Oecologia
DOI 10.1007/s00442-014-3117-1

SPECIAL TOPIC: NITROGEN DEPOSITION REASSESSED

# Soil denitrification fluxes from three northeastern North American forests across a range of nitrogen deposition

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Received: 27 September 2013 / Accepted: 14 October 2014


**Abstract** In northern forests, large amounts of missing N that dominate N balances at scales ranging from small watersheds to large regional drainage basins may be related to N-gas production by soil microbes. We measured denitrification rates in forest soils in northeastern North America along a N deposition gradient to determine whether N-gas fluxes were a significant fate for atmospheric N inputs and whether denitrification rates were correlated with N availability, soil $O_2$ status, or forest type. We quantified $N_2$ and $N_2O$ fluxes in the laboratory with an intact-core method and monitored soil $O_2$, temperature and moisture in three forests differing in natural and anthropogenic N enrichment: Turkey Lakes Watershed, Ontario; Hubbard Brook Experimental Forest, New Hampshire; and Bear Brook Watershed, Maine (fertilized and reference plots in hardwood and softwood stands). Total N-gas flux estimates ranged from <1 in fertilized hardwood uplands at Bear Brook to >100 kg N $ha^{-1}$ $year^{-1}$ in hardwood wetlands at Turkey Lakes. N-gas flux increased systematically with natural N enrichment from soils with high nitrification rates (Bear Brook < Hubbard Brook < Turkey Lakes) but did not increase in the site where N fertilizer has been added since 1989 (Bear Brook). Our results show that denitrification is an important and underestimated term (1–24 % of atmospheric N inputs) in N budgets of upland forests in northeastern North America, but it does not appear to be an important sink for elevated anthropogenic atmospheric N deposition in this region.

**Keywords** Nitrification · Northern forests · Nitrogen deposition · Nitrous oxide · Nitrogen enrichment · Soil oxygen

Communicated by Hormoz BassiriRad.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00442-014-3117-1) contains supplementary material, which is available to authorized users.

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## Introduction

Understanding the N cycle at landscape and regional scales is a great challenge in environmental science. Humans have doubled the annual global production of reactive N, leading to degradation of air and water quality as well as coastal ecosystems in many areas (Galloway et al. 2004). The development of management strategies and policies to address N pollution problems has been hindered by uncertainties about the fate and transport of different N forms (Davidson et al. 2012).

Uncertainties about the N cycle arise from large amounts of missing N (30–40 % of N inputs) that dominate N balances at all scales—from small headwater catchments to

Published online: 19 November 2014

large regional drainage basins (Boyer et al. 2006; Creed and Beall 2009; Seitzinger et al. 2006). Inputs of N through atmospheric deposition, fertilizer, and sewage are well constrained and are substantially higher than hydrologic outputs of N at many scales (Groffman 2008). There is much uncertainty about the fate of this excess N (Van Breemen et al. 2002)—is it stored in soils or vegetation? Is it converted to gas, and if so, in which forms? This uncertainty is particularly relevant in northeastern North America, where, despite reductions in emissions of nitrogen oxides and declines in atmospheric N deposition, atmospheric N deposition remains elevated (Environment Canada 2012). In this region, $^{15}N$ tracer studies indicate that while some of this N is taken up by vegetation, more ends up in soil (Nadelhoffer et al. 1999), but changes in soil N stocks are difficult to detect (Magill et al. 2004). At the same time, N concentrations in streams in many forested watersheds are declining (Bernal et al. 2012; Goodale et al. 2003; Martin et al. 2000), counter to expectations based on prevailing theories of N saturation and ecosystem development (Aber et al. 1998).

Uncertainty about N balances has led to increased interest in soil N-gas production as an important flux of N (Kulkarni et al. 2008). The gases NO, $N_2O$ and $N_2$ are produced by microbial processes (primarily nitrification and denitrification) in soils and sediments. These processes can convert reactive N to unreactive $N_2$ or to more reactive gases (NO, $N_2O$) that contribute to the creation of $O_3$ in the troposphere, to the greenhouse effect, and/or to the destruction of $O_3$ in the stratosphere (Groffman 1991; Ravishankara et al. 2009). Gas fluxes are perhaps the least well-understood component of the N cycle. They are difficult to quantify because of problematic measurement techniques (especially for $N_2$), high spatial and temporal variability, and a lack of methods for spatial and temporal scaling (Groffman et al. 2006a, b). Historically, denitrification has not been considered a major N flux in most terrestrial ecosystems (Bowden 1986), partly because measurements have been scarce, but also because the anoxic conditions that promote this anaerobic microbial metabolic pathway are not widespread in uplands. However, small areas (hotspots) and brief periods (hot moments) of suboxic conditions can be of great importance, particularly in N-rich environments, and can support significant amounts of N-gas flux activity (Groffman et al. 2009; McClain et al. 2003). In a previous study at Hubbard Brook Experimental Forest, New Hampshire, denitrification gas fluxes were estimated using a combination of methods and were found to range widely between 4 and 100 kg N $ha^{-1}$ $year^{-1}$ (Kulkarni et al. 2013). Recent applications of a new method for measuring soil denitrification have yielded estimates of 60–130 kg N $ha^{-1}$ $year^{-1}$ in riparian forests (Burgin and Groffman 2012), or 16–17 % of atmospheric N inputs (Duncan et al. 2013).

