text{-}NH_4^+$ from microbial biomass; and (2) when using highly enriched isotopic tracers the isotopic composition of the N_2O evolved is not significantly affected by fractionation.

2.3.3 Gross N turnover rates

The experimental setup allowed us also to calculate gross mineralization, gross nitrification rates and N consumption for VP and BP with the isotope pool dilution method (Kirkham and Bartholomew 1954). We applied the pool dilution method *in situ*, coupled with our virtual core technique and following the protocol suggested by Rütting *et al.* (2011).

The gross N transformation rates were calculated from data from T1 ($^{15}N\text{-}NO_3^-$; nitrification) and T2 ($^{15}N\text{-}NH_4^+$; ammonification) between time points 24 and 72 hours (3 d) after labeling. This time-period was selected because (1) gross nitrification rates for BP were constant during this period (Figure S3) and constant process rates are a prerequisite for estimating gross N transformation rates by Kirkham and Bartholomew, 1954 (2) the changes in 15N at % excess of NO3- from day 5 (120 hours) in BP surfaces, suggest quick cycles of abiotic fixation and release of NO3- (Figure 3 and S4), therefore shorter time period for the calculations is recommended to minimize errors due recycling of the label by assimilation to microbial biomass and remineralization (Braun et al., 2018) (3) the first time point of measurement (between 1 hour and 1 day after label application) could not be included in the calculations since that

resulted often in negative gross N transformation, most likely because the label was not yet evenly distributed in the soil.

The Eq. 8 and Eq. 9 of Kirkham and Bartholomew (1954) were used for the estimation of the gross mineralization/nitrification rate (m) and the gross NH_4^+/NO_3^- consumption rate (c):

$$m = \frac{(M_0 - M_1)}{t}\ x\ \frac{\log\left(\frac{H_0 M_1}{H_1 M_0}\right)}{log\left(\frac{M_0}{M_1}\right)} \quad (8)$$

$$c = \frac{M_0 - M_1}{t}\ x\ \frac{log\left(\frac{H_0}{H_1}\right)}{log\left(\frac{M_0}{M_1}\right)} \quad (9)$$

where M_0= initial $^{14+15}N$ pool; M_1 = $^{14+15}N$ pool at time 1, H_o= initial $^{15}N_{excess}$ pool, H_1 = $^{15}N_{excess}$ pool at time 1; t= time. All in µg N g^{-1} dry soil.

Kirkham and Bartholomew (1954) methodology rely on the assumptions: (1) mineralization and immobilization rates remain constant during the interval between successive measurements, (2) the ratio $^{15}N/^{14}N$ in the efflux is proportional to that of the labelled pool, and (3) immobilized labeled N is not remobilized during the experimental period (as mentioned above).

2.3.4 Water filled pore space (WFPS)

Soil water filled pore space (WFPS) in the topsoil was calculated using equation (11). For this, soil moisture sensor data (in mV) measured with a ML3 ThetaProbe (Delta-T Devices, Cambridge, UK) was converted to volumetric water content (θv), applying a sensor calibration as instructed by the manufacturer (see Gil *et al.,* 2017, supplementary material). Bulk density (BD) was measured in the field from volumetric soil samples. Particle density (PD) was estimated from soil organic matter content (SOM) as previously described (Marushchak *et al.* 2011). The total porosity (TP) was calculated using equation (10):

$$TP = 1 - \left(\frac{BD}{PD}\right) \quad (10)$$

$$WFPS = \frac{\theta v}{TP} \quad (11)$$

2.4 Statistical Analyses

Data was first tested for normal distribution using the normality test available in the Sigma Plot software (Systat, San Jose, CA). Since most of the data was not normally distributed, Kruskal-Wallis test was used to determine the significance of the experimental factors (surface type, ^{15}N treatment, soil properties, air temperature) on N_2O emissions. The Kruskal-Wallis test was followed by Mann-Whitney pairwise test significant difference in the ^{15}N recovery between treatments ($^{15}N\text{-}NO_3^-$ and $^{15}N\text{-}NH_4^+$) in each component (soil, plants and N_2O) and among N transformation rates between soil surface types. To explorer the role of soil characteristics, mineral N content and N transformation rates as drivers of in situ N_2O fluxes we used Spearman correlation analysis (IBM SPSS statistics software (version 23.0) and JMP®, Version Pro 14. SAS Institute Inc).

3 Results

3.1 Physicochemical characteristics of the soils

Physicochemical characteristics of BP and VP surfaces are summarized in Table 1. BP surfaces had higher bulk density than the VP surfaces, and particularly higher N content, resulting in much lower C/N ratios in BP as compared to VP. Both soils had similar low pH (mean = 3.4 ± 0.3). Water content was highly variable but on average higher in BP, with WFPS values ranging from 42% to 81% (mean 67 ± 5 %). In VP surfaces WFPS values ranged from 10 % to 57%, with a mean of 30 ± 7%. In BP, the nitrate and ammonium contents (60 ± 11 mg N kg^{-1} dry soil and 116 ± 39 mg N kg^{-1} dry soil, respectively) were higher than in VP (11 ± 4 mg N kg^{-1} dry soil and 35 ± 6 mg N kg^{-1} dry soil, respectively) (all values are mean ± SE).

3.2 N_2O emissions

Total N_2O and $^{15}N\text{-}N_2O$ fluxes followed approximately similar patterns across all BP plots, with the highest N_2O fluxes measured between day of the year (DOY) 210 and 215 (between three and nine days after ^{15}N application) (Figure 1 and 3c). This peak in N_2O fluxes was observed when temperatures of air (~ 18°C) and topsoil (5cm; 13°C) were highest. Bare peat surfaces showed net N_2O release throughout the experimental period, ranging between 0.1 and 31.8 mg N_2O m^{-2} d^{-1} (mean 9.8 ± 1.8 mg N_2O m^{-2} d^{-1}, n= 44) and were on average about 3 times higher than those from adjacent, non-labelled bare peat areas (mean 3.2 ± 0.5 mg N_2O m^{-2} d^{-1}, n= 34; Figure 1). The highest $^{15}N\text{-}N_2O$ flux in BP was measured from the T3 treatment ($^{15}NH_4{}^{15}NO_3$) ($p < 0.05$) (Figure 1; Figure 3c). The N_2O fluxes from the VP surfaces

were low throughout the sampling period and showed frequent uptake of N_2O (negative fluxes). The N_2O fluxes in VP from the ^{15}N labelled plots ranged from –1.6 to 4.3 mg N_2O m^{-2} d^{-1} (mean –0.02 ± 0.14 mg N_2O m^{-2} d^{-1}; n= 55) and were not significantly different from zero, and not significantly different from adjacent non-labelled VP areas (data not shown).

N_2O fluxes correlated positively with air T (R^2= 0.357; $p < 0.005$), NH_4^+ concentration in soil (R^2=0.423; $p < 0.001$) and CO_2 fluxes (R^2=0.399; $p < 0.005$). $^{15}N_2O$ fluxes from labelled plots showed similar positive correlation with air T as N_2O fluxes ($R^2 = 0.391$, $p < 0.005$).

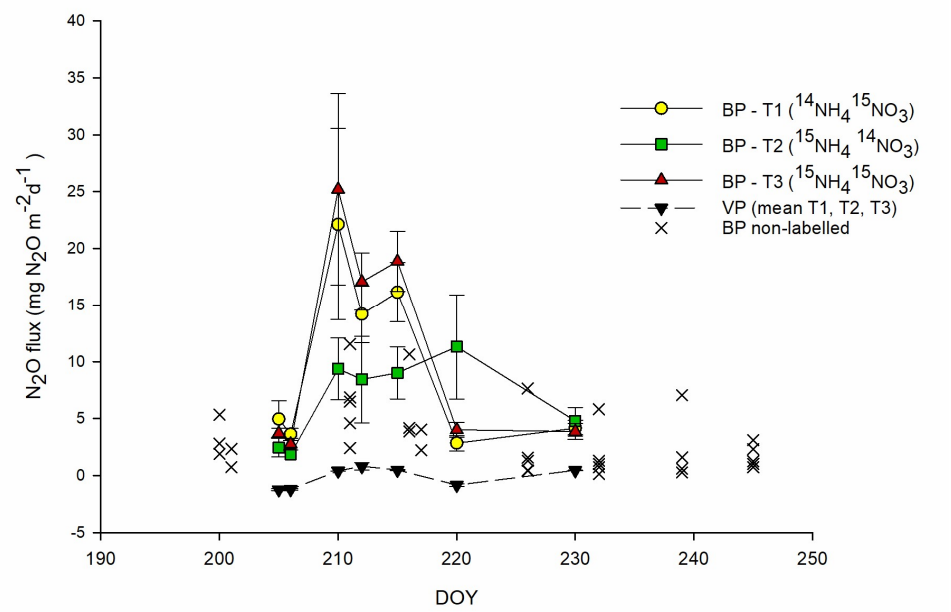

Figure 1. Total N_2O fluxes from labeled plots. For bare peat soils (BP) (in color) N_2O fluxes are mean values ± SE (n = 3) for each treatment while for vegetated peat soils (VP) (black triangles) the mean N_2O flux of three plots and three treatments is shown. For comparison, N_2O fluxes from BP non-labeled plots (x) located nearby are also shown (long-term experiment; permanent chambers, multiple sampling points). DOY = day of the year. T1 = treatment 1 ($^{14}NH_4^{15}NO_3$); T2 = treatment 1 ($^{15}NH_4^{14}NO_3$) and T3 = treatment 3 ($^{15}NH_4^{15}NO_3$). Error bars for VP data points are smaller than the scale.

