



Denitrification at two nitrogen-polluted, ombrotrophic *Sphagnum* bogs in Central Europe: Insights from porewater N₂O-isotope profiles

Martin Novak^{a,*}, Gerhard Gebauer^b, Michael Thoma^b, Jan Curik^a, Marketa Stepanova^a, Ivana Jackova^a, Frantisek Buzek^a, Jiri Barta^c, Hana Santruckova^c, Daniela Fottova^a, Ales A. Kubena^d

^a Czech Geological Survey, Department of Environmental Geochemistry and Biogeochemistry, Geologicka 6, 152 00 Prague 5, Czech Republic

^b University of Bayreuth, BayCEER – Laboratory of Isotope Biogeochemistry, 95440 Bayreuth, Germany

^c University of South Bohemia, Department of Ecosystems Biology, Branisovska 31, 370 05, Ceske Budejovice, Czech Republic

^d Institute of Information Theory and Automation, Czech Academy of Sciences, Pod Vodarenskou Vezi 4, 182 00 Prague 8, Czech Republic

ARTICLE INFO

Article history:

Received 29 May 2014

Received in revised form

25 September 2014

Accepted 22 October 2014

Available online 12 November 2014

Keywords:

Wetland

Denitrification

Nitrous oxide

Nitrogen isotopes

Ombrotrophic bog

Sphagnum

Porewater

ABSTRACT

Measurements of nitrogen isotope ratios ($\delta^{15}\text{N}$) along vertical porewater profiles are instrumental in localizing sources and sinks of nitrous oxide (N₂O) in wetland ecosystems. We present the first N₂O concentration and isotope data for porewaters of ombrotrophic, mountain-top bogs. Our two study sites, situated in the Czech Republic at elevations higher than 1000 m, have been affected by atmospheric N pollution for more than 100 years. In recent decades, the northeastern site KB received over 30 kg N ha⁻¹ yr⁻¹ via atmospheric deposition, the southwestern site BS was 3 times less polluted. Both peat bogs were surrounded by defoliated, dead spruce stands. We hypothesized that elevated nitrate (NO₃⁻) inputs in rain-fed bogs may cause sizeable N₂O emissions. Porewater N₂O concentrations in the studied ombrotrophic bogs were 400–800 times higher, compared to fens in nearby Bavaria (Germany). The highest N₂O concentrations and the lowest $\delta^{15}\text{N}$ -N₂O values were found at the bottom of the sampled porewater profiles, 40–60 cm below surface. Low $\delta^{15}\text{N}$ -N₂O values resulted from a N isotope fractionation associated with denitrification. The site with the lower historical N pollution, BS, exhibited up to 7 times higher porewater N₂O concentrations than KB, possibly due to a higher denitrification potential determined as abundance of *nirK* and *nirS* genes. Upcore, N₂O abundance decreased and $\delta^{15}\text{N}$ -N₂O increased, indicating N₂O reduction. Processes of N₂O formation and consumption at different depths were integrated by measuring N₂O fluxes across the peat – atmosphere interface. These fluxes were minute (<0.02 μmol N₂O m⁻² h⁻¹). We even observed scavenging of air-borne N₂O by the bog substrate. Nitrogen pollution of ombrotrophic bogs led to vigorous N cycling, manifested by extremely high peat porewater N₂O concentrations. However, effective *in-situ* consumption of the produced N₂O greatly reduced its warming potential.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Nitrous oxide (N₂O) is a potent greenhouse gas and a major ozone-depleting substance (Ravishankara et al., 2009). At present, N₂O concentration in the atmosphere is 324 ppb, 18% higher compared to pre-industrial times (Heil et al., 2014). N₂O contributes about 6% to the global warming potential, surpassed only by carbon dioxide (CO₂; 63%) and methane (CH₄; 18%; Frolking et al., 2011). Increased production of N₂O by soil microorganisms is a

consequence of a higher release of reactive nitrogen (nitrate, NO₃⁻, and ammonium, NH₄⁺) by human activities, mainly industry and agriculture (Morkved et al., 2006, 2007). The largest anthropogenic sources of reactive nitrogen (N_r) are fossil fuel burning, application of artificial fertilizers and planting of crop that can fix atmospheric N₂ (Billings, 2008). The two main biological processes that produce N₂O are nitrification and denitrification. High rates of N₂O production are more commonly associated with denitrification (Zhu et al., 2013). During denitrification, nitrate (NO₃⁻) or nitrite (NO₂⁻) are reduced to the gaseous nitrogen species, NO, N₂O, and N₂. At the cellular level, denitrification rates are controlled by the availability of NO₃⁻, labile organic carbon (C), and molecular oxygen (O₂). For microbial respiration, organic matter serves as an electron donor.

* Corresponding author.

E-mail address: martin.novak@geology.cz (M. Novak).

For microbial respiratory NO_3^- reduction, anoxic conditions are required (Firestone and Davidson, 1989). However, simultaneous respiration of O_2 and NO_3^- has been observed in oxygenated soils following anoxic periods (Morley et al., 2008).

The amount of N_2O escaping from wet soils into the atmosphere depends on the rate of its production and the rate of its biological reutilization within the soil (Wieder and Vitt, 2006). The balance is controlled by diffusion conditions and the enzymatic activity of the denitrifying community (Andert et al., 2012). Denitrification is the only biological process that consumes N_2O and NO (Holtan-Hartwig et al., 2000). Once N_2O reaches the atmosphere, its mean residence time is relatively long (114 years; Frohking et al., 2011). In contrast, the atmospheric lifetime of NO , also a greenhouse gas, is extremely short (hours to days). The final product of denitrification, N_2 , has no warming potential.

For efficient N_2O mitigation strategies, a quantitative understanding of the source/sink relationships is needed. As yet, the global atmospheric N_2O budget is poorly constrained. The discrepancy between the known sources and sinks of N_2O is almost 30% (Billings, 2008). Current budgets either overestimate N_2O sources, or underestimate N_2O sinks. A number of recent studies have quantified N_2O emissions from biological sources (Limpens et al., 2006). The main difficulty is that denitrification rates are highly variable across ecosystem types, both spatially and temporally (Maljanen et al., 2012). Soils, mostly agricultural, contribute 50–60% of global N_2O emissions (IPCC, 2014). Acidic soils generate more N_2O , relative to NO and N_2 (Palmer et al., 2010). Forest soils have also traditionally been viewed as a source of N_2O (Davidson, 1991). Recently, Goldberg and Gebauer (2009) and Berger et al. (2013b) have reported net N_2O consumption at the soil surface over much of the forest's growing season. This phenomenon may partly explain the discrepancy in the global atmospheric N_2O budget by lowering the source flux estimates (Chapuis-Lardy et al., 2007). Measurable N_2O emissions have been reported from wetlands (Palmer et al., 2012). Wetlands contain as much as 16% of the world's soil nitrogen (Limpens et al., 2006). Following draining, minerotrophic wetlands (N-rich fens) may produce relatively large emissions of N_2O . In contrast, N-poor ombrotrophic (rain-fed) bogs are characterized by relatively small N_2O emissions both under wet conditions, and after draining/re-wetting (Moore, 1994).

The relative role of denitrification as a pathway of N_r removal from ecosystems can be studied using stable isotope ratios $^{15}\text{N}/^{14}\text{N}$, expressed in the $\delta^{15}\text{N}$ notation as a relative per mil deviation from an agreed-upon standard (Søvik and Mørkved, 2008). Several authors have used ^{15}N -enriched NO_3^- to trace the origin of N_2O emissions from wetlands (Zhu et al., 2013). Natural-abundance N isotopes also help to disentangle the production/consumption relationships in vertical soil profiles, both in upland and wetland areas (Novak et al., 1999, 2003; Velthof et al., 2000; Kohzu et al., 2003; Hobbie and Ouimet, 2009). During denitrification, nitrogen of the product, N_2O , becomes isotopically lighter (lower $\delta^{15}\text{N}$), while nitrogen of the remaining substrate (NO_3^-) becomes progressively enriched in the heavier isotope (higher $\delta^{15}\text{N}$; Perez, 2005). A number of laboratory studies have quantified the N isotope effect associated with denitrification under controlled conditions (Mariotti et al., 1981). This effect is often reported as an isotope enrichment factor ϵ , defined as $\delta_{\text{product}} - \delta_{\text{reactant}}$. A higher denitrification rate due to a higher temperature or a higher supply of electron donors lowers the N isotope effect (Firestone and Davidson, 1989). Two studies have reported vertical $\delta^{15}\text{N}-\text{N}_2\text{O}$ profiles in minerotrophic wetlands (Goldberg et al., 2008, 2010). No N isotope data have been reported thus far for vertical N_2O profiles in mountain-top, ombrotrophic bogs. Here we present N_2O concentration and isotope data for two ombrotrophic bogs, situated in the Czech Republic (Central Europe). In the late 20th century, this

region experienced high atmospheric N_r loads (Oulehle et al., 2008; Novak et al., 2014). Between 1970 and 1996, Norway spruce died back on 1000 km² at elevations higher than 700 m, due to severe acidification (Erbanova et al., 2008). High NO_x emissions were typical of coal-fired powerplants. Our study sites, while rain-fed, were historically also nitrogen-rich, due to high N_r deposition. Our first objective was to quantify N_2O emissions from these ombrotrophic bogs and link their magnitude to between-site differences. Our second objective was to assess N_2O source/sink relationships along vertical peat profiles using $\delta^{15}\text{N}-\text{N}_2\text{O}$. We hypothesized that, due to the elevated N_r supply in the past, both bogs would exhibit high N_2O productions, but possibly also relatively high N_2O emissions.