Although low soil pH has been found to adversely affect denitrification activity in some environments (Philippot et al. 2011; Van den Heuvel et al. 2011), substantial denitrification rates have been measured in acidic soils in a variety of ecosystems (Butterbach-Bahl et al. 2002; Morse and Bernhardt 2013; Parkin et al. 1985; Simek and Cooper 2002). Furthermore, while few comparable measurements of direct $N_2$ and $N_2O$ fluxes from upland forest soils have been made, results from a well-described (45 kg N $ha^{-1}$ $year^{-1}$ in atmospheric N deposition and losses of 8 kg N $ha^{-1}$ $year^{-1}$ through fluxes of $N_2O$ and $N_2$, thus accounting for 18 % of N inputs) long-term research study in a chronically N-loaded spruce ecosystem (Höglwald Forest, Germany) suggest a strong capacity for denitrification despite acidic surface soils ranging in pH from 2.9 to 4.0 (Butterbach-Bahl et al. 2002; Kreutzer et al. 2009).

The uncertainty about N-gas fluxes, and N balances, in northeastern North America, is relevant to both air and water quality issues in this region. There are active legislative and management efforts to address the effects of N pollution on tropospheric $O_3$ levels, coastal eutrophication and drinking water quality, and to determine "critical loads" for the effects of atmospheric N deposition on terrestrial and aquatic ecosystems (Baron et al. 2011; Burns et al. 2008; Pardo et al. 2011). Gas fluxes are central to all of these questions, and therefore influence the integrity of water bodies as well as the drinking water and air quality of the most densely populated regions of the US and Canada (Driscoll et al. 2003).

The main objective of this study was to understand the importance and the drivers of soil denitrification (the anaerobic reduction of $NO_3^-$ to $N_2O$ and $N_2$) in three northeastern North American forests ranging in N availability. We hypothesized that:

1. Denitrification is a significant flux (up to 50 % of atmospheric N deposition) in ecosystem N budgets in northeastern North America.
2. This process increases in response to natural and/or anthropogenic N enrichment.
3. Variation in denitrification is driven by ecosystem N richness (elevated atmospheric N inputs and/or elevated N mineralization and nitrification rates) and soil $O_2$ dynamics, which are controlled by landscape position, rainfall, soil texture, and forest floor structure.

## Materials and methods

### Study sites

This study was conducted in three northern forest sites ranging in N availability from atmospheric N deposition,

fertilizer inputs, and soil N cycling rates (Fig. S1): Turkey Lakes Watershed (TLW), Hubbard Brook Experimental Forest (HBEF), and the Bear Brook Watershed in Maine (BBWM). The TLW is located in the Algoma Highlands on the Canadian Shield, Ontario, Canada (47°03′N, 84°25′W). The climate is cool and continental, with mean annual precipitation of 1,200 mm and mean annual temperature of 5.0 °C. There is a great deal of topographic heterogeneity at TLW; a representative catchment is composed of frequently dry uplands (60 %), critical transition zones (15 %, including 10 % toeslopes), and wetlands (25 %, with 15 % outer wetland and 10 % inner wetland) (Webster et al. 2008). Soils at TLW are stony, shallow silty-loam Spodosols developed in ablation till, with pH ranging from 3.5 to 4.0 in surface horizons and humified organic deposits in wetlands (Creed and Beall 2009; Hazlett et al. 2011). Northern hardwood forests in TLW are old-growth dominated by sugar maple (90 %; *Acer saccharum*) and yellow birch (*Betula allegheniensis*) (Webster et al. 2008). This is the most N-rich site, given mean annual total N deposition of 11 kg $ha^{-1}$ [wet and dry deposition, 1983–2009 (Creed, unpublished data)]. In addition, high abundance of sugar maple tends to result in higher rates of soil nitrification rates and higher N availability, potentially as a result of a low C:N ratio in leaf litter (Lovett and Mitchell 2004; Spoelstra et al. 2001).

The second forest, HBEF, is located in the White Mountains of New Hampshire, USA (43°56′N, 71°45′W). The HBEF has moderate N availability, with lower total atmospheric N inputs [7 kg N $ha^{-1}$ $year^{-1}$ (Yanai et al. 2013)] and lower abundance of sugar maple compared to TLW. It too has a continental climate, with mean annual precipitation of 1,400 mm and mean annual temperature of 5.3 °C. Soils in this steep terrain are derived from glacial till and dominated by acidic Spodosols [pH 3.9–4.5 (Soil Survey Staff 2006)]. The vegetation is typical of northern hardwood forests, composed primarily of American beech (*Fagus grandifolia*), sugar maple, and yellow birch, with increased presence of red spruce (*Picea rubens* Sarg.) and balsam fir (*Abies balsamea* L.) at higher elevations (Schwarz et al. 2003).

