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

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ABSTRACT

Measurements of nitrogen isotope ratios (\(\delta^{15}N\)) along vertical porewater profiles are instrumental in localizing sources and sinks of nitrous oxide (N\(_2\)O) in wetland ecosystems. We present the first N\(_2\)O 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 (NO\(_3\)) inputs in rain-fed bogs may cause sizeable N\(_2\)O emissions. Porewater N\(_2\)O concentrations in the studied ombrotrophic bogs were 400–800 times higher, compared to fens in nearby Bavaria (Germany). The highest N\(_2\)O concentrations and the lowest \(\delta^{15}N\)–N\(_2\)O values were found at the bottom of the sampled porewater profiles, 60–80 cm below surface. Low \(\delta^{15}N\)–N\(_2\)O 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 N\(_2\)O concentrations than KB, possibly due to a higher denitrification potential determined as abundance of nirK and nirS genes. Upcore, N\(_2\)O abundance decreased and \(\delta^{15}N\)–N\(_2\)O increased, indicating N\(_2\)O reduction. Processes of N\(_2\)O formation and consumption at different depths were integrated by measuring N\(_2\)O fluxes across the peat — atmosphere interface. These fluxes were minute (<0.02 \(\mu\)mol N\(_2\)O m\(^{-2}\) h\(^{-1}\)). We even observed scavenging of air-borne N\(_2\)O by the bog substrate. Nitrogen pollution of ombrotrophic bogs led to vigorous N cycling, manifested by extremely high peat porewater N\(_2\)O concentrations. However, effective in-situ consumption of the produced N\(_2\)O greatly reduced its warming potential.

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1. Introduction

Nitrous oxide (N\(_2\)O) is a potent greenhouse gas and a major ozone-depleting substance (Ravishankara et al., 2009). At present, N\(_2\)O concentration in the atmosphere is 324 ppb, 18% higher compared to pre-industrial times (Heil et al., 2014). N\(_2\)O contributes about 6% to the global warming potential, surpassed only by carbon dioxide (CO\(_2\); 63%) and methane (CH\(_4\); 18%; Froliking et al., 2011). Increased production of N\(_2\)O by soil microorganisms is a consequence of a higher release of reactive nitrogen (nitrate, NO\(_3\), and ammonium, NH\(_4\)) 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 N\(_2\) (Billings, 2008). The two main biological processes that produce N\(_2\)O are nitrification and denitrification. High rates of N\(_2\)O production are more commonly associated with denitrification (Zhu et al., 2013). During denitrification, nitrate (NO\(_3\)) or nitrite (NO\(_2\)) are reduced to the gaseous nitrogen species, NO, N\(_2\)O, and N\(_2\). At the cellular level, denitrification rates are controlled by the availability of NO\(_3\), labile organic carbon (C), and molecular oxygen (O\(_2\)). For microbial respiration, organic matter serves as an electron donor.
For microbial respiratory NO₃ reduction, anoxic conditions are required (Firestone and Davidson, 1989). However, simultaneous respiration of O₂ and NO₃ has been observed in oxygenated soils following anoxic periods (Morley et al., 2008).

The amount of N₂O 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 N₂O and NO (Holttan-Hartwig et al., 2000). Once N₂O reaches the atmosphere, its mean residence time is relatively long (114 years; Frolking 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, N₂, has no warming potential.

For efficient N₂O mitigation strategies, a quantitative understanding of the source/sink relationships is needed. As yet, the global atmospheric N₂O budget is poorly constrained. The discrepancy between the known sources and sinks of N₂O is almost 30% (Billings, 2008). Current budgets either overestimate N₂O sources, or underestimate N₂O sinks. A number of recent studies have quantified N₂O emissions from biological sources (Limpens et al., 2006). The major 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 N₂O emissions (IPCC, 2014). Acidic soils generate more N₂O, relative to NO and N₂ (Palmer et al., 2010). Forest soils have also traditionally been viewed as a source of N₂O (Davidson, 1991). Recently, Goldberg and Gebauer (2009) and Berger et al. (2013b) have reported net N₂O consumption at the soil surface over much of the forest’s growing season. This phenomenon may partly explain the discrepancy in the global atmospheric N₂O budget by lowering the source flux estimates (Chapuis-Lardy et al., 2007). Measurable N₂O emissions have been reported from wetlands (Palmer et al., 2012). Wetlands contain as much as 16% of the world’s soil nitrogen (Klimpens et al., 2006). Following draining, minerotrophic wetlands (N-rich fens) may produce relatively large emissions of N₂O. In contrast, N-poor ombrotrophic (rain-fed) bogs are characterized by relatively small N₂O emissions both under wet conditions, and after draining/re-wetting (Moore, 1994).