2. Material and methods

2.1. Study sites

The Blatenska slat (BS) and Kunstatska kaple (KB) bogs (Fig. 1, Table 1) are *Sphagnum*-dominated, ombrotrophic peatlands, situated on mountain plateaus at elevations above 1000 m. BS is part of the Sumava National Park in the southwestern Czech Republic, near the border with Bavaria (Germany; Bufkova et al., 2010). KB is located in the Orlické hory Natural Preserve Area in the north-eastern Czech Republic, near the border with Silesia (Poland; Dohnal et al., 1965). Heavy industry and regional air pollution characterize the northern part of the country, while the land use in the southern Czech Republic has been dominated by agriculture. In the north, atmospheric deposition of acidifying sulfur (S) was close to 160 kg S ha⁻¹ yr⁻¹ in the mid-1980's, and decreased thereafter (Novak et al., 2007). In the south, atmospheric S deposition was 12 kg ha⁻¹ yr⁻¹ in 1995, and exhibited a decreasing trend during the following decade (Fottova, 2013). Toxic trace elements (As, Be, Zn, Pb) were studied in atmospheric deposition of the Czech Republic by Erbanova et al. (2008), Bohdalkova et al. (2014), and Voldrichova et al. (2014). These authors reported higher pollution in the north, compared to the south.

The two sites differ in peat depth and age of the basal peat layer. While over 7 m of peat substrate have accumulated at BS, peat thickness at KB is only 60 cm. *Sphagnum* lawns in the centers of both study sites, where sampling took place, are characterized by a high water table level. For at least 10 months per year, only 2- to 5-cm high capitula of living *Sphagnum* come out of small water pools. A late summer water table drawdown rarely reaches the depth of

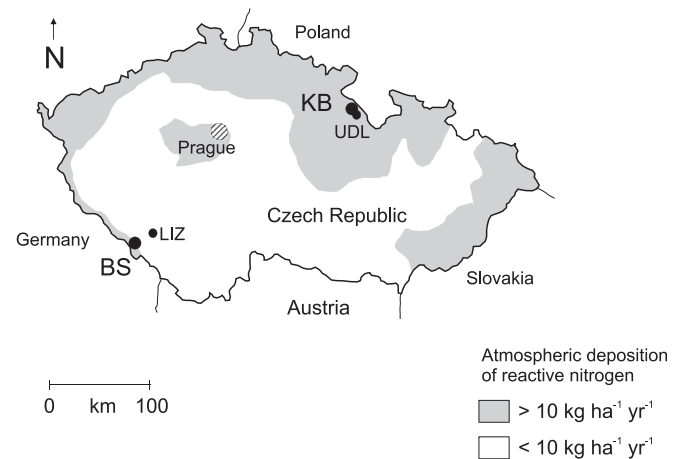


Fig. 1. Study sites. BS and KB are ombrotrophic bogs, UDL and LIZ are small forested catchments where atmospheric deposition of reactive N has been measured. Pollution contours are based on data by the Czech Hydrometeorological Institute, Prague.

Table 1
Study site characteristics.

Site	Location	Elevation (m)	Annual precipitation (mm)	Mean annual temperature (°C)	Bog area (ha)	Peat depth (cm)	Year of spruce die-back
Blatenska slat (BS)	48°58'33,474" N 13°27'22,978" E	1260	1150	3.5	7	700	1999
Kunstatska kaple bog (KB)	50°14'47,997" N 16°27'07,539" E	1040	1320	4.5	0.16	60	1985

10 cm below surface (cf., Bohdalkova et al., 2013). In the vicinity of the unforested centers of both bogs, defoliated mature spruce stands are situated. The reasons for the tree die-back, however, differ. Acid rain, dominated by H₂SO₄ and HNO₃, killed the trees near KB around 1985, whereas a bark-beetle infestation damaged the BS ecosystem in 1999. At both sites, margins of the bogs are overgrown by healthy dwarf pine.

2.2. Sampling

N₂O sampling was carried out between June 12 and 20, 2013. A custom-designed sampling device (Goldberg et al., 2008) was vertically inserted into the peat substrate on day 1, and allowed to equilibrate. N₂O dissolved in peat porewater was extracted on day 7 and taken to the laboratory for concentration and isotope analysis. In the field, N₂O fluxes at the peat surface were measured on days 1 and 7. The porewater gas sampling device used silicone tubes with a highly gas-permeable surface, submerged in the waterlogged peat. The cylindrical device (7 cm in diameter, 60 cm in length) consisted of 6 vertically positioned cylindrical cells with perforations (diameter of 0.5 cm in a 2.5 cm wide band in the center of each cell). In each cell, a 5-m long silicone tube (diameter of 3 mm) was coiled. The total sampling volume per cell was 35 mL. On day 1, a hole with a diameter of 6 cm was cored vertically in the peat to a depth of 60 cm using a gouge in which the gas sampling device was inserted. On day 7, one end of a sampling tube was connected to an evacuated gas vessel, and opened. The gas, extracted from the peat porewater, was transported to the surface by a gas-impermeable polyurethane tube (diameter of 1.8 mm). The extracted volume was filled with N₂ at ambient pressure from an attached air bag (Berger et al., 2013a). Evacuated glass bottles (100 mL) were used for sample storage. Porewater N₂O was collected separately from the depths of 10, 20, 30, 40, 50 and 60 cm below peat surface. Four replicate porewater gas sampling devices were used at each study site, installed randomly at distances of 10–30 m.

Atmospheric deposition of N_r was measured in the hydrological year 2013. Cumulative rainfall was sampled in 1-month intervals in an open area ("bulk deposition") and under the canopy of living Norway spruce ("throughfall"). Rainfall collectors were polyethylene (PE) funnels (area 122 cm²) fitted to 1 L PE bottles placed 120 cm above ground. In winter, the funnels were replaced by plastic vessels (area 380 cm²) with PE bags to collect snow. N_r deposition was directly measured at BS. Three throughfall collectors installed at distances of 10 m in a triangle, and two collectors of open-area deposition, were used. N_r deposition at KB was approximated by data from the small catchment UDL, located 4 km from the bog. UDL is part of a nation-wide hydrogeochemical monitoring network GEOMON (Oulehle et al., 2008). At UDL, a 10 × 10 m grid of nine collectors was used to collect spruce throughfall, and two collectors were used to sample open-area deposition. δ¹⁵N of NO₃⁻ and NH₄⁺ of both canopy throughfall and open-area deposition were measured in 30-day samples from BS (March 2013). Nitrogen isotope values for KB were approximated by analyzing throughfall and open-area deposition at Torfowisko

Zielenc in the Orlické Mts., situated 12 km from KB. Again, 30-day cumulative rainfall samples were analyzed (March 2013).

Peat substrate samples were collected from the depth of 0–2 and 40–42 cm at BS and KB for the determination of bulk density, ash content, C and N concentrations, and δ¹⁵N values. Peat porewater was collected from the same depths for the determination of pH, NO₃⁻ and NH₄⁺, Ca²⁺, Mg²⁺ and SO₄²⁻ concentrations. Three replicate bog water samplings were performed within the N₂O sampling plot.

Peat substrate samples were collected in triplicate from the depths of 15–25 cm and 50–60 cm for a microbiological analysis. The sampling dates were August 21, 2014 (KB), and August 26, 2014 (BS). These peat samples were stored anaerobically in ZipLoc bags at a temperature of –20 °C.

2.3. Analysis

Vertical N₂O flux rates at the peat – atmosphere interface were measured at each site by a closed chamber technique using a photoacoustic infrared gas analyzer (Multigas Monitor 1312 INNOVA, Denmark), installed *in situ*. N₂O concentrations were measured in five chambers (diameter of 19 cm, height of 30 cm) per site inserted into the peat substrate. The headspace in each chamber was approximately 1700 cm³. Changing N₂O concentrations in the headspace were monitored over 1 h (5 measurements per chamber). A single *in-situ* N₂O concentration measurement was reproducible within 15 ppb. Samples of ambient air were taken 0.5 m above ground. Gas fluxes were calculated from the linear increase or decrease in gas concentrations.

The gas samples collected from vertical porewater profiles were analyzed for N₂O concentrations and δ¹⁵N in the laboratory. A gas chromatograph (GC) coupled with an isotope ratio mass spectrometer (IRMS) and a pre-GC concentration interface (PreCon, Finnigan MAT, Hewlett-Packard GC 5890 series II and Delta V plus IRMS Thermo Fisher Scientific) were used for the analysis. A N₂O gas (99.999%, Linde) was used as a laboratory standard. The internal reproducibility of the instrumental set-up was ±0.15‰. Porewater N₂O concentrations were calculated from the total sample size and the peak area on mass 44, using a calibration line. The reproducibility of porewater N₂O concentration analysis was 4 ppb.