The third forest, BBWM in eastern Maine, USA (44°52′N; 68°6′W) comprises two watersheds. A control watershed (East Bear) has the lowest N availability, with an estimated annual total atmospheric N input of 4.5 kg N $ha^{-1}$ $year^{-1}$ (Rustad et al. 1994). A paired experimental watershed (West Bear), fertilized since 1989 with an additional 25 kg N $ha^{-1}$ $year^{-1}$, has the overall highest N availability (Fernandez et al. 2010). Mean annual temperature at BBWM is 4.9 °C, and mean annual precipitation is 1,400 mm. Soils in BBWM are acidic, coarse-loamy Spodosols (Typic Haplorthods) derived from basal till (Fernandez et al. 2010; Jefts et al. 2004). Only 5 % of land cover in both watersheds is represented by wetlands, which occur as an isolated wetland in the upper elevations of East Bear. Soil pH has a wider range in West Bear than East Bear, with lower values in the O horizon compared to the upper B horizon in both watersheds [pH 3.0–4.0 in West Bear vs. pH 3.1–3.8 in East Bear (Fernandez et al. 2010)]. The vegetation at BBWM features stands of hardwoods (similar in composition to HBEF; dominated by American beech and sugar maple) and softwoods, with higher elevations dominated by red spruce, with a minor component of balsam fir, and Eastern hemlock (*Tsuga canadensis*) (Jefts et al. 2004).

## Experimental design and monitoring

In each forest site, we established 10-m-diameter sampling plots early in the growing season (May/June 2011). At TLW, two transects spanning a topographical gradient were established, each with three habitats (toeslope, outer wetland, and inner wetland) (Webster et al. 2008). In HBEF, we located plots in two habitats: high (539–595 m) and low (375–511 m) elevation zones ($n = 3$ per elevation zone) within sugar maple-dominated stands. In each of the paired watersheds at BBWM, we located one plot in a hardwood stand and one in a softwood stand, for a total of four habitat types in this forest.

At each plot, we installed two diffusion-head soil oxygen sensors (sensor diameter 3.5 cm, sensor body 6.8 cm long, diffusion head 3.5 cm long; SO-100 series; Apogee Instruments, Logan, UT) and two soil moisture and temperature sensors (sensor body 3.2 cm wide, sensor body 5 cm long, sensing prongs 5 cm long; 5TM series; Decagon Devices, Pullman, WA) in the forest floor at 10-cm depth. The $O_2$ sensors were thus integrating $O_2$ concentrations from 6.8- to 10-cm depth; the soil moisture and temperature sensors were integrating from 5- to 10-cm depth. We connected the sensors to a Campbell Scientific data logger (CR800 or CR10X) to record hourly measurements of environmental variables. Data loggers were deployed at HBEF and BBWM on 2 June and at TLW on 17 June in inner wetland locations and on 17 July in outer wetland and toeslope locations. Data collection ended late in the growing season in 2011 (10 September at BBWM and HBEF, and 5 October at TLW). At BBWM, data loggers were deployed only in the hardwood and softwood plots in the control watershed (East Bear). At TLW, battery failures resulted in data gaps that were filled based on regression to nearby installations in similar landscape positions. The soil $O_2$ data presented here from HBEF are a subset of the data presented in Morse et al. (accepted) that extends over 2 years with intensive sampling during the snowmelt period. We converted soil moisture (volumetric water content) to water-filled pore space (WFPS) to account for differences in bulk density and soil porosity across sites.

### Soil sampling and analyses

We collected four surface soil samples (0- to 15-cm depth, primarily organic horizons, excluding leaf litter; 4-cm diameter) in each plot at the beginning and end of the sampling period in each forest site. Two cores were used for denitrification analyses and two for determination of other soil parameters. We estimated soil organic matter content by loss-on-ignition at 450 °C for 4 h (Nelson et al. 1986). We determined soil inorganic N ($NH_4^+$ and $NO_2^- + NO_3^-$) after 10-g soil samples were extracted with 40 mL of 2 M KCl (1 h at 125 r.p.m.) and analyzed with a colorimetric flow injection analyzer (Quikchem 8100; Lachat Instruments, Milwaukee, WI). Bulk density was calculated by dividing the dry weight by the volume of the soil samples.

Potential net N mineralization and nitrification and microbial respiration were measured in 10-day incubations of field moist soils in the laboratory using the chloroform fumigation-incubation method (Jenkinson and Powlson 1976) as described by Durán et al. (2013). Potential net N mineralization was quantified from the production of total inorganic N ($NH_4^+ + NO_2^- + NO_3^-$) and potential net nitrification was quantified from the production of $NO_3^-$ over the course of the incubation. Potential microbial respiration was quantified from the production of $CO_2$ [measured by gas chromatography with thermal conductivity detector (GC-TCD)] over the course of the incubation. The respiration assays also provide estimates of labile or readily respirable C pools.