3.3 ^{15}N recovery

The total amount of ^{15}N recovered in the soil, vegetation and N_2O were calculated for treatments T1 (^{15}N-NO_3^-) and T2 (^{15}N-NH_4^+). In general, the total recovery was close to 100% for the first 24 hours after labelling and gradually decreased to 42% for BP and 75% for VP in the end of the experiment of 24 days

(Figure 1S). At day 3, total recovery of ^{15}N was lower than expected and although we have no explanation for these findings, this low recovery did not significantly impact on the main results which were calculated from ^{15}N in mineral nutrient pools (more details below). Immediately after labelling (24h), 92% (VP) and 100% (BP) of the applied ^{15}N was recovered in the bulk peat soil. By the end of the experiment, still most of the label across VP and BP was found in the bulk peat soil in both treatments, as shown in the relative proportion of each component (Figure 2).

In VP the proportion of ^{15}N recovered in plants 24 days after labeling was on average 6 ± 2 % (n = 42) in both T1 (^{15}N-NO_3^-) and T2 (^{15}N-NH_4^+), with no significant difference between the treatments. The relative proportion of the label recovered in vegetation did not show a consistent trend over the experimental period, varying from 1 to 9% (Figure 2c-d). Most of the ^{15}N in vegetation was retained in mosses and lichens (3 to 4 %), followed by roots of higher plants (2%) and aboveground parts of higher plants (0.2%) (Figure S2).

Since the N_2O emissions from VP were negligible (Figure 1), the ^{15}N-enrichment of N_2O flux was not determined there. In BP, the ^{15}N-enrichment of the N_2O flux was detected three days after labeling, with cumulative increase with time (Figure 2a-b). The maximum ^{15}N-recovery in the cumulative N_2O flux from BP was observed toward the end of the experiment (day 24) from T1 (^{15}N-NO_3^-) (24 ± 9 %; n = 3). On average, the label recovered in ^{15}N-N_2O was higher in the T1 (^{15}N-NO_3^-) plots (13 ± 2 %; n = 3) compared to T2 (^{15}N-NH_4^+) plots (6 ± 1%; n = 3, $p < 0.05$). The maximum relative amount of ^{15}N-recovery in N_2O in BP surfaces was about 3.5 and 1.5 times higher than the maximum ^{15}N recovery in plants in VP for the treatments T1 and T2, respectively.

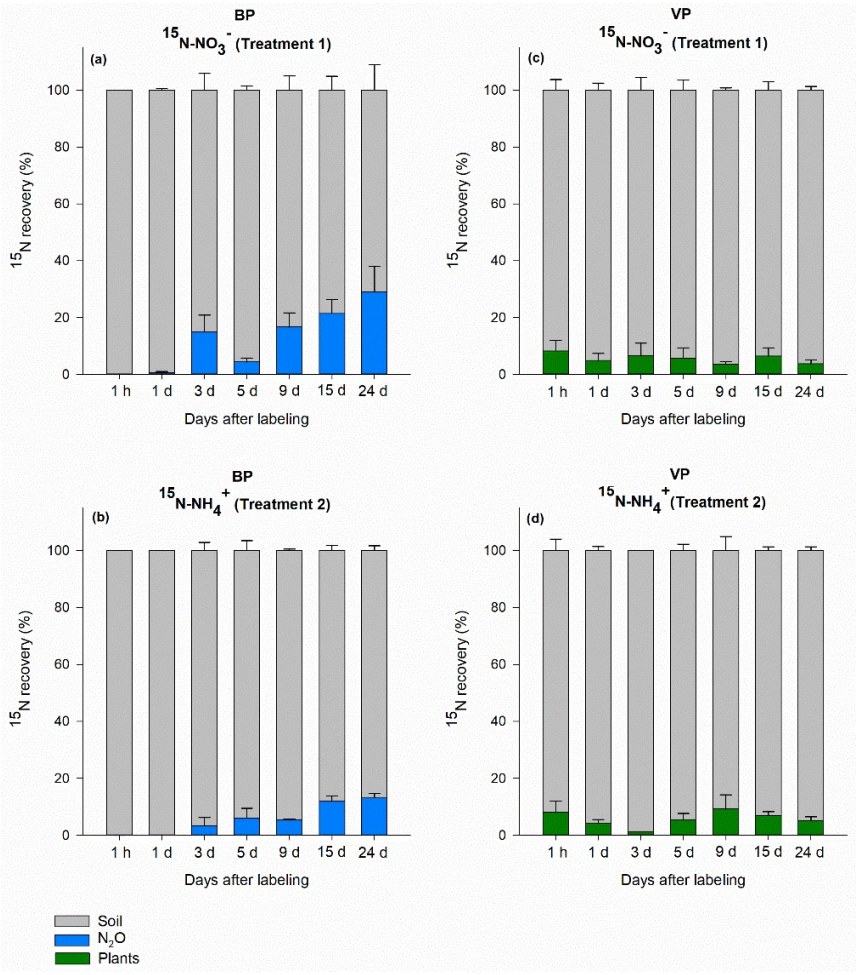

Figure 2. Relative distribution of the ^{15}N label recovered from the bare peat and vegetated peat soil for treatment 1 ($NH_4{}^{15}NO_3$) and treatment 2 ($^{15}NH_4NO_3$). Data are mean values ± SE (n = 3).

3.4 ^{15}N-concentrations of inorganic N pools and N_2O, and microbial sources of N_2O emitted from bare permafrost peatlands

In the labeling treatment T1 ($^{15}N\text{-}NO_3^-$), the highest $^{15}N\text{-}NO_3^-$ concentration was measured one day after labeling (0.8 ± 0.5 mg $^{15}N\text{-}NO_3^-$ kg^{-1} of dry soil) (Fig. 3b). In the same treatment, the ^{15}N concentration of the NH_4^+ pool was negligible during the 24 days of experiment (~ 0.1 mg $^{15}N\text{-}NH_4^+$ kg^{-1} dry soil), indicating there was no reduction of nitrate to ammonium.

In the treatment T2 ($^{15}N\text{-}NH_4^+$), the concentration of $^{15}N\text{-}NH_4^+$ decreased exponentially over time (Figure 3a). In the same treatment, $^{15}NO_3^-$ gradually increased during the first nine days after labeling and thereafter decreased until the end of the experiment.

In the treatment T3 ($^{15}NH_4^{15}NO_3$), the ^{15}N-NO_3 concentration of soil showed a similar trend as in T1 but the ^{15}N-concentrations were higher (Figure 3b). The $^{15}NH_4^+$ concentrations in T3 showed a similar trend as in T2, but the ^{15}N concentrations were lower in T3. In nearly all treatments, a second smaller peak was detected in ^{15}N concentrations of the added substrate on day 5, 9 or 15.

The ^{15}N concentration in N_2O showed similar patterns for all treatments. In T3, the highest ^{15}N-N_2O flux (7 ± 3 mg ^{15}N-N_2O m^{-2} d^{-1}) was measured on day 3 after labeling (Figure 3c). The same was true also for T1, but the ^{15}N flux was lower (3 ± 1 mg ^{15}N-N_2O m^{-2} d^{-1}). In T2, the peak in $^{15}N_2O$ flux (1.6 ± 0.5 mg ^{15}N-N_2O m^{-2} d^{-1}) was lower and occurred later, between the days 3 and 5 after the application of the label. In all treatments, a second smaller peak in $^{15}N_2O$ was observed, but it occurred (about 2 days) earlier in T1 and T3 than in T2. The $^{15}N_2O$ values correlated positively with the $^{15}NO_3^-$ values from all treatments ($R^2 = 0.5453$; $p < 0.05$, Figure S3), but not correlation between $^{15}NH_4^+$ and $^{15}N_2O$ was observed.