Water samples were analyzed for NH₄⁺ concentrations by spectrophotometry (Perkin-Elmer 200 Hitachi; 2 SD of 4.4%), and for NO₃⁻ concentrations by liquid chromatography (Knauer 1000; 2 SD of 3.8%). For N isotope analysis, 500 mL of atmospheric deposition were concentrated on Dowex 50W and Dowex 1 × cation and anion exchange resins, and separated into NH₄⁺ and NO₃⁻. Solution aliquots were made alkaline with MgO and steam distilled into a small excess of diluted H₂SO₄ (Bremner, 1965). Prior to distillation, Devardas alloy was added for NO₃⁻ analysis. The resulting ammonium sulfate was dried and analyzed for N isotopes. A Fisons 1108 analyzer was connected to a Finnigan MAT 251 mass spectrometer. The reproducibility (2 SD) of the N isotope analysis of water samples was 0.2‰. Analysis of Ca²⁺, Mg²⁺ and SO₄²⁻ (AAS and HPLC) were performed according to Kram et al. (1997).

Peat was dried at 60 °C and homogenized. A 10-mg aliquot of each sample was placed in a tin capsule and combusted in a Fisons

1108 elemental analyzer at 1040 °C. Nitrogen and carbon concentrations in peat were determined with a reproducibility of 1.5 and 1.0%, respectively. The reproducibility of N isotope analysis of dried peat was 0.35‰.

The denitrification potential was determined by an evaluation of the marker genes of denitrifying bacteria. Three replicates of each soil sample (0.25 g) were taken for DNA extraction using Power Soil DNA Isolation kit (MoBio Laboratories Inc., Carlsbad, CA, U.S.A.) according to the manufacturer's instructions with some modifications. A mini Bead-Beater (BioSpec Products, Inc.), at a speed of 6 m s⁻¹ for 45 s, was used for better disruption of cell walls. DNA was stored in 1.5 mL Eppendorf microtubes in a freezer (-20 °C) until analyses. Quality of the extracted DNA was verified by electrophoresis (1% w/v, 8 V/cm, 45 min). Total DNA was quantified using SYBR Green (Leininger et al., 2006). The amount of total bacteria and denitrifiers was measured by qPCR targeting 16S rRNA, nirK and nirS genes (Barta et al., 2010). Briefly, qPCR was performed using SYBR Green as the detection system in a reaction mixture of 20 µL. Fluorescence was measured after each extension step. Melting curve and agarose electrophoresis (1.5% w/v, 110 V, 45 min) was performed for quality verification of the PCR product after each qPCR. Three independent qPCRs were performed for each gene and soil replicate. Standard curves were obtained with serial 10-fold dilutions of a known amount of PCR amplicon prepared from pure bacterial strains containing 16S rDNA, nirK and nirS genes, respectively. The detection limit of assays was determined as the standard deviation of the most diluted standard in the calibration curve. The lower limits of quantification were ≤10 gene copies per µL of DNA extract.

Statistical analysis of the geochemical data was performed using the PASW (Version 18) software by SPSS. ANOVA with LSD *post-hoc* tests was used for multiple comparisons of δ¹⁵N values. A General

Linear Model with LSD *post hoc* tests was used for comparisons of N₂O fluxes. Factorial ANOVA (site and soil depth as independent variable), followed by the Tukey HSD test was used to test the differences of 16S rRNA, nirK and nirS gene copies between soil depths of the two sampling sites (Statistica 10.0, StatSoft).

3. Results

3.1. Atmospheric deposition

In the hydrological year 2013, KB was more N-polluted than BS (Fig. 2). Total N_r deposition underneath spruce canopy was 19.3 kg ha⁻¹ at KB and 10.4 kg ha⁻¹ at BS. Total N_r deposition in an open area ("bulk") was 17.1 kg ha⁻¹ at KB and 12.5 kg ha⁻¹ at BS. According to Lamers et al. (2000), medium-polluted sites are characterized by a deposition of 6–18 kg N_r ha⁻¹ yr⁻¹, an input of more than 18 kg N_r ha⁻¹ yr⁻¹ is typical of highly polluted sites. In this classification, BS belongs to medium-polluted sites, KB is a medium-to-highly polluted site.

At KB, both throughfall and open-area deposition contained more NO₃⁻-N than NH₄⁺-N. At BS, NH₄⁺-N was more abundant in open-area deposition than in throughfall (Fig. 2).

3.2. Physical and chemical parameters of the studied bogs

Shallow peat from the depth of 0–2 cm below surface was compared with deeper peat, collected at the depth of 40–42 cm (Table 2). At both sites, bulk peat density was higher at the greater depth. Ash content was lower than 4 wt. %, except for the 40–42 cm depth at KB, indicating a minerotrophic base of the KB peat profile. The concentration of total N in bulk peat substrate was similar at both sites, and increased from the topmost peat layer to the depth of 40–42 cm, approximately three times. The C/N ratio was slightly lower at KB than at BS at the peat surface (57 vs. 61, respectively), but similar at the depth of 40–42 cm (20). The C/N ratio at the peat surface fell within the range of C/N ratios reported from other sites worldwide (30–120; see Wieder and Vitt, 2006 for review). The C/N ratio at the 40–42 cm depth was lower than values previously reported from other sites (Wieder and Vitt, 2006).

Relatively low pH values (4.0–4.8) and low Ca, Mg concentrations in bog porewater were typical of both sites (Table 2). BS had slightly more acidic porewater than KB, but less sulfate at the bog surface than KB. NO₃⁻ concentrations were higher in KB surface bog water (0.7 mg L⁻¹) than in BS surface water (0.4 mg L⁻¹). BS deeper (40 cm) porewater was richer in NO₃⁻ (0.9 mg L⁻¹) than KB deeper porewater (0.3 mg L⁻¹). NH₄⁺ concentrations in bog water were lower than 0.3 mg L⁻¹ in all studied samples.

3.3. δ¹⁵N of atmospheric deposition and peat substrate

Cumulative rainfall samples from a 1-month period were analyzed for N isotope composition (Table 3). All measured δ¹⁵N values were lower than -4.0‰. For a specific sample type (NH₄⁺, NO₃⁻; open-area deposition, throughfall), δ¹⁵N differed by less than 2.6‰ between the two sites. Across the sites, the δ¹⁵N-NH₄⁺ values (average of -11.3 and -12.3‰ for open-area deposition and throughfall, respectively) were lower than the δ¹⁵N-NO₃⁻ values (-6.9 and -4.5‰, respectively).

Across the sites, δ¹⁵N values of the peat substrate averaged -2.2‰ (Table 2). At both sites, peat N was isotopically slightly heavier at the depth of 40–42 cm than at the bog surface. There was no overlap between stable N isotope composition of atmospheric deposition (isotopically lighter), and peat substrate (isotopically heavier, but still negative on the δ¹⁵N scale).

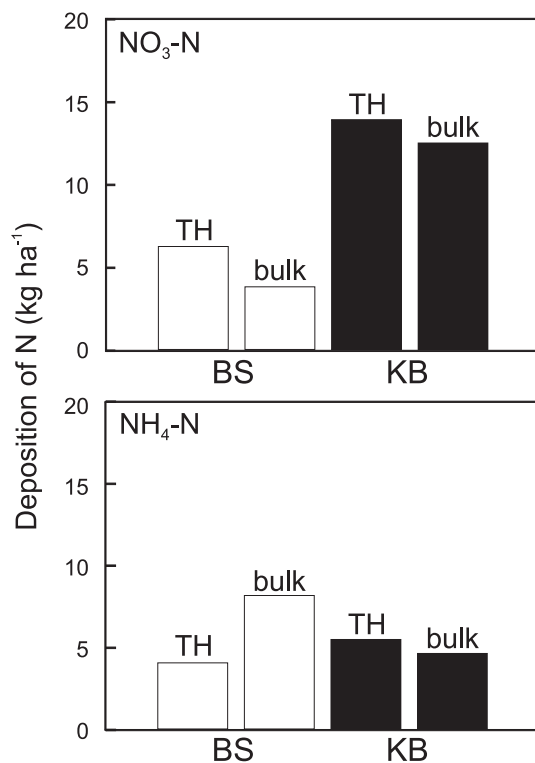


Fig. 2. Atmospheric deposition of reactive nitrogen in 2013. TH = spruce canopy throughfall, bulk = open-area deposition. Data are based on monthly cumulative rainfall sampling.

Table 2
Physical and chemical parameters of the bogs.