The relative role of denitrification as a pathway of N₂O removal from ecosystems can be studied using stable isotope ratios 15N/14N, expressed in the δ15N notation as a relative per mil deviation from an agreed-upon standard (Sovik and Morkved, 2008). Several authors have used 15N-enriched NO₃ to trace the origin of N₂O 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; Veitho et al., 2000; Kohzu et al., 2003; Hobbie and Ouiimette, 2009). During denitrification, nitrogen of the product, N₂O, becomes isotopically lighter (lower δ15N), while nitrogen of the remaining substrate (NO₃) becomes progressively enriched in the heavier isotope (higher δ15N; 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 ε = (δ_{product} − δ_{reactants}). 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 δ15N–N₂O profiles in minerotrophic wetlands (Goldberg et al., 2008, 2010). No N isotope data have been reported thus far for vertical N₂O profiles in mountain-top, ombrotrophic bogs. Here we present N₂O 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 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 NO₃ emissions were typical of coal-fired powerplants. Our study sites, while rain-fed, were historically also nitrogen-rich, due to high N deposition. Our first objective was to quantify N₂O emissions from these ombrotrophic bogs and link their magnitude to between-site differences. Our second objective was to assess N₂O source/sink relationships along vertical peat profiles using δ15N–N₂O. We hypothesized that, due to the elevated N supply in the past, both bogs would exhibit high N₂O productions, but possibly also relatively high N₂O emissions.

2. Material and methods

2.1. Study sites

The Blatenska slat (BS) and Kunststaka 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 Orlice hory Natural Preserve Area in the northeastern 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 capilatus of living Sphagnum come out of small water pools. A late summer water table drawdown rarely reaches the depth of...
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 N₂O 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. N₂O 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. N₂O 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 hydrogeochronological monitoring network GEOMON (Oulehle et al., 2008). At UDL, a 10 × 10 m grid of nine collectors was used to collect throughfall, and two collectors were used to sample open-area deposition. ¹⁵N 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 Zieleneč in the Orlice 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, Devadas 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 deposition underneath spruce canopy was 19.3 kg ha⁻¹ at KB and 10.4 kg ha⁻¹ at BS. Total N 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 ha⁻¹ yr⁻¹, an input of more than 18 kg N 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. 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.
3.4. N₂O fluxes across the bog – atmosphere interface

The two measurements of N₂O 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 N₂O flux measurements (Fig. S1). During the N₂O flux measurements, ambient air temperatures were high (up to 30 °C). The water table level in the bogs, however, remained very close to Sphagnum capillita during these hot days (June 12–20, 2013).

All measured N₂O 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 μmol N₂O m⁻² h⁻¹ was recorded at both BS and KB. In contrast, on day 7, a consumption of atmospheric N₂O in the peat substate was recorded at both sites (−13 and −21 μmol m⁻² N₂O h⁻¹ at BS and KB, respectively). The consumption of N₂O 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 N₂O on day 7 was larger than the magnitude of N₂O release on day 1. Overall, both bogs were slight consumers of airborne N₂O.

3.5. Peat-porewater N₂O concentration and isotope profiles

N₂O concentrations were higher at the southwestern less polluted site BS (Fig. 4a). The highest N₂O concentrations at BS were recorded at a depth of 60 cm (140 ppm). N₂O concentrations at the more polluted northeastern site KB were below 20 ppm (Fig. 4a). N₂O concentrations in the deepest horizon at KB were 7 times lower, compared to BS. The peat porewater N₂O concentrations were up to 430 times higher than the present-day N₂O concentrations in the ambient air (324 ppb). They were also higher compared to N₂O concentrations in minerotrophic fens, upland forest soils and rice paddies (Goldberg et al., 2008, 2010; Berger et al., 2013a).

Vertical N₂O concentration profiles, as well as δ¹⁵N profiles, exhibited clear spatial trends (Fig. 4a,b). Between 10 and 60 cm below peat surface at both sites, N₂O concentrations in peat porewaters increased with increasing depth. δ¹⁵N–N₂O values exhibited opposite vertical trends, relative to N₂O concentrations. With increasing depth, δ¹⁵N–N₂O of peat porewaters decreased.

Across the sites, δ¹⁵N–N₂O in porewaters ranged between −4.5 and +4.5%. With one exception, δ¹⁵N–N₂O values in BS porewaters were higher, compared to those at KB. The site with higher N₂O porewater concentrations (BS), also had higher δ¹⁵N–N₂O values. A significant negative correlation between N₂O concentrations and δ¹⁵N–N₂O was observed at KB (p = 0.036; Fig. 4c). At BS, the correlation was close to significant (p = 0.058).