The results of the source partitioning of N_2O emissions from BP (Figure 4) show that denitrification was the primary source, contributing on average by $79 \pm 6\%$ ($n = 21$) to the total ^{15}N-N_2O emissions. In T2 ($^{15}NH_4^{14}NO_3$), there was ^{15}N in the NO_3^- pool indicating that the applied ^{15}N-$NH4^+$ was nitrified and released as ^{15}N-N_2O in coupled nitrification-denitrification process. The contribution of ammonia oxidation to the overall N_2O flux was ~20%. During the period of high N_2O fluxes (3 days after ^{15}N application), the contribution of nitrification was particularly low. However, when N_2O emissions were low towards the end of the experiment, nitrification reached a maximum contribution of 55%.

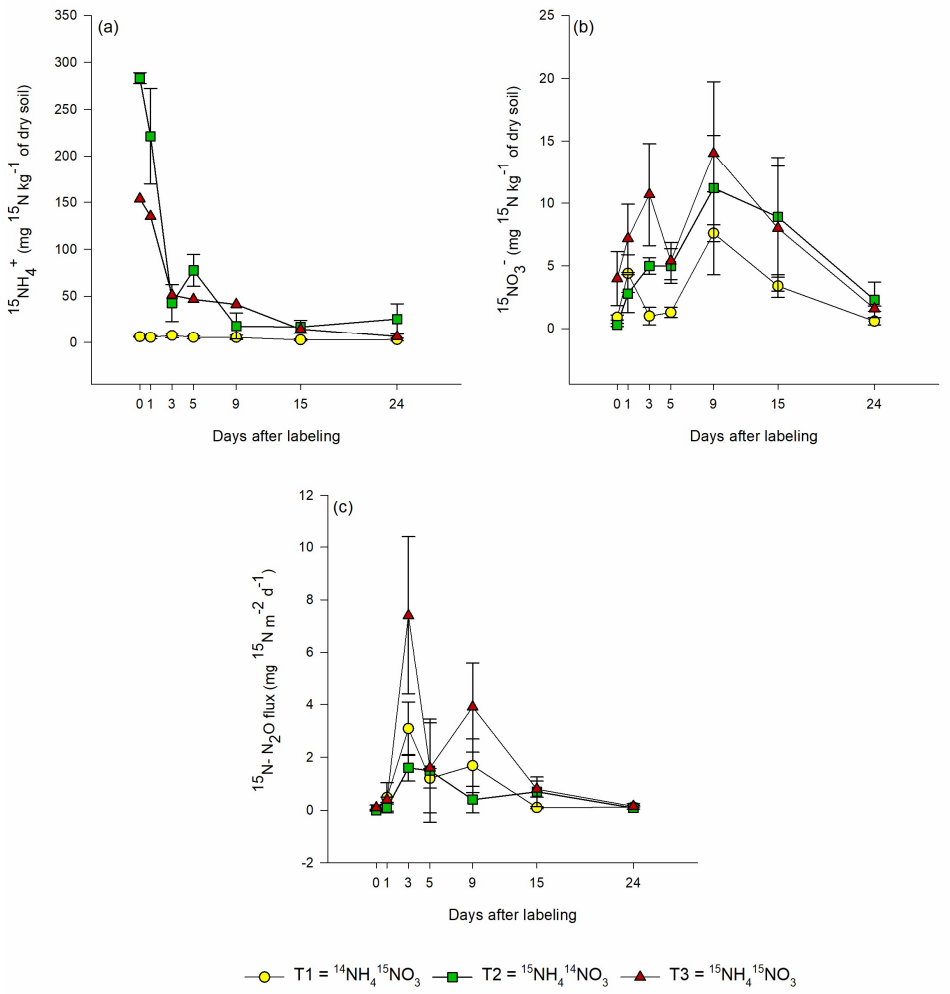

Figure 3. Evolution of ^{15}N concentration in (a-b) extractable inorganic N pools (NH_4^+ and NO_3^-) and (c) N_2O emissions from bare peat (BP) soil during the 24-day of the experiment for all labelling treatments. T1 = treatment 1 ($^{14}NH_4{}^{15}NO_3$); T2 = treatment 1 ($^{15}NH_4{}^{14}NO_3$) and T3 = treatment 3 ($^{15}NH_4{}^{15}NO_3$). Day 0 = 1hour after labeling. Values are mean ± 1 SE (n = 3).

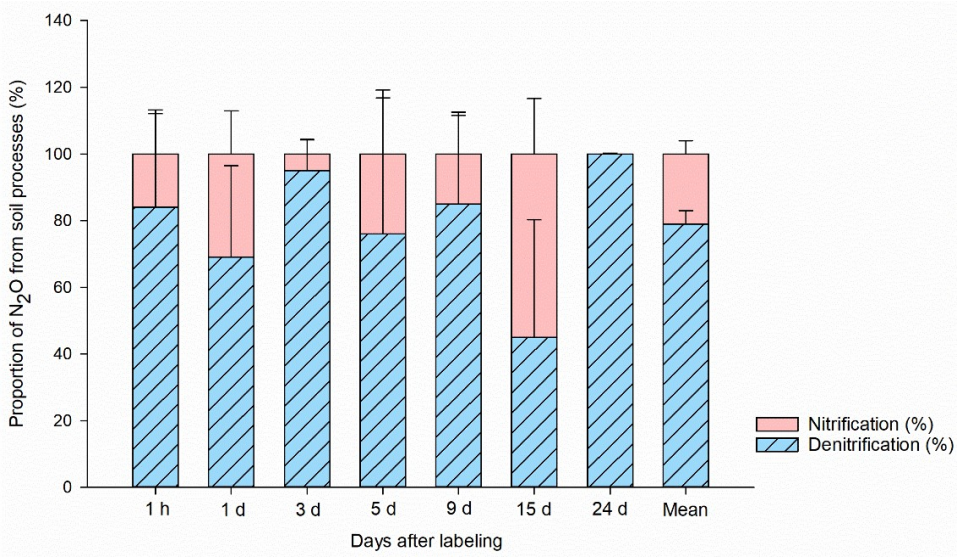

Figure 4. Proportion of N_2O (%) produced in the bare peat (BP) soil by denitrification and nitrification during the study period. The source partitioning was done following the previously described single and double ^{15}N-labeled ammonium nitrate method (Baggs *et al.,* 2003). The source contribution is calculated from $^{15}N_2O$ emitted and $^{15}NO_3^-$ pool in the soil, in relation to the total amount of ^{15}N label applied to the soil (Wrage *et al.,* 2005). The calculation was done individually by plot (n=3) and sampling point (n=7) and the average of three plots is reported by days after labeling. The mean value for the entire sapling period of 24 days is also shown.

3.5 Gross N turnover rates

As described above in the methods section 2.3.3, we chose to report gross mineralization and nitrification rates for the period between 24 and 72 hours. During this time period, the gross mineralization and nitrification rates were constant and positive, and we could assume negligible recycling of the ^{15}N label via microbial biomass back to the mineral N pool (Braun et al., 2018). For method comparison purposes, we have shown the gross mineralization and nitrification rates calculated for different periods in table S1 and S2 in supplementary material. We note that variability in the results depending on the time period chosen for the calculations was higher for gross nitrification rates than for gross mineralization rendering higher uncertainties in the nitrification data. However, the comparison between VP and BP, which is the focus here, is independent of the chosen calculation method and is valid. Further, high variability of gross N turnover rates is quite common in field labeling studies (e.g., Cookson et al., 2002; Harty et al., 2017). The high variability in our data could also simply reflect the spatial variation at the site between the subplots destructively sampled at different time-points.

Gross mineralization and nitrification rates in BP were higher than in VP ($p < 0.01$) (Table 2). In BP, gross mineralization rates were four times higher than gross nitrification rates. Gross nitrification rates in VP surfaces were negligible. NH_4^+ consumption rates were similar to gross mineralization rates for both surface types and higher in BP, while NO_3^- consumption only took place in BP surface and not in VP. See Table S1 and S2 in supplementary material, for gross N transformation rates calculated on a soil weight basis.

Table 2. Gross N transformation rates from bare peat (BP) and vegetated peat (VP) calculated from mineral N pools in the soil.

Surface type	Mineralization ($\mu g\ N\ cm^{-3}\ d^{-1}$)	NH_4^+ consumption ($\mu g\ N\ cm^{-3}\ d^{-1}$)	Nitrification ($\mu g\ N\ cm^{-3}\ d^{-1}$)	NO_3- consumption ($\mu g\ N\ cm^{-3}\ d^{-1}$)
BP	3.3±1.1[a]	3.5±1.6[a]	0.9±0.5	0.9±0.3
VP	0.5±0.6[b]	0.4±0.6[b]	0.0±0.0	0.0±0.0

Values are mean ± 1 S.E; n=3
Different letters indicate statistically significant differences between the surface types (P <0.05).