Site	Depth	Solid substrate						Bog water					
		Bulk density	Ash content	C content	N content	C/N	$\delta^{15}\text{N}_{\text{solid}}$	pH	NO_3^-	NH_4^+	Ca^{2+}	Mg^{2+}	SO_4^{2-}
		g cm^{-3}	wt. %	wt. %	wt. %		‰		mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}
BS	0 cm	0.23	2.2	46.3	0.76	61.0	-2.6	3.97 ± 0.020	0.37 ± 0.091	0.24 ± 0.022	3.07 ± 0.638	0.51 ± 0.103	0.97 ± 0.207
	40 cm	0.62	3.7	50.4	2.52	20.0	-1.6	4.15 ± 0.023	0.93 ± 0.089	0.16 ± 0.033	5.48 ± 0.645	0.99 ± 0.108	2.00 ± 0.146
KB	0 cm	0.30	2.0	45.3	0.79	57.4	-3.1	4.21 ± 0.055	0.70 ± 0.445	0.11 ± 0.003	2.66 ± 0.358	0.72 ± 0.133	2.90 ± 1.503
	40 cm	0.55	9.2	47.0	2.38	19.8	-1.4	4.75 ± 0.085	0.33 ± 0.081	0.09 ± 0.028	4.51 ± 0.557	2.15 ± 0.081	1.11 ± 0.363

3.4. N_2O fluxes across the bog – atmosphere interface

The two measurements of N_2O fluxes at the bog surfaces were carried out 7 days apart in an early summer season. The weather conditions were unique in that heavy rains, in lowlands accompanied by flash floods, preceded the N_2O flux measurements (Fig. S1). During the N_2O flux measurements, ambient air temperatures were high (up to 30 °C). The water table level in the bogs, however, remained very close to *Sphagnum capitata* during these hot days (June 12–20, 2013).

All measured N_2O fluxes at the bog – atmosphere interface were minute. A similar temporal trend was observed at both sites (Fig. 3). On day 1, a small emission $<0.03 \mu\text{mol N}_2\text{O m}^{-2} \text{h}^{-1}$ was recorded at both BS and KB. In contrast, on day 7, a consumption of atmospheric N_2O in the peat substrate was recorded at both sites (-13 and $-21 \mu\text{mol m}^{-2} \text{N}_2\text{O h}^{-1}$ at BS and KB, respectively). The consumption of N_2O in the waterlogged peat on day 7 was larger at KB than at BS ($p = 0.007$; Fig. 3). The magnitude of the consumption of atmospheric N_2O on day 7 was larger than the magnitude of N_2O release on day 1. Overall, both bogs were slight consumers of airborne N_2O .

3.5. Peat-porewater N_2O concentration and isotope profiles

N_2O concentrations were higher at the southwestern less polluted site BS (Fig. 4a). The highest N_2O concentrations at BS were recorded at a depth of 60 cm (140 ppm). N_2O concentrations at the more polluted northeastern site KB were below 20 ppm (Fig. 4a). N_2O concentrations in the deepest horizon at KB were 7 times lower, compared to BS. The peat porewater N_2O concentrations were up to 430 times higher than the present-day N_2O concentrations in the ambient air (324 ppb). They were also higher compared to N_2O concentrations in minerotrophic fens, upland forest soils and rice paddies (Goldberg et al., 2008, 2010; Berger et al., 2013a).

Vertical N_2O concentration profiles, as well as and $\delta^{15}\text{N}$ profiles, exhibited clear spatial trends (Fig. 4a,b). Between 10 and 60 cm below peat surface at both sites, N_2O concentrations in peat porewaters increased with increasing depth. $\delta^{15}\text{N}-\text{N}_2\text{O}$ values exhibited opposite vertical trends, relative to N_2O concentrations. With increasing depth, $\delta^{15}\text{N}-\text{N}_2\text{O}$ of peat porewaters decreased.

Across the sites, $\delta^{15}\text{N}-\text{N}_2\text{O}$ in porewaters ranged between -4.5 and $+4.5\text{‰}$. With one exception, $\delta^{15}\text{N}-\text{N}_2\text{O}$ values in BS porewaters were higher, compared to those at KB. The site with higher N_2O porewater concentrations (BS), also had higher $\delta^{15}\text{N}-\text{N}_2\text{O}$ values. A significant negative correlation between N_2O concentrations and $\delta^{15}\text{N}-\text{N}_2\text{O}$ values was observed at KB ($p = 0.036$; Fig. 4c). At BS, the correlation was close to significant ($p = 0.058$).

3.6. Comparison of $\delta^{15}\text{N}$ among sample types

Across the sites, porewater N_2O contained the isotopically heaviest N in the entire ecosystem. N in peat substrate had intermediate $\delta^{15}\text{N}$ values, while atmospheric deposition supplied the

isotopically lightest N ($\delta_{\text{N}_2\text{O}} > \delta_{\text{substrate}} > \delta_{\text{input}}$). Across the sites, the mean $\delta^{15}\text{N}$ values were $+1.4$, -2.2 , and -8.8‰ for porewater N_2O , peat substrate and atmospheric deposition, respectively ($p = 0.033$, $p = 0.0006$).

3.7. Denitrification potential

Denitrification potential was determined by estimating the *nirK* and *nirS* gene copy numbers. *NirK* denitrifiers were the dominant group of denitrifying bacteria comprising up to 55% of bacteria calculated as a relative proportion of *nirK* gene copies per 16S rRNA gene copies (Table S1). Both *nirK* and *nirS* denitrifiers were more abundant at BS, and in shallow peat (15–25 cm below surface; Fig. 5b, c).

4. Discussion

4.1. Nitrogen pollution at BS and KB

In 2013, N_r deposition in an open area was 1.4 times higher at the northeastern site KB than at the southwestern site BS (Fig. 2). Underneath spruce canopy, the difference was larger, with 1.9 times higher N_r input at KB than at BS. As seen in Fig. 4a, the N_2O concentrations in peat porewater were higher at BS, the site with lower present-day N_r input. That was counter-intuitive: higher N_r pollution should result in higher NO_3^- supply for biological nitrogen cycling. The previous history of atmospheric deposition of N_r in both regions could provide an additional insight. Fig. S2a gives a time-series of coal production in the northern Czech Republic for the past 110 years. Coal mining, coal burning in large thermal power plants, and NO_x emissions, peaked in 1987. Since then, a steady decrease in coal burning has been recorded. A historic perspective on total N_r emissions for the Czech Republic was published by Kopacek and Vesely (2005). The area in the north, received higher N_r inputs than the south, but the relative magnitude of N_r pollution near KB and BS remained unknown. In 1994, seven years after the peak N_r pollution, we started to monitor monthly atmospheric deposition of N_r in two small forested catchments in the vicinity of the studied peatlands: LIZ is situated near BS, while UDL is close to KB (Fig. 1; catchments of the GEOMON network). Fig. S2 gives cumulative N_r inputs at UDL and LIZ for the most recent 18 years (Fottova, 2013). This pollution time-series is consistent with Fig. 2, and confirms higher pollution at KB than at BS. The difference, however, appears larger than in Fig. 2: the cumulative N_r input, according to Fig. S2, was 3 times higher in the northeast than in the southwest of the Czech Republic. We conclude that even in the historical perspective, KB received higher N_r inputs, compared to BS.

4.2. N_r availability in peat

As seen in Table 2, bog water N_r concentrations were not always higher at the atmospherically more polluted site KB. N_r availability at the bog surface may be patchy. The main reasons are: (i) uneven evapotranspiration (Bragazza and Limpens, 2004); (ii) variable

Table 3
Isotope composition of reactive N species in atmospheric deposition.

Site	$\delta^{15}\text{N}$ (‰)			
	Open-area deposition		Spruce throughfall	
	NH_4^+	NO_3^-	NH_4^+	NO_3^-
BS	-10.4	-6.8	-11.1	-4.8
KB	-12.2	-7.0	-13.6	-4.2

biological activity, including floral and microbial N uptake and mineralization (Braker et al., 2011); (iii) lateral mixing of through-fall and open-area deposition (Novak et al., 2014). Another major control of N_r availability near the ombrotrophic peat surface is downward leaching and export of nitrogen under high pollution (Lamers et al., 2000; Novak et al., 2014). All these mechanisms may have contributed to nearly identical C/N ratios in solid peat at KB and BS, found at the 40 cm depth (Table 2).

4.3. Why was there higher N_2O production under lower N inputs?

Because higher N_2O concentrations (Fig. 4a) were observed at the less polluted site (BS), parameters other than external NO_3^- input must have controlled N_2O production. These parameters may have included different pH and different peat depths. As seen in Table 2, pH was lower at BS than at KB. Previous studies have shown that NO_3^- production via nitrification is extremely slow in acidic bogs (Limpens et al., 2006). If NO_3^- supply via nitrification played a major role, we would expect higher N_2O production at the less acidic site, KB. The opposite was true (Fig. 4a). pH was not likely the main control of the relative N_2O production at our study sites. We also note that the optimum pH for denitrification is 4.7–5.2 (Palmer et al., 2010). This again would point to better conditions for N_2O production at KB.