3.6. Comparison of δ¹⁵N among sample types

Across the sites, porewater N₂O contained the isotopically heaviest N in the entire ecosystem. N in peat substrate had intermediate δ¹⁵N values, while atmospheric deposition supplied the isotopically lightest N (δ¹⁵N > δsubstrate > δinput). Across the sites, the mean δ¹⁵N values were +1.4, −2.2, and −8.8‰ for porewater N₂O, 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, N₃ 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 N₃ input at KB than at BS. As seen in Fig. 4a, the N₂O concentrations in peat porewater were higher at BS, the site with lower present-day N₃ input. That was counter-intuitive: higher N₃ pollution should result in higher NO₃ supply for biological nitrogen cycling. The previous history of atmospheric deposition of N₃ 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 NOX emissions, peaked in 1987. Since then, a steady decrease in coal burning has been recorded. A historic perspective on total N₃ emissions for the Czech Republic was published by Kopacek and Vesely (2005). The area in the north, received higher N₃ inputs than the south, but the relative magnitude of N₃ pollution near KB and BS remained unknown. In 1994, seven years after the peak N₃ pollution, we started to monitor monthly atmospheric deposition of N₃ 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 N₃ 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 N₃ 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 N₃ inputs, compared to BS.

4.2. N₃ availability in peat

As seen in Table 2, bog water N₃ concentrations were not always higher at the atmospherically more polluted site KB. N₃ availability at the bog surface may be patchy. The main reasons are: (i) uneven evapotranspiration (Bragazza and Limpens, 2004); (ii) variable
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 Nr 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 N2O production under lower N inputs?

Because higher N2O concentrations (Fig. 4a) were observed at the less polluted site (BS), parameters other than external NO3\textsuperscript{-}/C0 input must have controlled N2O 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 NO3\textsuperscript{-} production via nitrification is extremely slow in acidic bogs (Limpens et al., 2006). If NO3\textsuperscript{-} supply via nitrification played a major role, we would expect higher N2O production at the less acidic site, KB. The opposite was true (Fig. 4a). pH was not likely the main control of the relative N2O 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 N2O production at KB.

Obviously, abiotic factors alone cannot explain the higher N2O 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, and therefore their proportion can change seasonally, according to a changing input of available C, N and P. NirK denitrifiers have higher affinity to NO3\textsuperscript{-}, therefore they can efficiently and rapidly transform it to N2O (Parsonage et al., 1985). Moreover, relative emissions of N2O versus N2 might increase when nitrate concentrations are periodically elevated (Palmer et al., 2010). Therefore, both the dominance of nirK denitrifiers and higher concentration of NO3\textsuperscript{-} could lead to higher N2O concentration at BS.

Previous studies have indicated that low C/N ratios of around 20 are associated with maximum N2O production (Maljanen et al.,

Table 3

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<tr>
<th>Site</th>
<th>δ\textsuperscript{15}N (%)</th>
<th>Open-area deposition</th>
<th>Spruce throughfall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NH\textsubscript{4}\textsuperscript{+}</td>
<td>NO\textsubscript{3}</td>
<td>NH\textsubscript{4}</td>
</tr>
<tr>
<td>BS</td>
<td>–10.4</td>
<td>–6.8</td>
<td>–11.1</td>
</tr>
<tr>
<td>KB</td>
<td>–12.2</td>
<td>–7.0</td>
<td>–13.6</td>
</tr>
</tbody>
</table>

Fig. 3. N2O fluxes across the peat – atmosphere boundary.

Fig. 4. Vertical N2O profiles in peat porewaters. (a) N2O concentrations, (b) δ\textsuperscript{15}N of porewater N2O, (c) negative correlations between N2O concentrations and δ\textsuperscript{15}N. BS, KB – peat bogs.
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 isotope enrichment factor of denitrification varies between −9 and −30‰ (Hobbie and Ouimette, 2009; Snider et al., in press). In our case, the N2O may come either from atmospheric deposition, or from mineralization of organically-bound N (Kalbitz and Geyer, 2002). The latter source of N2O 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 δ15N 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 δ15N of freshly formed N2O to be lower than −11‰. In actual fact, the mean δ15N-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 δ15N 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 Morkved, 2008). Because δ15N of both potential sources of NO3 were lower than δ15N-N2O, we rule out this scenario.