4 Discussion

4.1 N_2O flux rates from bare and vegetated Peat soils

Similar to previous studies at the study site, the N_2O fluxes from unlabeled reference plots were higher from BP (mean 3.2 ± 0.5 mg $N_2O\ m^{-2}\ d^{-1}$) than from VP, where N_2O fluxes were negligible throughout the sampling period (mean –0.02 ± 0.14 mg $N_2O\ m^{-2}\ d^{-1}$). The emission rates were highest at warmest air temperatures (R^2= 0.357; $p < 0.005$). Nitrous oxide fluxes from BP are comparable to the emissions generally reported from drained boreal peatlands used for agriculture (0.1 – 15.1 mg $N_2O\ m^{-2}.\ d^{-1}$) (Maljanen *et al.,* 2010) and from tropical forests (0.09 – 2.5 mg $N_2O\ m^{-2}\ d^{-1}$). Tropical forests are among the most important natural terrestrial ecosystems in terms of N_2O emissions (Werner *et al.,* 2007), while it was generally assumed that N_2O emissions from Arctic soils are negligible. Contrary to this general pattern, the results here confirm the earlier findings that there are surfaces in the Arctic, namely bare peat soils on permafrost peatlands, with the potential to emit substantial amounts of N_2O (Repo *et al.,* 2009; Marushchak *et al.,* 2011; Voigt *et al.,* 2017a).

The bulk N_2O fluxes from the ^{15}N-labelling subplots were on average 3 times higher than those from adjacent, non-labelled bare peat areas. The concentration of inorganic N was at most doubled by adding labelled NO_3^- and/or NH_4^+, but the final nutrient content never exceeded maximum content of native

NO_3^- or NH_4^+ observed in the soil (data not shown). The bulk N_2O fluxes from the labelled plots (~10 mg N_2O m^{-2} d^{-1}) were still within the range of N_2O fluxes observed in previous years from BP surfaces (1.9–31 mg N_2O $m^{-2}d^{-1}$) (Repo *et al.,* 2009; Marushchak *et al.,* 2011). The differences in the N_2O fluxes from BP labelled and non-labelled plots could be also attributed to the natural spatial variation in the N_2O fluxes within the BP surfaces, which can be large even on small spatial scales (< 1m, personal observation. data not shown). The N_2O emissions from labelled and non-labelled plots had similar responses to changes in temperature ($R^2 = 0.391$, $p < 0.005$), which was likely the major factor controlling the temporal variation in the N_2O fluxes from BP surfaces during the study period. Even if some stimulation occurred, this likely did not affect the relative contribution of different microbial pathways to the total N_2O emissions because BP surfaces were not N limited during the study period (see discussion below).

4.2 Gross mineralization and nitrification rates from Bare and Vegetated Peat soils

Gross mineralization and nitrification rates were higher in BP than in VP (Table 2) This can be explained by the lower C/N ratio in BP (Booth *et al.*, 2005) together with higher WFPS, which seemed to favor N turnover supporting the third hypothesis of this study. The low NO_3^- consumption in BP, suggests that microbial demands are met in BP surfaces. This suggestion agrees with the findings of Diáková *et al.,* (2016) where significantly higher net N mineralization rates were observed in BP compared to VP, indicating that microbial communities in BP had a surplus of available N. Gross mineralization, nitrification and NO_3^- consumption in VP are negligible indicating severe N limitations in VP.

Gross N mineralization rates in BP (3.3 ± 1.1 µg N cm^{-3} d^{-1}) were higher compared to the gross mineralization rates reported for boreal peatlands (1 to 2 µg N cm^{-3} d^{-1}; Westbrook & Devito, 2004) and within the range reported for mineral tundra soils (mineral and organic horizon; 0.1 to 9 µg N cm^{-3} d^{-1}) (Biasi *et al.,* 2005, Meyer *et al.,* 2006; Buckeridge *et al*., 2007; Marushchak *et al.,* 2011) and organic layers of spruce forest soil (1 to 4 µg N cm^{-3} d^{-1}) (Brüggemann *et al.,* 2005; Zeller *et al.,* 2008). The gross N mineralization and nitrification rates of BP expressed per g dry weight (Table S1b; 12.3 ± 4.2 and 3.2 ± 1.9 µg N g^{-1} d^{-1}) were also comparable to rates found in boreal, temperate and tropical soils (Booth *et al.,* 2005), and were in line with results from previous studies from Arctic ecosystems and permafrost-affected soils (e.g., Kaiser *et al.,* 2007; Wild *et al.,* 2015, Ramm *et al.,* 2022). These relatively high N turnover rates contradict the general idea that organic N cycling dominates in cold ecosystems and mineral N cycling is of low importance (Schimel *et al.,* 2004). Instead, it seems that gross N

mineralization rates and gross nitrification rates can be high in arctic and sub-arctic ecosystems, if conditions are favorable (e.g., low C/N ratio, high %N, suitable water content; Booth *et al.,* 2005; Ramm *et al.,* 2022). In VP, the gross N turnover rates were negligible and, thus, lower than rates reported from BP or from other Arctic ecosystems (Alves *et al.,* 2013). The fact that the largest part of ^{15}N was found in the bulk soil immediately after label addition (see below) suggest quick cycles of abiotic fixation and release of NO_3^- in the peat soils, which could have made detection of gross nitrification rates in soils with low turnover rates, such as VP, difficult. Nevertheless, it is clear that the differences in mineral N cycling are an important factor explaining the differences in N_2O fluxes between BP and VP.

4.3 Fate of mineral N and factors affecting N_2O production

The total recovery of applied ^{15}N within 24 hours was close to 100% in both studied surface types. The recovery percentage decreased during the course of experiment in both VP and BP, which might be a consequence of lateral and vertical leaching of N forms within the soils, particularly in the case of $^{15}NO_3^-$ (Clough *et al.,* 2001). Also, part of the label could have been lost as gaseous fluxes of NO and N_2, which were not measured here. Both downward leaching and gaseous N losses as NO and N_2 were likely higher in BP than in VP because of effective plant N uptake and microbial immobilization in VP. Indeed, the total recovery of ^{15}N was higher in VP than in BP surfaces during the whole 24-day experiment (~79% vs. ~62%, respectively). It is also likely that the ^{15}N might increasingly accumulated as ^{15}N-N_2O and ^{15}N-N_2 in pore water/gas in BP. Soil gas concentrations of N_2O can be very high (up to 4500 ppb) particularly in BP (Gil *et al.,* 2017). However, since more than 60% and 80% of ^{15}N was recovered in VP and BP, respectively, we did account for all the major sinks of NO_3^- and NH_4^+ in both soils throughout the 24-day experiment.

In both VP and BP, the largest relative proportion of ^{15}N label after 24 days of experiment was observed in bulk peat (71–92% of total ^{15}N recovered), comprising physically adsorbed, dissolved, chemically or electro-chemically fixed and microbially immobilized ^{15}N. Peatlands are known to be able to efficiently retain nutrients to deal with low N inputs, which has given them ecological functions as nutrient buffers (Vikman et al, 200). Recovery of ^{15}N in bulk peat was higher for $^{15}NH_4^+$ than for $^{15}NO_3^-$ (Figure 2a and 2b). This suggests that fixation of nutrients to SOM is one of the main reasons for the high retention of ^{15}N, since soil particles are negatively charged (Schlesinger, 1997) and since fixation capacity is high under acidic conditions (Huber, Oberhauser & Kreutzer, 2002). This is supported by other studies that have found evidence for similarly high fixation of nutrients, particularly of $^{15}NH_4^+$, to organic peat

material (e.g., Munchmeyer *et al*., 2000). Microbial immobilization likely is another reason for high ^{15}N recovery in bulk soil in BP and VP, since NH_4^+ and NO_3^- consumption rates were as high as production rates in both soils as obtained from the pool dilution approach (Table 2; see discussion below). Rapid uptake of ^{15}N by microbes in soils with low N in from arctic and sub-arctic ecosystems has been documented during the first days after the addition of label in previous experiments (Nordin, Schmidt & Shaver, 2004; Sørensen *et al.,* 2008).