### Laboratory fluxes of $N_2$, $N_2O$, and $CO_2$ under varying $O_2$ atmospheres

We estimated soil gas fluxes of $N_2$, $N_2O$, and $CO_2$ under varying $O_2$ concentrations (0, 5, 10, or 20 % $O_2$) by incubating two intact soil cores from each site and plot in gas-tight chambers in the N-free air recirculation method (N-FARM) measurement system described by Burgin et al. (2010) and Burgin and Groffman (2012). Prior to the start of incubations, a 16-h period of alternating vacuum/flush cycles (every 90 s) removed background atmospheric $N_2$ from the chamber headspaces, replacing it with a mixture of $O_2$ and ultra-high purity He. Soils were incubated for 24 h in this $N_2$-free atmosphere, producing $N_2$, $N_2O$, and $CO_2$; the concentration of these gases in the headspace of each chamber was measured at three time points (generally 0, 7, and 24 h) using Shimadzu GC8-A GCs (GC-TCD and gas chromatography with electron capture detector) in-line with the N-FARM system. Two replicate soil cores from each plot and forest site were sequentially incubated at 10, 5, and then 20 % $O_2$. Samples from TLW were additionally incubated at 0 % $O_2$, since soil $O_2$ concentrations in these plots did drop below 5 % $O_2$ during the experiment. Data from HBEF are a subset of those presented in Morse et al. (accepted), which corresponds to 2 years of sampling with a special focus on the snowmelt period.

For each plot and $O_2$ concentration, we determined soil denitrification rate as the net production of $N_2O$ and $N_2$ during the incubation period, and we calculated the $N_2O$ yield as $[N_2O/(N_2O + N_2)]$. To establish a lower limit of gas fluxes attributable to biological activity, we "killed" soil cores by autoclaving soil cores from each plot (three times at 134 °C for 1 h), and then incubated these cores at 0 % $O_2$ and measured $N_2$, $N_2O$, and $CO_2$ production after 0, 24, and 48 h. Production of gases during these killed core incubations provide estimates of residual degassing and/or leakage for the N-FARM method (Burgin and Groffman 2012; Burgin et al. 2010). Based on results of denitrification kinetics in northern forest soils (Holtan-Hartwig et al. 2002), we adjusted laboratory gas flux measurements to reflect ambient temperatures at the time of field sampling, using $Q_{10}$ values of 2 for $CO_2$ fluxes and 3.4 for $N_2O$ and $N_2$ fluxes.

To examine variation in soil characteristics, C and N availability, and gas fluxes among forest sites and habitats, we first conducted paired *t*-tests to look for differences between samples collected at the beginning and at the end of the monitoring period. Finding no significant differences at $p = 0.05$, we took the mean of the two sampling events and replicate cores for each of the sampled plots to avoid pseudoreplication, before performing nonparametric ANOVA tests with permutation ($df = 2$), with adjusted *p*-values for multiple comparisons. To determine the variation in these parameters within each forest site as a function of habitat, we used mean values over the two sampling events and performed nonparametric analysis of variance tests with permutation ($df = 1$–3, depending on forest site), with adjusted *p*-values for multiple comparisons. We used correlation analysis to explore relationships between environmental variables, soil C and N availability, and laboratory-measured gas fluxes. R statistical software was used for all statistical tests [(R Development Core Team 2009); R package lmPerm (Wheeler 2010)].

## Results

### Soil properties and soil microbial activity

Soils from TLW had significantly higher KCl-extractable $NO_3^-$ and significantly lower bulk density than those from HBEF and BBWM; differences in organic matter content were significant only between TLW and HBEF (Table 1; $p < 0.001$). Potential net nitrification rates were also significantly higher at TLW ($p < 0.001$; Fig. 1a). In BBWM,

**Table 1** Soil characteristics by forest site [Turkey Lakes Watershed (*TLW*), Hubbard Brook Experimental Forest (*HBEF*); Bear Brook Watershed, Maine (*BBWM*)] and habitat

| Site | Habitat | Bulk density (g $cm^{-3}$) | Organic matter (%) | $NH_4$–N (mg N g $soil^{-1}$) | $NO_3$–N (mg N g $soil^{-1}$) |
|---|---|---|---|---|---|
| TLW | Inner wetland | 0.109 ± 0.008 a | 74.2 ± 3.6 a | 7.51 ± 1.5 b | 25.3 ± 4.3 a |
| | Outer wetland | 0.134 ± 0.005 a | 61.0 ± 8.7 a | 7.75 ± 3.4 b | 21.5 ± 2.4 a |
| | Toeslope | 0.207 ± 0.024 a | 72.4 ± 6.2 a | 9.15 ± 2.8 b | 32.4 ± 5.2 a |
| HBEF | Low elevation | 0.456 ± 0.031 b | 20.9 ± 3.8 b | 15.6 ± 5.2 ab | 6.39 ± 2.4 b |
| | High elevation | 0.438 ± 0.034 b | 23.4 ± 2.9 b | 6.52 ± 1.2 ab | 6.04 ± 1.9 b |
| BBWM | Hardwood | 0.586 ± 0.039 b | 13.4 ± 1.8 ab | 12.3 ± 1.2 a | 1.46 ± 1.1 b |
| | Hardwood + N | 0.314 ± 0.066 b | 23.4 ± 1.1 ab | 28.7 ± 12 a | 11.8 ± 2.8 b |
| | Softwood | 0.449 ± 0.14 b | 72.1 ± 20 ab | 31.9 ± 9.2 a | 3.43 ± 2.6 b |
| | Softwood + N | 0.184 ± 0.018 b | 78.0 ± 14 ab | 166 ± 39 a | 12.9 ± 4.6 b |