Obviously, abiotic factors alone cannot explain the higher N_2O concentrations in BS pore waters, relative to KB. Therefore, we determined denitrification potential as the abundance of two marker genes of denitrification, *nirK* and *nirS*. Both *nirK* and *nirS* denitrifiers were more abundant at BS than at KB at the 50–60 cm depth. The denitrification potential was approximately 50% higher at BS. *NirK* denitrifiers clearly dominated at both sites, being one order higher than *nirS* denitrifiers. This contrasts with recent estimates of these groups in acidic peat and spruce forest soils (Barta et al., 2010; Palmer et al., 2012) where *nirS* denitrifiers dominated. *NirK* and *nirS* denitrifiers respond differently to changes in available nutrients,

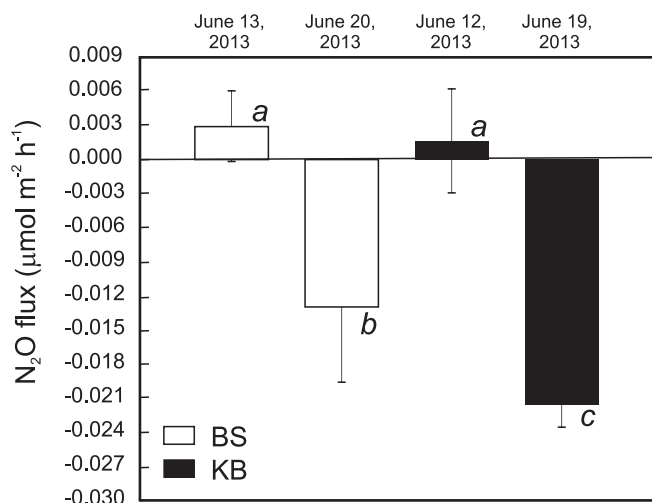


Fig. 3. N_2O fluxes across the peat – atmosphere boundary.

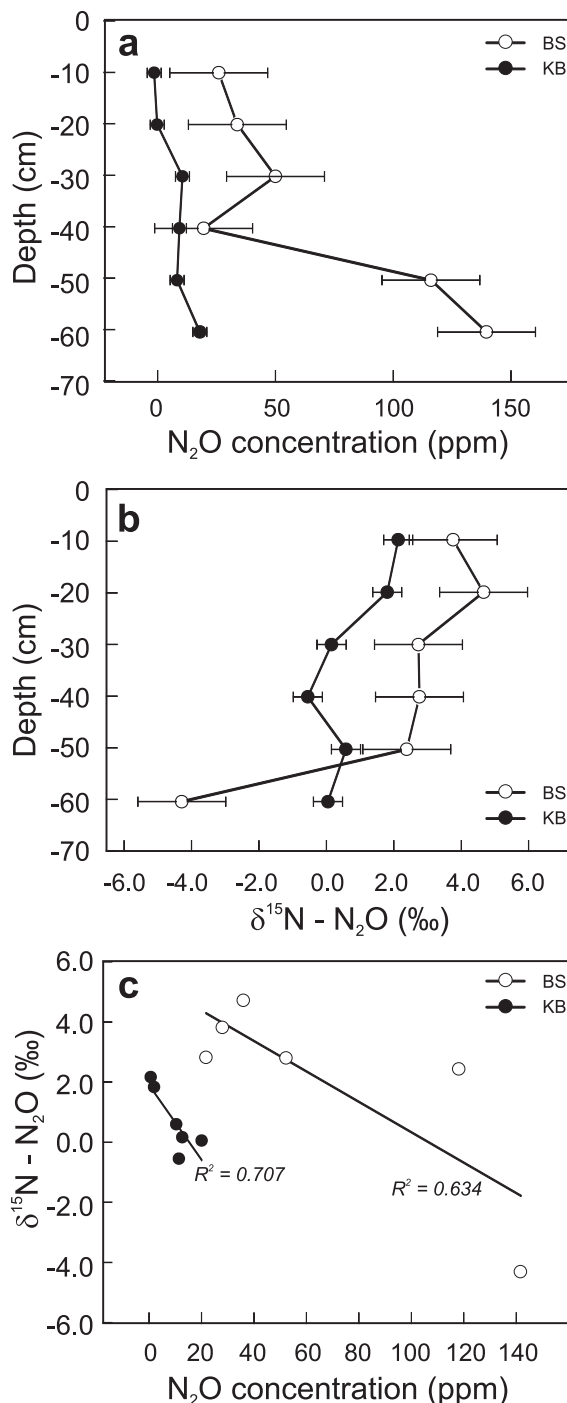


Fig. 4. Vertical N_2O profiles in peat porewaters. (a) N_2O concentrations, (b) $\delta^{15}\text{N}$ of porewater N_2O , (c) negative correlations between N_2O concentrations and $\delta^{15}\text{N}$. BS, KB – peat bogs.

and therefore their proportion can change seasonally, according to a changing input of available C, N and P. *NirK* denitrifiers have higher affinity to NO_3^- , therefore they can efficiently and rapidly transform it to N_2O (Parsonage et al., 1985). Moreover, relative emissions of N_2O versus N_2 might increase when nitrate concentrations are periodically elevated (Palmer et al., 2010). Therefore, both the dominance of *nirK* denitrifiers and higher concentration of NO_3^- could lead to higher N_2O concentration at BS.

Previous studies have indicated that low C/N ratios of around 20 are associated with maximum N_2O production (Maljanen et al.,

2012). Such C/N ratios are typical of the depth of 40–42 cm below peat surface at both BS and KB (Table 2). At BS, more than 6 m of peat accumulated below such depth. In contrast, at KB, there was no peat deeper than 60 cm. Previous studies have shown that at depths greater than 40–50 cm, peat is progressively depleted in labile organic C, needed for microbial respiration (Schlesinger, 2005). Further downcore, N₂O production decreases. If BS and KB are compared (Tables 1 and 2), contrasting total peat depth is the most striking difference. We suggest that a contribution of N₂O from peat strata deeper than 60 cm at BS caused that this site was richer in N₂O along shallow porewater profiles. In contrast to our sites, C/N ratios at pristine bogs are higher than 30 (Wieder and Vitt, 2006); their N₂O porewater concentration profiles have not been reported.

4.4. N isotope effect of denitrification

Laboratory and field studies have indicated that N₂O production in soils and waters can result in large N isotope fractionations: The

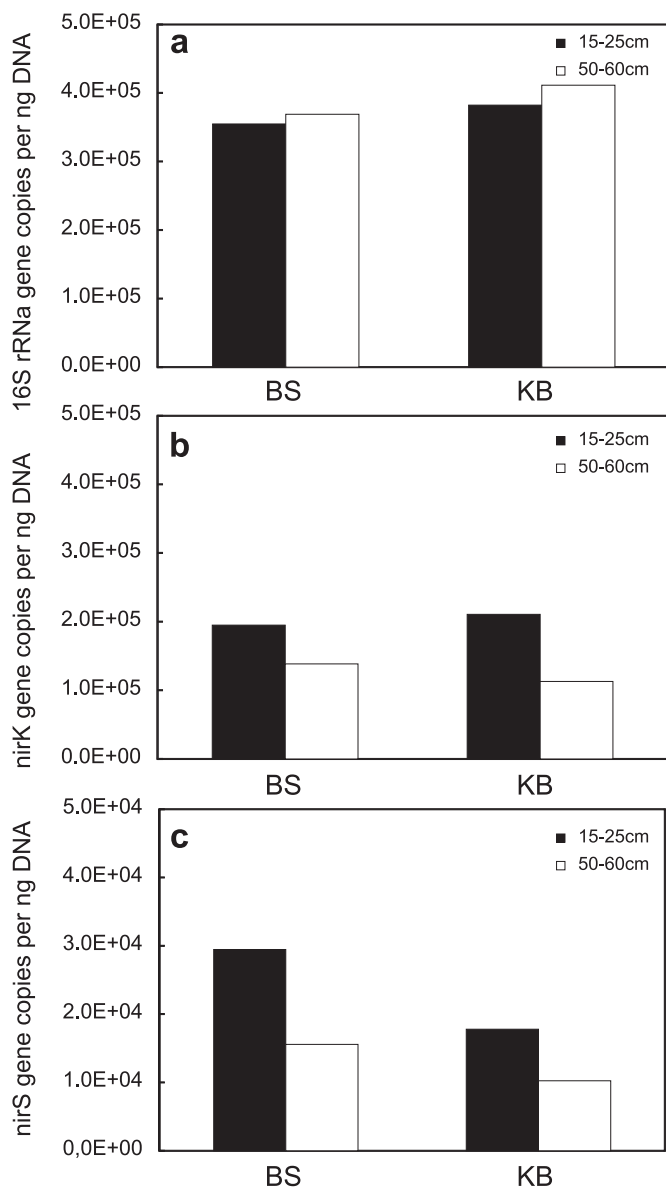


Fig. 5. Comparison of abundances of bacteria (a), *nirK* (b) and *nirS* (c) denitrifiers in two different soil depths (15–25 cm, 50–60 cm) at BS and KB.

isotope enrichment factor of denitrification varies between -9 and -30% (Hobbie and Ouimette, 2009; Snider et al., in press). In our case, the NO₃⁻ may come either from atmospheric deposition, or from mineralization of organically-bound N (Kalbitz and Geyer, 2002). The latter source of NO₃⁻ is believed to be relatively small at ombrotrophic sites, while its N isotope signature may or may not be shifted relative to organic N (Nadelhoffer and Fry, 1988). Based on $\delta^{15}\text{N}$ data for atmospheric deposition and peat substrate (Tables 2 and 3), and using the magnitude of the N isotope effect of N₂O production via denitrification from the literature (Hobbie and Ouimette, 2009; Snider et al., in press), we would expect $\delta^{15}\text{N}$ of freshly formed N₂O to be lower than -11% . In actual fact, the mean $\delta^{15}\text{N}$ -N₂O value across the study sites was 1.4% , and never became lower than -4.5% . We conclude that none of the porewater gas samples (Fig. 4b) contained unaltered, freshly formed N₂O. All the analyzed gas samples contained residual N₂O following partial N₂O consumption. This conclusion is corroborated by the negative N₂O isotope – concentration relationship in Fig. 4c. During denitrification, small to negligible N isotope fractionation might theoretically be possible in the case of a substrate excess, and a high N₂O formation rate (Søvik and Mørkved, 2008). Because $\delta^{15}\text{N}$ of both potential sources of NO₃⁻ were lower than $\delta^{15}\text{N}$ -N₂O, we rule out this scenario.