It has been shown that in the short term plants compete poorly for available soil N, but this competition depends on the season and many other factors (Grogan & Jonasson, 2003; Nordin, Schmidt & Shaver, 2004). In our 3-week study period, the average ^{15}N uptake by the plants (vascular plants + mosses) of ~6% was of the same order of magnitude as in reports from other arctic ecosystems (1 to 5 % within 4 h up to 12 weeks; Grogan & Jonasson, 2003; Nordin, Schmidt & Shaver, 2004). The fact that ^{15}N in plants did not constantly increase in our experiment (Figure S2) suggests that the label, once incorporated into the soil, is only slowly released in plant-available forms, as suggested also by others (Sorensen *et al.,* 2008). Generally, following the soil most of the label was recovered in mosses (3–4%), followed by roots (~1 to 3%) and aboveground vascular plant parts (< 1%) in VP. The relatively large difference in ^{15}N observed between mosses and vascular plants might be related to the difference in their mechanism for nutrient acquisition. Mosses derive N principally from atmospheric deposition (e.g., wet deposition) but also from soil N, and their nutrient acquisition is passive and is thought to relate to the pattern of water uptake (Ayres *et al.,* 2006). Since the ^{15}N tracers were added in water solution this should have facilitated the uptake of the ^{15}N label by the mosses in VP surfaces, which penetrate the upper soil column where the label was injected.

It has been shown that plants from different ecosystems, including Arctic ecosystems, can show N uptake flexibility between forms of N (organic N, NO_3^-, NH_4^+) based on environmental conditions and species competition (McKane *et al.,* 2002; Gao *et al.*, 2020). In our study, there was no difference in the plant uptake of ^{15}N-NO_3^- and ^{15}N-NH_4^+ in VP surfaces. The ^{15}N in plants was determined for the bulk and not for individual species and is possible that discrimination between the N forms based on species-specific preferences could take place (Gao *et al.*, 2020).

In BP, where plants were absent, 24% of the applied ^{15}N was detected in the cumulative N_2O emission at the end of the experiment. The recovery of the label in N_2O in BP is thus up to threefold larger than the relative portion of label observed in plants in VP (maximum value ~ 9%). This confirms our second hypothesis, that a higher proportion of the added ^{15}N is released in gaseous form in BP than taken up by

plants and immobilized in VP. It suggests that competition for N is an important regulator of N_2O in these peatlands, and that plants control to some extent the emissions of this strong greenhouse gas. This has been observed before for a restored boreal peatland with various levels of nitrate addition and plant coverage and for a drained forested peatland, where presence of roots halved N_2O emission (Silvan *et al.,* 2005; Holz *et al.,* 2016). It is likewise supported by recent results from a mesocosms study which show that presence of vegetation limits N_2O emissions from a permafrost peatland by ~90% (Voigt *et al.,* 2017b). On the other hand, in BP where plants are absent, microbes are not N limited and excess mineral N is highly available for microbial N_2O production processes, such as nitrification and denitrification (Schimel & Bennett*,* 2004). The differences in N_2O emissions between BP and VP are further a direct consequence of variable production rates of mineral N forms, with much lower gross N mineralization and nitrification rates in VP than in BP likely due to higher C/N ratios of the soils. Another important factor limiting N_2O production in VP is likely the low WFPS (29 % ± 1 %; Table 1) and thus high aeration status of peat in VP, which deceases denitrification potential (Firestone and Davidson, 1989.). The higher WFPS in BP, on the other hand, creates ideal conditions for mineralization, nitrification and denitrification to take place (Liimatainen *et al.,* 2018). To conclude, in VP with low C/N ratio and high aeration status, N_2O production is limited by low mineralization, nitrification and denitrification rates together with plant N uptake and immobilization of N. We thus find strong support for the second hypothesis in this study.

4.4 Microbial source of N_2O emitted from the bare peat surfaces

The source partitioning approach suggests a general dominance of denitrification processes (~79%) as a source of N_2O in BP surfaces. The results of the source partitioning approach are also corroborated by the higher ^{15}N-N_2O fluxes after application of ^{15}N-NO_3^- compared to application of ^{15}N-NH_4^+. The soil properties and N dynamics hint also at denitrification pathways being dominant in BP surfaces, where the high NO_3^- content and the intermediate to high soil moisture conditions cause high N_2O emissions via denitrification, as also suggested previously (Repo *et al.,* 2009; Palmer *et al.,* 2011; Marushchak *et al.,* 2011). Palmer *et al.* (2011) detected a high number of functional genes involved in denitrification in these soils and high potential for denitrification. Few, highly specialized taxa using acetate as their energy source, mostly belonging to the family of Burkholderiaceae (co-occurring with Rhodanobacter sp.*)*, seem to be responsible for most of the denitrification occurring in these acidic soils (Hetz & Horn, 2021). The first hypothesis of this study was thus supported by our labelling study and the source partitioning

approach. On a side note, we cannot clearly explain the second peak which we found in $^{15}N_2O$ and several inorganic ^{15}N pools in BP, but this could be due to immobilization and later recycling of added ^{15}N by microbes and by abiotic fixation (Braun *et al.,* 2018).

Despite the clear dominance of denitrification, the relative contribution of total nitrification to the N_2O emissions from the BP surfaces (~20%) was still significant and could be particularly important during drier summers (low soil water content) and at the end of the growing season when the N_2O emissions are generally lower, as shown here and also in Gil *et al.* (2017). In 2011, we found evidence for nitrification derived N_2O via ^{15}N natural abundance approaches in an exceptionally dry year in Seida, where WFPS of BP was almost 20% less than in 2010 (this study) and N_2O emissions were much lower (Gil et al., 2017). Nitrifier denitrification can be, however, ruled out as a possible source of N_2O in these soils since we know now that in Seida peat ammonia oxidizing archaea (AOA) are responsible for ammonia oxidation and ammonia oxidizing bacteria (AOB) are lacking there (Siljanen *et al*., 2019; Hetz & Horn, 2021). AOA are not capable for denitrification in contrast to AOB. However, nitrite produced by AOA could allow abiotic production of N_2O by chemical denitrification, where nitrite reacts with SOM in acidic conditions, prevailing in studied peat soils (Kappelmeyer *et al.,* 2003).

Since physical and chemical conditions in the studied permafrost peat surfaces are favorable for both nitrification and denitrification, it is possible that adjacent aerobic and anaerobic microhabitats enabled both ammonia oxidation and denitrification to occur and produce N_2O. Furthermore, nitrate/nitrite from nitrification is used as an electron acceptor in denitrification. Nitrous oxide production through a coupled nitrification-denitrification process is typical for C rich soils (Siljanen *et al,* 2019). This is supported by our data from T2, where ^{15}N label from $^{15}N\text{-}NH_4^+$ appeared in N_2O flux after a short time lag. Since the balance between nitrification and denitrification in soils influences N_2O emission strength with higher N_2O emissions associated with denitrification, increased soil water content as predicted for Alaska (Douglas *et al.,* 2020) might stimulate N_2O emissions from sites with high N availability.

5 Conclusions

The N_2O emission rates from the BP surfaces (mean 3 mg N_2O m^{-2} d^{-1}) were high, as hypothesized, while N_2O emissions from VP were negligible throughout the sampling period. In VP, N_2O production was limited by the low inorganic N content and low delivery of N from SOM, as opposed to BP. For both, VP and BP, most of the ^{15}N label was recovered in the bulk peat, followed by N_2O flux in BP and

plants in VP. The recovery of the label was larger in N_2O in BP than in plants in VP. This suggests that competition for mineral N between plants and microbes limits the N_2O release in VP, together with low mineralization and nitrification rates as a result of the high C/N ratio. In addition, low bulk density (high porosity) and low water content limit N_2O production by anaerobic denitrification in VP, while soil moisture content in BP is favorable for denitrification.

The source partitioning of N_2O from BP surfaces supports the role of denitrification as the dominant process behind the high N_2O emissions from BP during the study period. However, it also showed that nitrifying processes are taking place in BP and emit some N_2O. Thus, also nitrification is a key process involved in N_2O production in these soils both directly and indirectly through the NO_3^- supply for denitrification. With future warming, increased rainfall and permafrost thaw, anaerobic conditions might become more prevalent across the Arctic, which might cause increased N_2O release. In addition to soil moisture changes, abrupt permafrost thaw and thermokarst causes disturbance of the vegetation cover, which may improve the N availability for soil microbes, including those producing N_2O. On the other hand, overall trends towards increasing plant growth in a warming Arctic might slow down N_2O release in the long-term. The net effect of all these changes on N_2O emissions from permafrost regions are currently not known but need to be the focus of future studies. It is important to consider these processes in N cycling models for permafrost regions, which are currently being developed.

6 Data availability

Most of the data is provided in the figures and tables in the paper and the supplementary material, and any additional data may be obtained from J. Gil (email: jenie.gillugo@uef.fi).

7 Author contribution

C.B., E.M.B., T.R. and P.J.M. designed the study; J.G., M.E.M., C.B., T.T. and A.N., conducted the field work; D.K. and A.N. provided access to and expertise on the study sites and supported the project with field logistics, J.G. and C.B. conducted laboratory analysis and data processing; J.G. wrote the first version of the manuscript with contribution from C.B. and T.P., after which all co-authors provided input on manuscript text, figures, and discussion of scientific content.