*Different letters* indicate significant differences between forests ($p < 0.001$)

+*N* Fertilized treatment

$NH_4^+$ pools were significantly higher than in TLW (Table 1; $p < 0.001$). Soils in HBEF had relatively low potential microbial activity: net N mineralization rates were significantly lower in HBEF compared to TLW (Fig. 1b; $p = 0.021$), and respiration was significantly lower than in the other sites (Fig. 1c; $p < 0.001$). Within TLW, soil C and N availability did not differ significantly according to habitat type. At HBEF, high-elevation sites had higher potential net N mineralization and potential nitrification rates compared to low-elevation sites ($p < 0.05$; Fig. 1a–b), and there was greater variability across BBWM plots than in the other forest sites, with potential respiration significantly higher in softwood unfertilized plots ($p = 0.024$; Fig. 1a–c).

## Environmental variables

Monitoring results from data logger installations revealed similar soil temperature patterns across all forest sites and habitats during the growing season, with lows during the study period occurring in mid-June and early September (10–11 °C) and highs in late July (18–21 °C; Fig. 2a).

Soil moisture exhibited greater variation than temperature both across forests and habitats and throughout the growing season (Fig. 2b). At TLW, WFPS was high and steady (58–70 %) in toeslope and outer wetland locations throughout the growing season; whereas the inner wetlands experienced lower and more variable WFPS as the summer progressed. Plots in HBEF and BBWM experienced similar patterns of soil moisture: intermediate values (39–48 % WFPS) at the beginning of the growing season, with progressively drier conditions through early August (28–35 %), until soil moisture began to return to or exceed previous levels, driven by rain events (Fig. 2b).

Seasonal patterns in soil moisture were not reflected in soil $O_2$ concentrations in surface soils (Fig. 2c). Across all sites, only plots in TLW inner wetlands experienced suboxic conditions (<5 % $O_2$), dropping from near 15 % $O_2$ in early June (following installation) to 0 % $O_2$ for nearly 3 weeks, before conditions became drier and more oxygenated (~17 % $O_2$) by late July. Data on soil $O_2$ from TLW outer wetland or toe slope plots were not recorded from 10 June to 17 July due to battery failures. Mean $O_2$ concentration during the sampling period was 10 % in the TLW inner wetland and 18–20 % in all other sampling sites in the study (Fig. 2c).

## Laboratory fluxes of $N_2$, $N_2O$, and $CO_2$ under varying $O_2$ concentrations

Across all $O_2$ concentrations, $CO_2$ flux was significantly higher in TLW and BBWM compared to HBEF ($p = 0.009$; Fig. 3). Within TLW, inner wetland habitats had significantly higher $CO_2$ flux compared to toeslope habitats ($p < 0.05$), and fluxes from soils incubated at 0 % $O_2$ were significantly lower compared to other levels of $O_2$ availability ($p < 0.05$; Fig. 3). Within the other two forest sites, habitat was a significant factor in influencing soil $CO_2$ fluxes ($p < 0.05$), but there were no significant differences in $CO_2$ fluxes as a function of $O_2$ availability.

Fluxes of $N_2O$ were highly variable, spanning four orders of magnitude, with no significant differences found between forest sites or habitats within forests or as a function of $O_2$ availability (Fig. 4).

In contrast to $N_2O$ fluxes, $N_2$ fluxes varied significantly by forest site and by $O_2$ concentration ($p < 0.001$), with significant interactions between the two factors ($p = 0.029$; Fig. 5). $N_2$ fluxes measured at 10 % $O_2$ were consistently and significantly higher compared to fluxes at 5 and 20 % $O_2$ in all sites. Fluxes in TLW were significantly higher compared to BBWM and HBEF across all $O_2$ concentrations ($p < 0.001$). Within TLW, inner wetlands had significantly higher $N_2$ fluxes compared to other habitats ($p < 0.05$); $N_2$ fluxes within HBEF and BBWM did not vary as a function of habitat (Fig. 5).