4.5. The depth of N₂O consumption in peat

Goldberg et al. (2008) argued that the lowest $\delta^{15}\text{N}$ -N₂O values, accompanying the highest N₂O concentrations along vertical soil profiles, mark the layer of maximum N₂O production by denitrification. In a minerotrophic fen, these authors observed such coupled peaks at a depth of 30–50 cm. Both above and below this depth interval, N₂O concentrations generally decreased, and $\delta^{15}\text{N}$ -N₂O values increased. Both opposite trends resulted from a single process: biological consumption of N₂O. Nitrogen in the N₂O found upcore and downcore from the horizon of maximum N₂O production was an isotopically heavier residuum following N₂O reduction to N₂. In the current study, we suggest the same interpretation for patterns in the uppermost peat layers, shallower than 60 cm. With a decreasing peat depth, the lower N₂O concentrations and the higher $\delta^{15}\text{N}$ values result from advancing N₂O consumption. Upward diffusion of N₂O from the depths of maximum denitrification rates cannot *per se* explain the patterns seen in Fig. 4a,b. The process of diffusion discriminates against the heavy isotope ¹⁵N, resulting in progressively lower $\delta^{15}\text{N}$ -N₂O upcore from the main N₂O production zone (Goldberg et al., 2008). At the bottom of the sampled profiles, we did not reach the depths of decreasing N₂O concentrations and increasing $\delta^{15}\text{N}$ values that would be similar to Goldberg et al. (2008).

Somewhat higher $\delta^{15}\text{N}$ -N₂O values in BS peat profiles, compared to KB peat profiles (Fig. 4b), may indicate a higher percentage of consumed N₂O at BS. However, fractionation factors for both N₂O production and consumption often vary in time and place. The fractionation factors may have primarily differed between BS and KB (Perez, 2005). A straightforward interpretation of the found difference between $\delta^{15}\text{N}$ -N₂O at BS and KB (Fig. 4b) is thus hampered by the limitations of our sampling methodology: $\delta^{15}\text{N}$ values were known neither for the substrate (NO₃⁻) during N₂O production, nor for the product of N₂O consumption (N₂).

4.6. Comparison of $\delta^{15}\text{N}$ -N₂O in ombrotrophic and minerotrophic peatlands

In a minerotrophic fen, Goldberg et al. (2008) found porewater $\delta^{15}\text{N}$ -N₂O values between -10 and -20% . The mean $\delta^{15}\text{N}$ -N₂O value was close to -15% . In contrast, ombrotrophic bogs in the current study, exhibited a mean $\delta^{15}\text{N}$ values of porewater N₂O of 1.4% . Similar to the BS vs. KB comparison discussed above, we

cannot directly assess the relative proportion of consumed N_2O between the Czech sites (this study) and the German sites (Goldberg et al., 2008, 2010), *i.e.*, between bogs and fens. If we assumed similar field-based δ values for N_2O reduction in both studies, then the percentage of consumed porewater N_2O would be larger in N-rich ombrotrophic bogs, compared to N-rich minerotrophic fens.

4.7. N_2O emissions

Porewater N_2O concentrations in this study were much higher, compared to minerotrophic fens (Goldberg et al., 2008, 2010). The porewater N_2O concentrations at BS were up to 800 and 400 times higher than those at Schloppnerbrunnen (Germany). Porewater N_2O concentrations at KB were up to 110 and 55 times higher than those at the German sites (Goldberg et al., 2008, 2010). Despite that, N_2O fluxes across the bog – atmosphere boundary at BS and KB were minute. This finding agrees well with previous studies (Maljanen et al., 2012), reporting N_2O emission rates for individual soil types in the order agricultural soils > drained and rewetted peatlands > intact fens > intact bogs. Unvegetated wet soils generally exhibit higher N_2O emissions, while N_2O emissions from bare peat are negligible (Marushchak et al., 2011). Our measurements took place during the growing season. Emissions outside of the growing season may contribute less than 40% to the annual N_2O fluxes from wetlands (Maljanen et al., 2012). It is thus unlikely that winter-time N_2O fluxes at our sites exceed those in Fig. 3. Regional N_2O emissions of $0.3 \text{ g m}^{-2} \text{ yr}^{-1}$ were estimated for Nordic wetlands (Maljanen et al., 2010). The net warming potential of the combined fluxes of greenhouse gases (CO_2 , CH_4 , N_2O) in the Nordic countries is negative, because the wetland soils are still a net sink for CO_2 . Mountain-top bogs in the Czech Republic are probably also a net sink for the combined greenhouse gases (see, *e.g.*, CH_4 emission rates in Bohdalkova et al., 2013). Along north–south transects across Europe, with an increasing temperature, N_2O emissions from wetlands tend to increase (Danevcic et al., 2010). In Central Europe, low N_2O emissions were reported from Polish wetlands ($0.004 \text{ g m}^{-2} \text{ yr}^{-2}$). These emissions did not correlate with any measured site-specific parameters (Juszczak and Augustin, 2013). N_2O emissions in northern Europe correlated neither with the age of land use change, nor with peat bulk density (Maljanen et al., 2010).

Our sites were medium- to highly N_r polluted (*sensu* Lamers et al., 2000). The highest deposition flux of $38 \text{ kg N}_r \text{ ha}^{-1} \text{ yr}^{-1}$ was measured in the 1990s near KB (Fottova, 2013). We estimate that in the preceding years of peak industrial pollution (*ca.* 1987; Fig. S2a) the deposition fluxes probably exceeded $50 \text{ kg N}_r \text{ ha}^{-1} \text{ yr}^{-1}$. Several field manipulations studies have shown that very high additions of N_r to an ombrotrophic peatland ($30\text{--}56 \text{ kg ha}^{-1} \text{ yr}^{-1}$) did not lead to elevated N_2O emissions (Sheppard et al., 2013). Our data from intact, N_r -polluted bogs are consistent with these findings.

4.8. N_2O source/sink relationships at the bog – atmosphere interface

It has been shown previously that the same microbial communities are capable of generating N_2O , but also consuming N_2O (Palmer et al., 2010). Within 7 days, our study sites turned from very small N_2O emitters to very small N_2O consumers ($p < 0.05$). Removal of N_2O from the atmosphere by an ecosystem, and its consumption, have been reported from a number of other sites and soil types (Limpen et al., 2006). We currently do not know whether switching from N_2O production to N_2O consumption at BS and KB were linked to changes in the weather. Following heavy rains and

unseasonably low temperatures (daily maximum of $6 \text{ }^\circ\text{C}$), day 7 was much warmer ($30 \text{ }^\circ\text{C}$). Warmer, drier conditions accompanied scavenging of air-borne N_2O by the peat on day 7, compared to day 1 and the previous weeks. In general, an increasing $\text{N}_2/\text{N}_2\text{O}$ ratio during denitrification can result from decreasing O_2 availability and increasing temperature (Firestone and Davidson, 1989). In response to warmer air, the temperature of bog waters changes only to the depth of a few cm below water table (*i.e.*, temperature does not affect the deepest layers under study). If consumption of atmospheric N_2O by the wetland was, indeed, triggered off by warmer weather, N_2 production must have occurred in very shallow porewaters (0–5 cm below the water table).

4.9. Linkage between N and S cycling in the wetlands

In anaerobic sediments, the energy yield of nitrate reduction is higher than that of sulfate reduction. Accordingly, in a water-logged vertical peat profile, nitrate reduction should proceed at shallower depths and/or before sulfate reduction (Schlesinger, 2005). However, it is common that various metabolic pathways in microorganisms co-exist, due to a spatial variation in the abundance of the terminal electron acceptors (Schlesinger, 2005, and references therein). In our study, we found lower N_2O concentrations in peat in the northeast, a region known for more than 10 times higher historical S inputs compared to the southwest (Novak et al., 2007). Since the availability of substrates was not quantified in our study, competition for substrates cannot be invoked as an explanation for the observed lower N_2O abundance accompanying higher SO_4^{2-} inputs in the north. Clearly, the linkage between N and S cycling in stressed wetlands merits further study.