8 Competing interests

The authors declare that they have no conflict of interest.

9 Acknowledgment

This study was financially supported by the Academy of Finland, project CryoN 2010–2014 (Mechanisms underlying large N_2O emissions from cryoturbated peat surfaces in tundra; decision Nr. 132045). We acknowledge further funding from the European Union 7th Framework Program under project Page 21 (contract Nr. GA282700); Academy of Finland, project NOCA (decision Nr. 314630); EMPIR project SIRS (Metrology for stable isotope reference standards, project number 16ENV06 SIRS), and (DEFROST)-Nordic Centre of Excellence Program (Impacts of changing cryosphere – depicting ecosystem-climate feedbacks from permafrost, snow and ice). We are also grateful to the consortium of the ESF supported project CryoCARB for productive discussions. J. Gil acknowledges salary funding from doctoral program of University of Eastern Finland during 2014 and personal grant awarded by Finnish Cultural Foundation (2015) and Academy of Finland/Vilho, Yrjö ja Kalle Väisälän Foundation (2016). We also thank Jouko Pokela, Slava Hozainov, Igor Marushchak, Natalia Kaneva and Henry Mora for their invaluable help during the fieldwork. The authors would like to thank the anonymous reviewers for their insightful comments and suggestions that have contributed to improve our manuscript significantly.

10 References

Abbott, B. W. and Jones, J. B.: Permafrost collapse alters soil carbon stocks, respiration, CH4, and N2O in upland tundra, Global Change Biology, 21, 4570-4587, 10.1111/gcb.13069, 2015.

Alves, R. J. E., Wanek, W., Zappe, A., Richter, A., Svenning, M. M., Schleper, C. and Urich, T.: Nitrification rates in Arctic soils are associated with functionally distinct populations of ammonia-oxidizing archaea ISME J. 7 1620–31, 2013.

Ayres, E., van der Wal, R., Sommerkorn, M., and Bardgett, R. D.: Direct uptake of soil nitrogen by mosses, Biology Letters, 2, 286-288, 10.1098/rsbl.2006.0455, 2006.

Baggs, E. M.: Soil microbial sources of nitrous oxide: recent advances in knowledge, emerging challenges and future direction, Current Opinion in Environmental Sustainability, 3, 321-327, 10.1016/j.cosust.2011.08.011, 2011.

Baggs, E. M. and Philippot, L.: Microbial terrestrial pathways to nitrous oxide, in: Nitrous oxide and climate change, edited by: (ed.)., S. K., Earthscan. Routledge. Taylor & Francis., UK USA, 2010.

Baggs, E. M., Richter, M., Cadisch, G., and Hartwig, U. A.: Denitrification in grass swards is increased under elevated atmospheric CO2, Soil Biology & Biochemistry, 35, 729-732, 10.1016/s0038-0717(03)00083-x, 2003.

Biasi, C., Jokinen, S., Marushchak, M. E., Hamalainen, K., Trubnikova, T., Oinonen, M., and Martikainen, P. J.: Microbial Respiration in Arctic Upland and Peat Soils as a Source of Atmospheric Carbon Dioxide, Ecosystems, 17, 112-126, 10.1007/s10021-013-9710-z, 2014.

Biasi, C., Wanek, W., Rusalimova, O., Kaiser, C., Meyer, H., Barsukov, P., and Richter, A.: Microtopography and plant-cover controls on nitrogen dynamics in hummock tundra ecosystems in Siberia, Arctic Antarctic and Alpine Research, 37, 435-443, 10.1657/1523-0430(2005)037[0435:mapcon]2.0.co;2, 2005.

Booth, M. S., Stark, J. M., and Rastetter, E.: Controls on nitrogen cycling in terrestrial ecosystems: A synthetic analysis of literature data, Ecological Monographs, 75, 139-157, 10.1890/04-0988, 2005.

Borge, A. F., Westermann, S., Solheim, I., and Etzelmüller, B.: Strong degradation of palsas and peat plateaus in northern Norway during the last 60 years, The Cryosphere, 11, 1–16, https://doi.org/10.5194/tc-11-1-2017, 2017.

Braun, J., Mooshammer, M., Wanek, W., Prommer, J., Walker, T. W. N., Rutting, T., and Richter, A.: Full N-15 tracer accounting to revisit major assumptions of N-15 isotope pool dilution approaches for gross nitrogen mineralization, Soil Biology & Biochemistry, 117, 16-26, 10.1016/j.soilbio.2017.11.005, 2018.

Brüggemann, N., Rosenkranz, P., Papen, H., Pilegaard, K., and Butterbach-Bahl, K.: Pure stands of temperate forest tree species modify soil respiration and N turnover, Biogeosciences Discussions, 2, 303-331, 2005.

Buckeridge, K. M. and Jefferies, R. L.: Vegetation loss alters soil nitrogen dynamics in an Arctic salt marsh, Journal of Ecology, 95, 283-293, 10.1111/j.1365-2745.2007.01214.x, 2007.

Clough, T. J., Sherlock, R. R., Cameron, K. C., Stevens, R. J., Laughlin, R. J., and Muller, C.: Resolution of the N-15 balance enigma?, Australian Journal of Soil Research, 39, 1419-1431, 10.1071/sr00092, 2001.

Decock, C. and Six, J.: How reliable is the intramolecular distribution of N-15 in N2O to source partition N2O emitted from soil?, Soil Biology & Biochemistry, 65, 114-127, 10.1016/j.soilbio.2013.05.012, 2013.

Douglas, T. A., Turetsky, M. R., and Koven, C. D.: Increased rainfall stimulates permafrost thaw across a variety of Interior Alaskan boreal ecosystems, Npj Climate and Atmospheric Science, 3, 10.1038/s41612-020-0130-4, 2020.

Elberling, B., Christiansen, H. H., and Hansen, B. U.: High nitrous oxide production from thawing permafrost, Nature Geoscience, 3, 332-335, 10.1038/ngeo803, 2010.

Firestone, M. and Davidson, E.: Microbiological basis of NO and N2O production and consumption in soil, in: Exchange of Trace Gases between Terrestrial Ecosystems and the Atmosphere, edited by: Andreae MO, S. D., eds. John Wiley & Sons., New York, 7-21, 1989.

Fry, B.: Stable Isotope Ecology, Springer Science + Business Media, LLC2006.

Gao, L., Cui, X. Y., Hill, P. W., and Guo, Y. F.: Uptake of various nitrogen forms by co-existing plant species in temperate and cold-temperate forests in northeast China, Applied Soil Ecology, 147, 10, 10.1016/j.apsoil.2019.103398, 2020.

Gardner, J. B. and Drinkwater, L. E.: The fate of nitrogen in grain cropping systems: a meta-analysis of N-15 field experiments, Ecological Applications, 19, 2167-2184, 10.1890/08-1122.1, 2009.

Gil, J., Perez, T., Boering, K., Martikainen, P. J., and Biasi, C.: Mechanisms responsible for high N_2O emissions from subarctic permafrost peatlands studied via stable isotope techniques, Global Biogeochemical Cycles, 31, 172-189, 10.1002/2015gb005370, 2017.

Goldberg, S. D., Knorr, K. H., Blodau, C., Lischeid, G., and Gebauer, G.: Impact of altering the water table height of an acidic fen on N2O and NO fluxes and soil concentrations, Global Change Biology, 16, 220-233, 10.1111/j.1365-2486.2009.02015.x, 2010.

Grogan, P. and Jonasson, S.: Controls on annual nitrogen cycling in the understory of a subarctic birch forest, Ecology, 84, 202-218, 10.1890/0012-9658(2003)084[0202:coanci]2.0.co;2, 2003.

Harden, J. W., Koven, C. D., Ping, C. L., Hugelius, G., McGuire, A. D., Camill, P., Jorgenson, T., Kuhry, P., Michaelson, G. J., O'Donnell, J. A., Schuur, E. A. G., Tarnocai, C., Johnson, K., and Grosse, G.: Field information links permafrost carbon to physical vulnerabilities of thawing, Geophysical Research Letters, 39, 10.1029/2012gl051958, 2012.

Harrison, M. D., Groffman, P. M., Mayer, P. M., and Kaushal, S. S.: Nitrate removal in two relict oxbow urban wetlands: a N-15 mass-balance approach, Biogeochemistry, 111, 647-660, 10.1007/s10533-012-9708-1, 2012.

Heikkinen, J. E. P., Elsakov, V., and Martikainen, P. J.: Carbon dioxide and methane dynamics and annual carbon balance in tundra wetland in NE Europe, Russia, Global Biogeochemical Cycles, 16, 16, 10.1029/2002gb001930, 2002.