We produced estimates of annual N-gas fluxes by conservatively upscaling the laboratory measurements of $N_2O$ and $N_2$ fluxes assuming a 184-day growing season in the

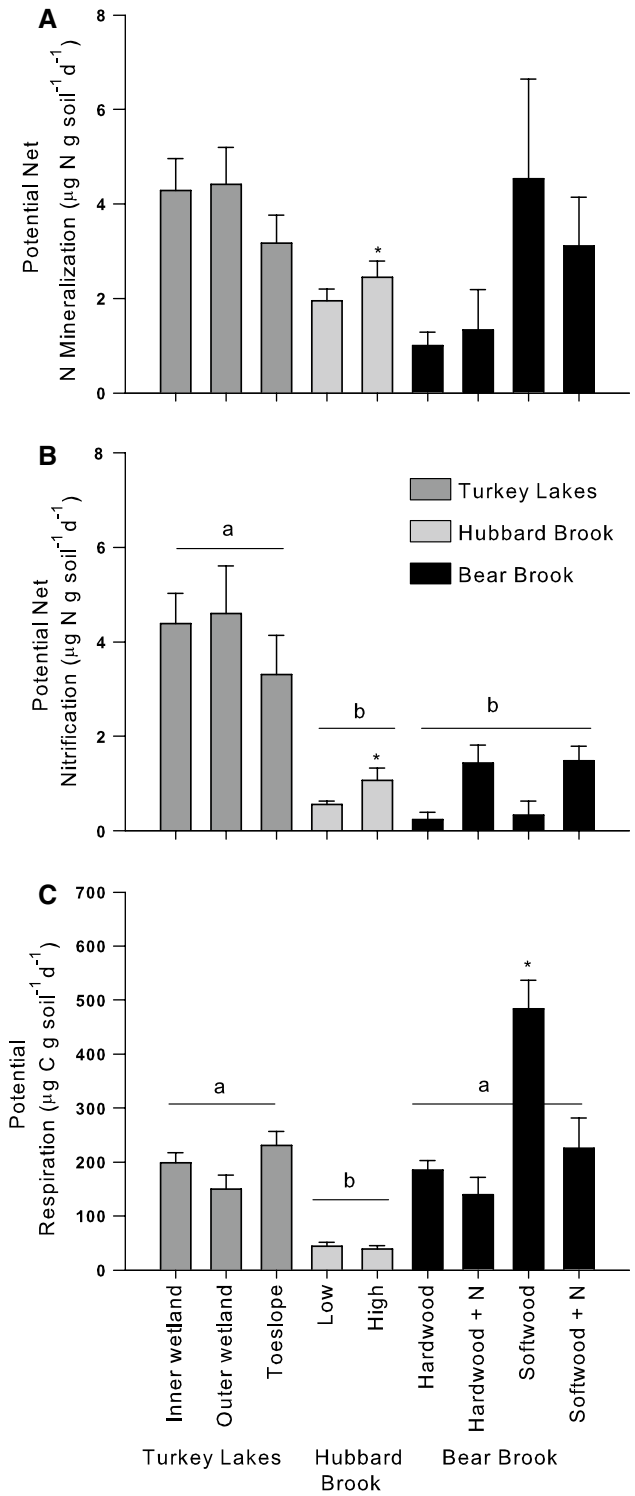


**Fig. 1** Soil assays for **a** potential net N mineralization, **b** potential nitrification, and **c** potential respiration, as indexes of soil C and N availability in three forest sites and contrasting habitats. Results are shown as means over the two sampling dates by forest site and habitat, with *different letters* indicating significant differences between forest sites ($p < 0.001$) and an *asterisk* indicating significant differences ($p < 0.001$) among habitats within a forest. Data from HBEF are a subset from a 2-year data set presented in Morse et al. (accepted). *d* Day