5. Conclusions

Denitrification is the largest environmental source of N_2O . In ombrotrophic peatlands, nitrate, the substrate for denitrifiers, can be supplied by three different processes: nitrification of NH_4^+ , N-fixation, and atmospheric deposition. The first process is unimportant in acidic conditions because nitrifiers are poor competitors for ammonium (Firestone and Davidson, 1989). The second process remains to be poorly quantified (Vile et al., *in press*). The third process, atmospheric deposition, may become an important NO_3^- supplier to bogs located in industrial regions. We present the first porewater N_2O concentration and isotope data for N-polluted ombrotrophic bogs. We hypothesized that sizeable atmospheric NO_3^- input into the bogs may result in relatively large N_2O emissions (Maljanen et al., 2012). Compared to a fen with a changing water table level (Goldberg et al., 2008), our polluted ombrotrophic site BS had 400 times higher N_2O concentrations in peat porewater. High inputs of atmospheric NO_3^- augmented N_2O production in ombrotrophic bogs. Downward mobility of NO_3^- in peat substrate (Novak et al., 2014) may have also contributed to the higher N_2O production. The depth of maximum N_2O production in bogs (30–60 cm) was similar to fens and rice paddies (Berger et al., 2013a). Part of the produced N_2O was reduced to N_2 , as documented by increasing $\delta^{15}\text{N}\text{--}\text{N}_2\text{O}$ values and lower N_2O concentrations upcore. A high degree of biological N_2O consumption in the peat was further corroborated by: (i) very low measured N_2O emissions, (ii) N isotope systematics; the found N_2O was enriched in the heavy isotope ^{15}N relative to both atmospheric deposition and solid peat, and (iii) high denitrification potential of both bogs. High- ^{15}N nitrous oxide at our sites can only be a residue of advanced N_2O reduction. We conclude that N pollution of ombrotrophic bogs in the temperate zone does not significantly contribute to climatic warming, due to efficient N_2O reduction in peat substrate, and low N_2O emissions. Even N-polluted bogs may

scavenge air-borne N₂O and reduce it to N₂ with no warming potential.

Acknowledgment

This study was supported by the Czech Science Foundation (Grant no. P504/12/1782 to M.N.). Skillful technical assistance in N₂O isotope abundance and concentration analysis by Isolde Baumann (BayCEER – Laboratory of Isotope Biogeochemistry, University of Bayreuth) is gratefully acknowledged. We thank Drs. Ivana Buřkova, Alžbeta Cejkova, Vladimír Cernohous and Jitka Richterová for scientific advice.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2014.10.021>.

References

- Anderj, J., Borjesson, G., Hallin, S., 2012. Temporal changes in methane oxidizing and denitrifying communities and their activities in a drained peat soil. *Wetlands* 32 (6), 1047–1055.
- Barta, J., Melichova, T., Vanek, D., Picek, T., Santruckova, H., 2010. Effect of pH and dissolved organic matter on the abundance of nirK and nirS denitrifiers in spruce forest soil. *Biogeochemistry* 101, 123–132.
- Berger, S., Jang, I., Seo, J., Kang, H., Gebauer, G., 2013a. A record of N₂O and CH₄ emissions and underlying soil processes of Korean rice paddies as affected by different water management practices. *Biogeochemistry* 115, 317–332.
- Berger, S., Jung, E., Köpp, J., Kang, H., Gebauer, G., 2013b. Monsoon rains, drought periods and soil texture as drivers of soil N₂O fluxes – soil drought turns East Asian temperate deciduous forest soils into temporary and unexpectedly persistent N₂O sinks. *Soil Biology & Biochemistry* 57, 273–281.
- Billings, S., 2008. Nitrous oxide in flux. *Nature* 456 (18), 888–889.
- Bohdalkova, L., Novak, M., Stepanova, M., Fottova, D., Chrastny, V., Mikova, J., Kubena, A.A., 2014. The fate of atmospherically derived Pb in central European catchments: insights from spatial and temporal pollution gradients and Pb isotope ratios. *Environmental Science and Technology* 48, 4336–4343.
- Bohdalkova, L., Curik, J., Kubena, A.A., Buzek, F., 2013. Dynamics of methane fluxes from two peat bogs in the Ore Mountains, Czech Republic. *Plant Soil and Environment* 59 (1), 14–21.
- Bragazza, L., Limpens, J., 2004. Dissolved organic nitrogen dominates in European bogs under increasing atmospheric N deposition. *Global Biogeochemical Cycles* 18 (4), GB4018.
- Braker, G., Dörsch, P., Bakken, L.R., 2011. Genetic characterization of denitrifier communities with contrasting intrinsic functional traits. *Federation of European Microbiological Societies* 79, 542–554.
- Bremner, J.M., 1965. Inorganic forms of nitrogen. In: Black, C.A. (Ed.), *Methods of Soils Analysis, Part 2, Agronomy*, vol. 9. American Society of Agronomy, Madison, WI, pp. 1179–1237.
- Buřkova, I., Štíbal, F., Mikulaskova, E., 2010. Restoration of drained mires in the Sumava National Park, Czech Republic. In: Eiseltova, M. (Ed.), *Restoration of Lakes, Streams, Floodplains, and Bogs in Europe: Principles and Case Studies*. Springer Verlag, pp. 331–354.
- Chapuis-Lardy, L., Wrage, N., Metay, A., Chotte, J.-L., Bernoux, M., 2007. Soil, a sink for N₂O? A review. *Global Change Biology* 13, 1–17.
- Danevcic, T., Mandic-Mulec, I., Stres, B., Stopar, D., Hacin, J., 2010. Emissions of CO₂, CH₄ and N₂O from Southern European peatlands. *Soil Biology & Biochemistry* 42 (9), 1437–1446.
- Davidson, E.A., 1991. Fluxes of nitrous oxide and nitric oxide from terrestrial ecosystems. In: Rogers, J., Whitman, W. (Eds.), *Microbial Production and Consumption of Greenhouse Gases: Methane, Nitrogen Oxides and Halomethanes*. American Society for Microbiology, Washington, DC, pp. 219–235.
- Dohnal, Z., Kunst, M., Mestrik, V., Raucina, S., Vydra, V., 1965. *Czechoslovak Peatlands*. Czechoslovak Academy of Sciences, Czechoslovakia (in Czech).
- Erbanova, L., Novak, M., Fottova, D., Dousova, B., 2008. Export of arsenic from forested catchments under easing atmospheric pollution. *Environmental Science and Technology* 42 (19), 7187–7192.
- Firestone, M.K., Davidson, E.A., 1989. Microbiological basis of NO and N₂O production and consumption in soil. In: Andraea, M.O., Schimml, D.S. (Eds.), *Exchange of Trace Gases Between Terrestrial Ecosystems and the Atmosphere*. John Wiley & Sons Ltd., S. Bernhard, Dahlem Konferenzen, pp. 7–21.
- Fottova, D., 2013. GEOMON Hydrochemical Database. Czech Geological Survey.
- Frolking, S., Talbot, J., Jones, M.C., Treat, C.C., Kauffman, J.B., Tuittila, E.S., Roulet, N., 2011. Peatlands in the Earth's 21st century climate system. *Environmental Reviews* 19, 371–396.
- Goldberg, S.D., Knorr, K.-H., Blodau, Ch., Lischeid, G., Gebauer, G., 2010. 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.
- Goldberg, S.D., Gebauer, G., 2009. Drought turns a Central European Norway spruce forest soil from an N₂O source to a transient N₂O sink. *Global Change Biology* 15, 850–860.
- Goldberg, S.D., Knorr, K.-H., Gebauer, G., 2008. N₂O concentration and isotope signature along profiles provide deeper insight into the fate of N₂O in soils. *Isotopes in Environmental and Health Studies* 44 (4), 377–391.
- Heil, J., Wolf, B., Brüggemann, N., Emmenegger, L., Tuzson, B., Vereecken, H., Mohn, J., 2014. Site-specific ¹⁵N isotopic signatures of abiotically produced N₂O. *Geochimica et Cosmochimica Acta* 139, 72–82.
- Hobbie, E.A., Ouimette, A.P., 2009. Controls of nitrogen isotope patterns in soil profiles. *Biogeochemistry* 95, 355–371.
- Holtan-Hartwig, L., Dörsch, P., Bakken, L.R., 2000. Comparison of denitrifying communities in organic soils: kinetics of NO₃⁻ and N₂O reduction. *Soil Biology & Biochemistry* 32, 833–843.
- IPCC: Intergovernmental Panel on Climate Change, Fifth assessment Report (AR5), 2014.
- Juszczak, R., Augustin, J., 2013. Exchange of the greenhouse gases methane and nitrous oxide between the atmosphere and a temperate peatland in Central Europe. *Wetlands* 33, 895–907.
- Kalbitz, K., Geyer, S., 2002. Different effects of peat degradation on dissolved organic carbon and nitrogen. *Organic Geochemistry* 33, 319–326.
- Kohzu, A., Matsui, K., Yamada, T., Sugimoto, A., Fujita, N., 2003. Significance of rooting depth in mire plants: evidence from natural ¹⁵N abundance. *Ecological Research* 18 (3), 257–266.
- Kopacek, J., Vesely, J., 2005. Sulfur and nitrogen emissions in the Czech Republic and Slovakia from 1850 till 2000. *Atmospheric Environment* 39, 2179–2188.
- Kram, P., Hruska, J., Wenner, B.S., Driscoll, Ch.T., Johnson, Ch.E., 1997. The biogeochemistry of basic cations on two forest catchments with contrasting lithology in the Czech Republic. *Biogeochemistry* 37, 173–202.
- Lamers, L.P.M., Bobbink, R., Roelofs, J.G.M., 2000. Natural nitrogen filter fails in polluted raised bogs. *Global Change Biology* 6 (5), 583–586.
- Leininger, S., Ulrich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G.W., Posser, J.J., Schuster, S.C., Schleper, C., 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* 442, 806–809.
- Limpens, J., Heijmans, M.P.D., Berendse, F., 2006. The nitrogen cycle in boreal peatlands. In: Wieder, R.K., Vitt, D.H. (Eds.), *Boreal Peatland Ecosystems*. Springer – Verlag, Berlin, pp. 195–230.
- Maljanen, M., Shurpali, N., Hytonen, J., Makiranta, P., Aro, L., Potila, H., Laine, J., Li, C.S., Martikainen, P.J., 2012. Afforestation does not necessarily reduce nitrous oxide emissions from managed boreal peat soils. *Biogeochemistry* 108, 1–3, 199–218.
- Maljanen, M., Sigurdsson, B.D., Guðmundsson, J., Óskarsson, H., Huttunen, J.T., Martikainen, P.J., 2010. Greenhouse gas balances of managed peatlands in the Nordic countries – present knowledge and gaps. *Biogeosciences* 7, 2711–2738.
- Mariotti, A., Germon, J.C., Hubert, P., Kaiser, P., Letolle, R., Tardieu, X., Tardieu, P., 1981. Experimental determination of nitrogen kinetic isotope fractionation: some principles; illustration for the denitrification and nitrification process. *Plant and Soil* 62, 413–430.
- Marushchak, M.E., Pitkämäki, A., Koponen, H., Biasi, C., Seppälä, M., Martikainen, P.J., 2011. Hot spots for nitrous oxide emissions found in different types of permafrost peatlands. *Global Change Biology* 17, 2601–2614.
- Moore, T.R., 1994. Trace gas emissions from Canadian peatlands and the effect of climate change. *Wetlands* 14, 223–227.
- Mørkved, P.T., Dörsch, P., Bakken, L.R., 2007. The N₂O product ratio of nitrification and its dependence on long-term changes in soil pH. *Soil Biology & Biochemistry* 39, 2048–2057.
- Mørkved, P.T., Dörsch, P., Henriksen, T.M., Bakken, L.R., 2006. N₂O emissions and product ratios of nitrification and denitrification as affected by freezing and thawing. *Soil Biology & Biochemistry* 38, 3411–3420.
- Morley, N., Baggs, E.M., Dörsch, P., Bakken, L., 2008. Production of NO, N₂O and N₂ by extracted soil bacteria, regulation by NO₃⁻ and O₂ concentrations. *Federation of European Microbiological Societies* 65, 102–112.
- Nadelhoffer, K.J., Fry, B., 1988. Controls on natural nitrogen-15 and carbon-13 abundances in forest soil organic matter. *Soil Science Society of America Journal* 52, 1633–1640.
- Novak, M., Stepanova, M., Jackova, I., Vile, M.A., Wieder, R.K., Buzek, F., Adamova, M., Erbanova, L., Fottova, D., Komarek, A., 2014. Isotopic evidence for nitrogen mobility in peat bogs. *Geochimica et Cosmochimica Acta* 133, 351–361.
- Novak, M., Mitchell, M.J., Jackova, I., Buzek, F., Schweigstilova, J., Erbanova, L., Prikryl, R., Fottova, D., 2007. Processes affecting oxygen isotope ratios of atmospheric and ecosystem sulfate in two contrasting forest catchments in Central Europe. *Environmental Science and Technology* 41, 703–709.
- Novak, M., Buzek, F., Harrison, A.F., Prechova, E., Jackova, I., Fottova, D., 2003. Similarity between C, N and S stable isotope profiles in European spruce forest soils: implications for the use of δ³⁴S as a tracer. *Applied Geochemistry* 18, 765–779.
- Novak, M., Buzek, F., Adamova, M., 1999. Vertical trends in δ¹³C, δ¹⁵N and δ³⁴S ratios in bulk *Sphagnum* peat. *Soil Biology and Biochemistry* 31, 1343–1346.
- Oulehle, F., McDowell, W.H., Aitkenhead-Peterson, J.A., Kram, P., Hruska, J., Navratil, T., Buzek, F., Fottova, D., 2008. Long-term trends in stream nitrate concentrations and losses across watersheds undergoing recovery from acidification in the Czech Republic. *Ecosystems* 11 (3), 410–425.
- Palmer, K., Biasi, C., Horn, M.A., 2012. Contrasting denitrifier communities relate to contrasting N₂O emission patterns from acidic peat soils in arctic tundra. *ISME Journal* 6 (5), 1058–1077.