Herrmann, A., Willett, V. B., Stockdale, E. A., and Jones, D. L.: Interference by amino acids during the determination of N-15 ammonium in soil, Soil Biology & Biochemistry, 37, 1747-1750, 10.1016/j.soilbio.2005.02.008, 2005.

Hetz SA and Horn MA, Burkholderiaceae Are Key AcetateAssimilators During Complete Denitrification in Acidic Cryoturbated Peat Circles of the Arctic Tundra. Front. Microbiol. 12:628269.doi: 10.3389/fmicb.2021.62826, 2021.

Holz, M., Aurangojeb, M., Kasimir, A., Boeckx, P., Kuzyakov, Y., Klemedtsson, L., and Rutting, T.: Gross Nitrogen Dynamics in the Mycorrhizosphere of an Organic Forest Soil, Ecosystems, 19, 284-295, 10.1007/s10021-015-9931-4, 2016.

Huber, C., Oberhauser, A., and Kreutzer, K.: Deposition of ammonia to the forest floor under spruce and beech at the Hoglwald site, Plant and Soil, 240, 3-11, 10.1023/a:1015825024164, 2002.

Jung, M. Y., Gwak, J. H., Rohe, L., Giesemann, A., Kim, J. G., Well, R., Madsen, E. L., Herbold, C. W., Wagner, M., and Rhee, S. K.: Indications for enzymatic denitrification to N2O at low pH in an ammonia-oxidizing archaeon, Isme Journal, 13, 2633-2638, 10.1038/s41396-019-0460-6, 2019.

Kaiser, C., Meyer, H., Biasi, C., Rusalimova, O., Barsukov, P., and Richter, A.: Conservation of soil organic matter through cryoturbation in arctic soils in Siberia, Journal of Geophysical Research-Biogeosciences, 112, 10.1029/2006jg000258, 2007.

Kappelmeyer, U., Kuschk, P., and Stottmeister, U.: Model experiments on the influence of artificial humic compounds on chemodenitrification, Water Air and Soil Pollution, 147, 317-330, 10.1023/a:1024518027312, 2003.

Kaverin, D. A., Pastukhov, A. V., Lapteva, E. M., Biasi, C., Marushchak, M., and Martikainen, P.: Morphology and properties of the soils of permafrost peatlands in the southeast of the Bol'shezemel'skaya tundra, Eurasian Soil Science, 49, 498-511, 10.1134/s1064229316050069, 2016.

Kirkham, D. and Bartholomew, W.: Equations for following nutrient transformations in soil, utilizing tracer data, Soil Science Society of America Journal, 18, 33-34, 1954.

Kirkham, D. and Bartholomew, W.: Equations for following nutrient transformations in Soil, utilizing tracer data: II, Soil Science Society of America Journal, 19, 189-192, 1955.

Liimatainen, M., Voigt, C., Martikainen, P. J., Hytonen, J., Regina, K., Oskarsson, H., and Maljanen, M.: Factors controlling nitrous oxide emissions from managed northern peat soils with low carbon to nitrogen ratio, Soil Biology & Biochemistry, 122, 186-195, 10.1016/j.soilbio.2018.04.006, 2018.

Liu, H., Zak, D., Rezanezhad, F., and Lennartz, B.: Soil degradation determines release of nitrous oxide and dissolved organic carbon from peatlands, Environmental Research Letters, 14, 10.1088/1748-9326/ab3947, 2019.

Ma, W. K., Schautz, A., Fishback, L. A. E., Bedard-Haughn, A., Farrell, R. E., and Siciliano, S. D.: Assessing the potential of ammonia oxidizing bacteria to produce nitrous oxide in soils of a high arctic lowland ecosystem on Devon Island, Canada, Soil Biology & Biochemistry, 39, 2001-2013, 10.1016/j.soilbio.2007.03.001, 2007.

Maljanen, M., Sigurdsson, B. D., Guomundsson, J., Oskarsson, H., Huttunen, J. T., and Martikainen, P. J.: Greenhouse gas balances of managed peatlands in the Nordic countries – present knowledge and gaps, Biogeosciences, 7, 2010.

Marushchak, M. E., Pitkamaki, A., Koponen, H., Biasi, C., Seppala, M., and Martikainen, P. J.: Hot spots for nitrous oxide emissions found in different types of permafrost peatlands, Global Change Biology, 17, 2601-2614, 10.1111/j.1365-2486.2011.02442.x, 2011.

Marushchak, M. E., Kiepe, I., Biasi, C., Elsakov, V., Friborg, T., Johansson, T., Soegaard, H., Virtanen, T., and Martikainen, P. J.: Carbon dioxide balance of subarctic tundra from plot to regional scales, Biogeosciences, 10, 437-452, 10.5194/bg-10-437-2013, 2013.

Marushchak, M.E. , Kerttula, J. , Diáková, K. , *et al.*: Thawing Yedoma permafrost is a neglected nitrous oxide source. Nature Communications 12, 7107, https://doi.org/10.1038/s41467-021-27386-2, 2021.

McKane, R. B., Johnson, L. C., Shaver, G. R., Nadelhoffer, K. J., Rastetter, E. B., Fry, B., Giblin, A. E., Kielland, K., Kwiatkowski, B. L., Laundre, J. A., and Murray, G.: Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra, Nature, 415, 68-71, 10.1038/415068a, 2002.

Meyer, H., Kaiser, C., Biasi, C., Hammerle, R., Rusalimova, O., Lashchinsky, N., Baranyi, C., Daims, H., Barsukov, P., and Richter, A.: Soil carbon and nitrogen dynamics along a latitudinal transect in Western Siberia, Russia, Biogeochemistry, 81, 239-252, 10.1007/s10533-006-9039-1, 2006.

Miranda, K. M., Espey, M. G., and Wink, D. A.: A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite, Nitric Oxide-Biology and Chemistry, 5, 62-71, 10.1006/niox.2000.0319, 2001.

Mulvaney, R. L. and Khan, S. A.: Use of diffusion to determine inorganic nitrogen in a complex organic matrix, Soil Science Society of America Journal, 63, 240-246, 1999.

Münchmeyer, U., Russow, R., and Augusti, J.: Net and Gross Nitrogen Mineralization in Drained and Reflooded Fen Soils, Isotopes in Environmental and Health Studies, 36, 79-98, 10.1080/10256010008032934, 2000.

Nordin, A., Schmidt, I. K., and Shaver, G. R.: Nitrogen uptake by arctic soil microbes and plants in relation to soil nitrogen supply, Ecology, 85, 955-962, 10.1890/03-0084, 2004.

Palmer, K., Biasi, C., and Horn, M. A.: Contrasting denitrifier communities relate to contrasting N2O emission patterns from acidic peat soils in arctic tundra, Isme Journal, 6, 1058-1077, 10.1038/ismej.2011.172, 2011.

Pang, P. C. K.: Distribution and recovery of N-15 after fertilization of douglas-fir saplings with different nitrogen-sources, Plant and Soil, 84, 167-174, 10.1007/bf02143180, 1985.

Prosser, J. I., Hink, L., Gubry-Rangin, C., and Nicol, G. W.: Nitrous oxide production by ammonia oxidizers: Physiological diversity, niche differentiation and potential mitigation strategies, Global Change Biology, 26, 103-118, 10.1111/gcb.14877, 2020.

Ramm, E., Liu, C.Y., Ambus, P., Butterbach-Bahl, K., Hu, B,, Martikainen, P.J., Marushchak, M.E, Mueller, C.W., Rennenberg, H., Schloter, M., Siljanen, H.M.P., Voigt, C., Werner. C., Biasi, C., Dannenmann, M., :A review of the importance of mineral nitrogen cycling in the plant-soil-microbe system of permafrost-affected soils-changing the paradigm. Environmental Research Letters 17, 013004, https://doi.org/10.1088/1748-9326/ac417e, 2022.

Repo, M. E., Susiluoto, S., Lind, S. E., Jokinen, S., Elsakov, V., Biasi, C., Virtanen, T., and Martikainen, P. J.: Large N2O emissions from cryoturbated peat soil in tundra, Nature Geoscience, 2, 189-192, 10.1038/ngeo434, 2009.

Richardson, D., Felgate, H., Watmough, N., Thomson, A., and Baggs, E.: Mitigating release of the potent greenhouse gas N2O from the nitrogen cycle - could enzymic regulation hold the key?, Trends in Biotechnology, 27, 388-397, 10.1016/j.tibtech.2009.03.009, 2009.

Robinson, D.: delta N-15 as an integrator of the nitrogen cycle, Trends in Ecology & Evolution, 16, 153-162, 10.1016/s0169-5347(00)02098-x, 2001.