4.5. The depth of N2O consumption in peat

Goldberg et al. (2008) argued that the lowest δ15N-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 δ15N-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 δ15N 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 δ15N-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 δ15N values that would be similar to Goldberg et al. (2008).

Somewhat higher δ15N-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 δ15N-N2O at BS and KB (Fig. 4b) is thus hampered by the limitations of our sampling methodology: δ15N values were known neither for the substrate (NO3) during N2O production, nor for the product of N2O consumption (N2).

4.6. Comparison of δ15N-N2O in ombrotrophic and minerotrophic peatlands

In a minerotrophic fen, Goldberg et al. (2008) found porewater δ15N-N2O values between −10 and −20‰. The mean δ15N-N2O value was close to −15‰. In contrast, ombrotrophic bogs in the current study, exhibited a mean δ15N 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$_2$O 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 d values for N$_2$O reduction in both studies, then the percentage of consumed porewater N$_2$O would be larger in N-rich ombrotrophic bogs, compared to N-rich minerotrophic fens.

4.7. N$_2$O emissions

Porewater N$_2$O concentrations in this study were much higher, compared to minerotrophic fens (Goldberg et al., 2008, 2010). The porewater N$_2$O concentrations at BS were up to 800 and 400 times higher than those at Schloppnerbrunnen (Germany). Porewater N$_2$O 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$_2$O 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$_2$O 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$_2$O emissions, while N$_2$O emissions from bare peat are negligible (Maruschak 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$_2$O fluxes from wetlands (Maljanen et al., 2012). It is thus unlikely that winter-time N$_2$O fluxes at our sites exceed those in Fig. 3. Regional N$_2$O emissions of 0.3 g m$^{-2}$ 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$_2$O) 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$_2$O emissions from wetlands tend to increase (Danecvic et al., 2010). In Central Europe, low N$_2$O emissions were reported from Polish wetlands (0.004 g m$^{-2}$ yr$^{-2}$). These emissions did not correlate with any measured site-specific parameters (Juszczak and Augustin, 2013). N$_2$O 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$_2$ polluted (sensu Lamers et al., 2000). The highest deposition flux of 38 kg N$_2$ ha$^{-1}$ 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 kg N$_2$ ha$^{-1}$ yr$^{-1}$. Several field manipulations studies have shown that very high additions of N to an ombrotrophic peatland (30--56 kg ha$^{-1}$ yr$^{-1}$) did not lead to elevated N$_2$O emissions (Sheppard et al., 2013). Our data from intact, N$_2$-polluted bogs are consistent with these findings.

4.8. N$_2$O source/sink relationships at the bog -- atmosphere interface

It has been shown previously that the same microbial communities are capable of generating N$_2$O, but also consuming N$_2$O (Palmer et al., 2010). Within 7 days, our study sites turned from very small N$_2$O emitters to very small N$_2$O consumers (p < 0.05). Removal of N$_2$O from the atmosphere by an ecosystem, and its consumption, have been reported from a number of other sites and soil types (Limpens et al., 2006). We currently do not know whether switching from N$_2$O production to N$_2$O consumption at BS and KB were linked to changes in the weather. Following heavy rains and unseasonably low temperatures (daily maximum of 6 °C), day 7 was much warmer (30 °C). Warmer, drier conditions accompanied scavenging of air-borne N$_2$O by the peat on day 7, compared to day 1 and the previous weeks. In general, an increasing N$_2$/N$_2$O ratio during denitification 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$_2$O 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 Schlegel N$_2$O 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$_2$O 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$_2$O. 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$_2$O 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$_2$O 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$_2$O concentrations in peat porewater. High inputs of atmospheric NO$_3^-$ augmented N$_2$O production in ombrotrophic bogs. Downward mobility of NO$_3^-$ in peat substrate (Novak et al., 2014) may have also contributed to the higher N$_2$O production. The depth of maximum N$_2$O production in bogs (30–60 cm) was similar to fens and rice paddies (Berger et al., 2013a). Part of the produced N$_2$O was reduced to N$_2$, as documented by increasing $^{15}$N--N$_2$O values and lower N$_2$O concentrations upcore. A high degree of biological N$_2$O consumption in the peat was further corroborated by: (i) very low measured N$_2$O emissions, (ii) N isotope systematics; the found N$_2$O 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$_2$O reduction. We conclude that N$_2$O pollution of ombrotrophic bogs in the temperate zone does not significantly contribute to climatic warming, due to efficient N$_2$O reduction in peat substrate, and low N$_2$O emissions. Even N-polluted bogs may...
scavenge air-borne N$_2$O and reduce it to N$_2$ with no warming potential.

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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.

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