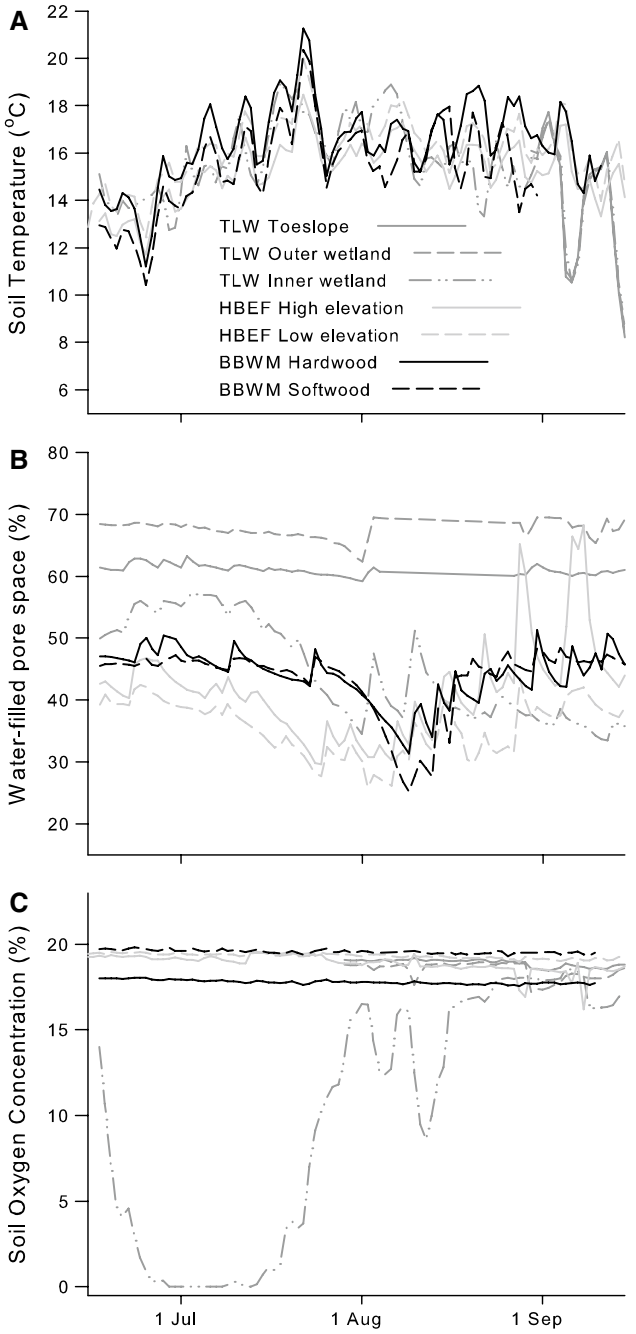


**Fig. 2** **a** Soil temperature, **b** soil moisture as water-filled pore space (%), and **c** soil $O_2$ concentrations in surface soils from three forest sites [Turkey Lakes Watershed (*TLW*), Hubbard Brook Experimental Forest (*HBEF*); Bear Brook Watershed, Maine (*BBWM*)] and contrasting habitats. Data from HBEF are a subset from a 2-year data set presented in Morse et al. (accepted)

absence of data from winter-time measurements (Kulkarni et al. 2013). For the HBEF, BBWM and TLW toeslope and outer wetland sites, we upscaled data from incubations at 20 % $O_2$ which reflect the mean soil $O_2$ concentrations measured in the field plots at these sites. For the TLW inner

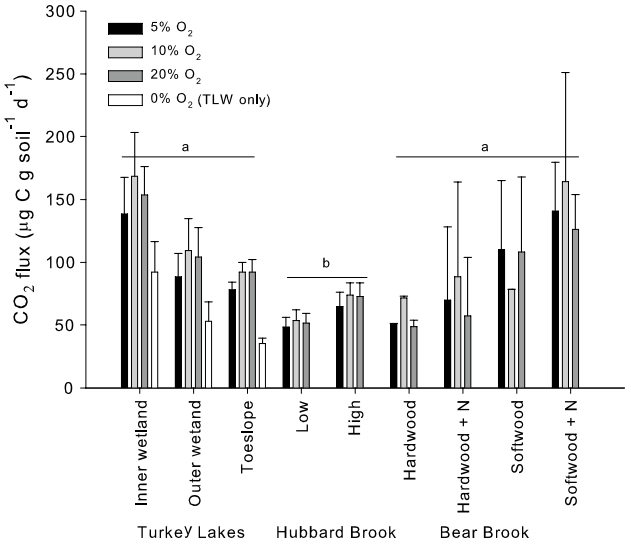


**Fig. 3** $CO_2$ flux over the two sampling dates by forest site and habitat (mean ± SE) from surface soils incubated at 0 % (TLW only) 5, 10, and 20 % $O_2$. Rates are adjusted for differences between field and lab temperatures using $Q_{10} = 2$. Results are shown by forest site and habitat, with *different letters* indicating significant differences between forest sites ($p < 0.01$). Data from HBEF are a subset of data presented in Morse et al. (accepted). For abbreviations, see Fig. 2

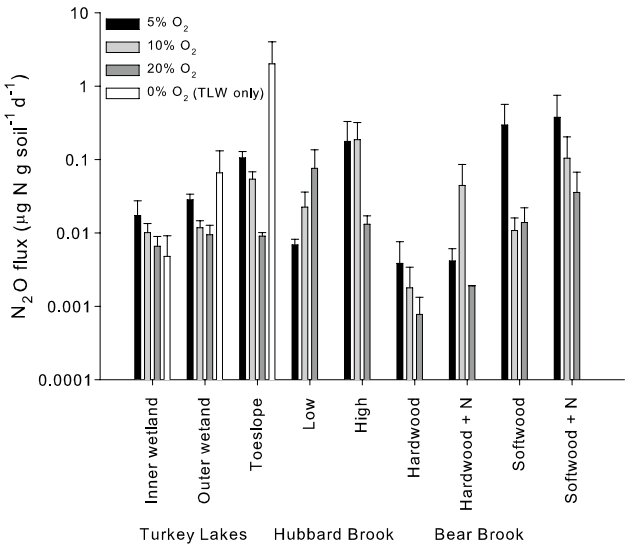


**Fig. 4** $N_2O$ flux over the two sampling dates by forest site and habitat (mean ± SE) from surface soils incubated at 0 % (TLW only) 5, 10, and 20 % $O_2$. Rates are adjusted for differences between field and lab temperatures using $Q_{10} = 3.4$. Data from HBEF are a subset from a 2-year data set presented in Morse et al. (accepted). For abbreviations, see Fig. 2

wetlands we upscaled data from incubations at 10 % $O_2$, which gave the mean $O_2$ concentration at this site. Total annual N-gas flux estimates ranged from 0.30 kg N $ha^{-1}$ in N-fertilized hardwood plots at BBWM to 102 kg N $ha^{-1}$ in