Rütting, T.: Ignoring isotopic fractionation does not bias quantifications of gross nitrogen transformations, Rapid Communications in Mass Spectrometry, 26, 1639-1640, 10.1002/rcm.6269, 2012.

Rütting, T., Huygens, D., Staelens, J., Muller, C., and Boeckx, P.: Advances in N-15-tracing experiments: new labelling and data analysis approaches, Biochemical Society Transactions, 39, 279-283, 10.1042/bst0390279, 2011.

Sannel, A. B. K., and Kuhry, P. : Warming-induced destabilization of peat plateau/thermokarst lake complexes, J. Geophys. Res., 116, G03035, doi:10.1029/2010JG001635, 2011.

Schadel, C., Bader, M. K. F., Schuur, E. A. G., Biasi, C., Bracho, R., Capek, P., De Baets, S., Diakova, K., Ernakovich, J., Estop-Aragones, C., Graham, D. E., Hartley, I. P., Iversen, C. M., Kane, E. S., Knoblauch, C., Lupascu, M., Martikainen, P. J., Natali, S. M., Norby, R. J., O'Donnell, J. A., Chowdhury, T. R., Santruckova, H., Shaver, G., Sloan, V. L., Treat, C. C., Turetsky, M. R., Waldrop, M. P., and Wickland, K. P.: Potential carbon emissions dominated by carbon dioxide from thawed permafrost soils, Nature Climate Change, 6, 950-+, 10.1038/nclimate3054, 2016.

Schimel, J. P. and Bennett, J.: Nitrogen mineralization: Challenges of a changing paradigm, Ecology, 85, 591-602, 10.1890/03-8002, 2004.

Schlesinger, W. H.: Biogeochemistry. An Analysis of Global Change, 2nd, Toronto: Academic Press., San Diego, London, Boston, New York, Sydney, Tokyo1997.

Schuur, E. A. G., Vogel, J. G., Crummer, K. G., Lee, H., Sickman, J. O., and Osterkamp, T. E.: The effect of permafrost thaw on old carbon release and net carbon exchange from tundra, Nature, 459, 556-559, 10.1038/nature08031, 2009.

Schuur, E. A. G., McGuire, A. D., Schadel, C., Grosse, G., Harden, J. W., Hayes, D. J., Hugelius, G., Koven, C. D., Kuhry, P., Lawrence, D. M., Natali, S. M., Olefeldt, D., Romanovsky, V. E., Schaefer, K., Turetsky, M. R., Treat, C. C., and Vonk, J. E.: Climate change and the permafrost carbon feedback, Nature, 520, 171-179, 10.1038/nature14338, 2015.

Siciliano, S. D., Ma, W. K., Ferguson, S., and Farrell, R. E.: Nitrifier dominance of Arctic soil nitrous oxide emissions arises due to fungal competition with denitrifiers for nitrate, Soil Biology & Biochemistry, 41, 1104-1110, 10.1016/j.soilbio.2009.02.024, 2009.

Siljanen, H. M. P., Alves, R. J. E., Ronkainen, J. G., Lamprecht, R. E., Bhattarai, H. R., Bagnoud, A., Marushchak, M. E., Martikainen, P. J., Schleper, C., and Biasi, C.: Archaeal nitrification is a key driver of high nitrous oxide emissions from arctic peatlands, Soil Biology & Biochemistry, 137, 10.1016/j.soilbio.2019.107539, 2019.

Silvan, N., Tuittila, E. S., Kitunen, V., Vasander, H., and Laine, J.: Nitrate uptake by Eriophorum vaginatum controls N2O production in a restored peatland, Soil Biology & Biochemistry, 37, 1519-1526, 10.1016/j.soilbio.2005.01.006, 2005.

Simek, M. and Cooper, J. E.: The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years, European Journal of Soil Science, 53, 345-354, 10.1046/j.1365-2389.2002.00461.x, 2002.

Seppälä, M. : Surface abrasion of palsas by wind action in Finnish Lapland, Geomorphology,Volume 52, Issues 1–2, Pages 141-148,ISSN 0169-555X, https://doi.org/10.1016/S0169-555X(02)00254-4, 2003.

Seppälä, M. :. Synthesis of studies of palsa formation underlining the importance of local environmental and physical characteristics. Quaternary Research, 75(2), 366-370. doi:10.1016/j.yqres.2010.09.007, 2011

Sorensen, P. L., Clemmensen, K. E., Michelsen, A., Jonasson, S., and Strom, L.: Plant and microbial uptake and allocation of organic and inorganic nitrogen related to plant growth forms and soil conditions at two subarctic tundra sites in Sweden, Arctic Antarctic and Alpine Research, 40, 171-180, 10.1657/1523-0430(06-114)[sorensen]2.0.co;2, 2008.

Stevens, R. J., Laughlin, R. J., Burns, L. C., Arah, J. R. M., and Hood, R. C.: Measuring the contributions of nitrification and denitrification to the flux of nitrous oxide from soil, Soil Biology & Biochemistry, 29, 139-151, 10.1016/s0038-0717(96)00303-3, 1997.

Takakai, F., Desyatkin, A. R., Lopez, C. M. L., Fedorov, A. N., Desyatkin, R. V., and Hatano, R.: CH(4) and N(2)O emissions from a forest-alas ecosystem in the permafrost taiga forest region, eastern Siberia, Russia, Journal of Geophysical Research-Biogeosciences, 113, 16, 10.1029/2007jg000521, 2008.

Toyoda, S., Yoshida, N., and Koba, K.: Isotopocule analysis of biologically produced nitrous oxide in various environments, Mass Spectrometry Reviews, 36, 135-160, 10.1002/mas.21459, 2017.

Vikman, A., Sarkkola, S., Koivusalo, H., Sallantaus, T., Laine, J., Silvan, N., Nousiainen, H., and Nieminen, M.: Nitrogen retention by peatland buffer areas at six forested catchments in southern and central Finland, Hydrobiologia, 641, 171-183, 10.1007/s10750-009-0079-0, 2010.

Voigt, C., Lamprecht, R. E., Marushchak, M. E., Lind, S. E., Novakovskiy, A., Aurela, M., Martikainen, P. J., and Biasi, C.: Warming of subarctic tundra increases emissions of all three important greenhouse gases - carbon dioxide, methane, and nitrous oxide, Global Change Biology, 23, 3121-3138, 10.1111/gcb.13563, 2017a.

Voigt, C., Marushchak, M. E., Abbott, B. W., Elberling, B., Siciliano, S. D., Sonnentag, O., Stewart, K. J., Yang, Y., and Martikainen, P. E.: Nitrous oxide emissions from permafrost-affected soils, *Nat Rev Earth Environ* **1**, 420–434, https://doi.org/10.1038/s43017-020-0063-9, 2020.

Voigt, C., Marushchak, M. E., Lamprecht, R. E., Jackowicz-Korczynski, M., Lindgren, A., Mastepanov, M., Granlund, L., Christensen, T. R., Tahvanainen, T., Martikainen, P. J., and Biasi, C.: Increased nitrous oxide emissions from Arctic peatlands after permafrost thaw, Proceedings of the National Academy of Sciences of the United States of America, 114, 6238-6243, 10.1073/pnas.1702902114, 2017b.

Werner, C., Butterbach-Bahl, K., Haas, E., Hickler, T., and Kiese, R.: A global inventory of N2O emissions from tropical rainforest soils using a detailed biogeochemical model, Global Biogeochemical Cycles, 21, 18, 10.1029/2006gb002909, 2007.

Westbrook, C. J. and Devito, K. J.: Gross nitrogen transformations in soils from uncut and cut boreal upland and peatland coniferous forest stands, Biogeochemistry, 68, 33-49, 10.1023/B:BIOG.0000025739.04821.8e, 2004.

Wild, B., Schnecker, J., Knoltsch, A., Takriti, M., Mooshammer, M., Gentsch, N., Mikutta, R., Alves, R. J. E., Gittel, A., Lashchinskiy, N., and Richter, A.: Microbial nitrogen dynamics in organic and mineral soil horizons along a latitudinal transect in western Siberia, Global Biogeochemical Cycles, 29, 567-582, 10.1002/2015gb005084, 2015.

Wrage, N., van Groenigen, J. W., Oenema, O., and Baggs, E. M.: A novel dual-isotope labelling method for distinguishing between soil sources of N(2)O, Rapid Communications in Mass Spectrometry, 19, 3298-3306, 10.1002/rcm.2191, 2005.

Zeller, B., Liu, J. X., Buchmann, N., and Richter, A.: Tree girdling increases soil N mineralization in two spruce stands, Soil Biology & Biochemistry, 40, 1155-1166, 10.1016/j.soilbio.2007.12.009, 200