- Palmer, K., Drake, H.L., Horn, M.A., 2010. Association of novel and highly diverse acid-tolerant denitrifiers with N₂O fluxes of an acidic fen. *Applied and Environmental Microbiology* 76 (4), 1125–1134.
- Parsonage, D., Greenfields, A.J., Ferguson, S.J., 1985. The high-affinity of paracoccus-denitrificans cells for nitrate as an electron-acceptor-analysis of possible mechanisms of nitrate and nitrite movement across the plasma-membrane and the basis for inhibition by added nitrite of oxidase activity in permeabilized cells. *Biochimica et Biophysica Acta* 807, 81–95.
- Perez, T., 2005. Factors that control the isotopic composition of N₂O from soil emissions. In: Flanagan, L.B., Ehleringen, J.R., Pataki, D.E. (Eds.), *Stable Isotopes and Biosphere-Atmosphere Interactions: Processes and Biological Controls*. Elsevier Academic Press, San Diego, pp. 69–84.
- Ravishankara, A.R., Daniel, J.S., Portmann, R.W., 2009. Nitrous oxide (N₂O): the dominant ozone-depleting substance emitted in the 21st century. *Science* 326, 123–125.
- Schlesinger, W.H., 2005. *Biogeochemistry: Treatise on Geochemistry*, vol. 8. Elsevier.
- Sheppard, L.J., Leith, I.D., Leeson, S.R., van Dijk, N., Field, C., Levy, P., 2013. Fate of N in a peatland, Whim bog: immobilisation in the vegetation and peat, leakage into pore water and losses as N₂O depend on the form of N. *Biogeosciences* 10, 149–160.
- Snider, D.M., Venkiteswaran, J.J., Schiff, S.L., Spoelstra, J., 2014. The stable isotope composition of nitrous oxide produced during denitrification in agricultural soils and stream sediment. *Geochimica et Cosmochimica Acta* (in press).
- Søvik, A.K., Mørkved, P.T., 2008. Use of stable nitrogen isotope fractionation to estimate denitrification in small constructed wetlands treating agricultural runoff. *Science of the Total Environment* 392, 157–165.
- Velthof, G.L., van Groenigen, J.W., Gebauer, G., Pietrzak, S., Jarvis, S.C., Pinto, M., Corré, W., Oenema, O., 2000. Temporal stability of spatial patterns of nitrous oxide fluxes from sloping grassland. *Journal of Environmental Quality* 29, 1397–1407.
- Vile, M.A., Wieder, R.K., Zivkovic, T., 2014. N₂-fixation by methanotrops sustains carbon and nitrogen accumulation in pristine peatlands. *Biogeochemistry*. <http://dx.doi.org/10.1007/s10533-014-0019-6> in press.
- Voldrichova, P., Chrastny, V., Sipkova, A., Farkas, J., Novak, M., Stepanova, M., Krachler, M., Veselovsky, F., Blaha, V., Prechova, E., Komarek, A., Bohdalkova, L., Curik, J., Mikova, J., Erbanova, L., Pacheroova, P., 2014. Zinc isotope systematic in snow and ice accretions in Central European mountains. *Chemical Geology* 388, 130–141.
- Wieder, R.K., Vitt, D.H. (Eds.), 2006. *Boreal Peatland Ecosystems*. Ecological Studies 188. Springer-Verlag, Berlin.
- Zhu, J., Mulder, J., Bakken, L., Dörsch, P., 2013. The importance of denitrification for N₂O emissions from an N-saturated forest in SW China: results from in situ ¹⁵N labeling experiments. *Biogeochemistry* 116, 103–117.