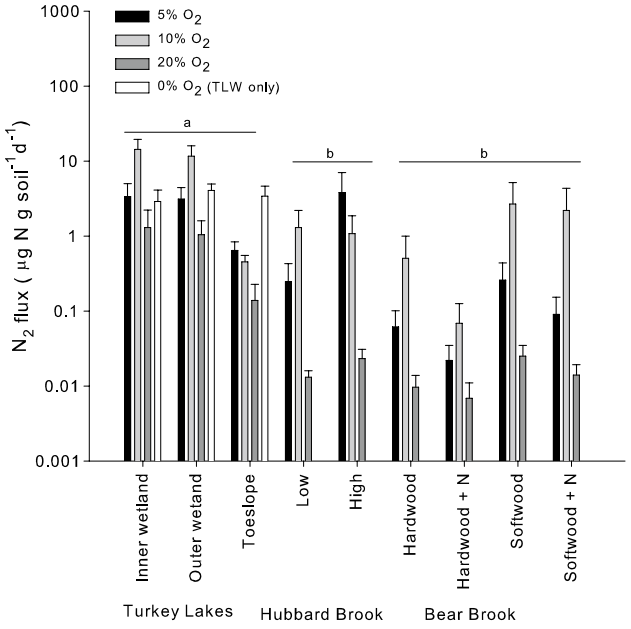


**Fig. 5** $N_2$ flux over the two sampling dates by forest site and habitat (mean ± SE) from surface soils incubated at 0 % (TLW only) 5, 10, and 20 % $O_2$. Rates are adjusted for differences between field and lab temperatures using $Q_{10} = 3.4$. Results are shown by forest site and habitat, with *different letters* indicating significant differences between forest sites ($p < 0.01$). Data from HBEF are a subset from a 2-year data set presented in Morse et al. (accepted). For abbreviations, see Fig. 2

inner wetland plots at TLW (Table 2). There was likewise a great range in $N_2O$ yields across sites, from <1 % in TLW inner wetlands to 50 % in low-elevation plots at HBEF (Table 2).

## Discussion

### N-gas fluxes and N availability in forests

In this study, we measured denitrification rates in northern forest soils along a N deposition gradient to determine whether N-gas fluxes were a significant fate for atmospheric N inputs and whether denitrification rates were correlated with N availability, soil $O_2$ status, or forest type. N availability, as characterized by elevated N deposition, high abundance of sugar maple, high extractable $NO_3^-$ pools, and high potential N mineralization and nitrification rates, was highest in TLW, where C availability was likewise high, and soil $O_2$ levels were lower than in the other two forests. As expected, these favorable conditions for denitrification resulted in higher denitrification rates in upland soils at TLW compared to BBWM and HBEF. While these results suggest that denitrification does vary coherently with patterns of N availability in forests, there were no

**Table 2** Estimated seasonal N-gas losses and $N_2O$ yield [$N_2O/(N_2 + N_2O)$] by habitat (TLW, HBEF, BBWM)

| Site | Atmospheric N deposition (kg N $ha^{-1}$ $year^{-1}$) | Habitat | $O_2$ % | Total N-gas flux (kg N $ha^{-1}$ $season^{-1}$) | % of atmospheric N inputs | $N_2O$ yield (%) |
|---|---|---|---|---|---|---|
| TLW | 11 | Inner wetland | 10 | 102 ± 20 | 930 | 0.28 ± 0.2 |
| | | Outer wetland | 20 | 9.2 ± 4 | 84 | 9.1 ± 5 |
| | | Toeslope | 20 | 1.7 ± 0.6 | 15 | 18 ± 6 |
| HBEF | 7 | Low elevation | 20 | 1.7 ± 0.9 | 24 | 50 ± 8 |
| | | High elevation | 20 | 0.90 ± 0.2 | 13 | 34 ± 8 |
| BBWM | 4.5 (+25 kg N) | Hardwood | 20 | 0.56 ± 0.2 | 12 | 6.6 ± 2 |
| | | Softwood | 20 | 0.97 ± 0.3 | 22 | 33 ± 20 |
| | | Hardwood + N | 20 | 0.30 ± 0.1 | 1.0 | 33 ± 9 |
| | | Softwood + N | 20 | 0.60 ± 0.4 | 2.0 | 45 ± 10 |

Results are based on a 184-day growing season and gas fluxes measured in lab incubations at $O_2$ concentrations representative of mean soil $O_2$ measurements in field plots. Atmospheric N deposition estimates are drawn from the literature. For abbreviations, see Table 1

significant correlations between atmospheric N deposition, potential net N mineralization and nitrification rates. It is important to note that we sampled on two dates only and that, while we did expose samples from these dates to a wide range of $O_2$ levels, it is possible that the conditions on the two days we sampled were not generally representative. Still, these results suggest that expectations for N-gas loss in forests can reasonably be based on factors that control inherent N availability such as overstory composition, soil C:N ratio and soil pH (Lovett et al. 2002). Our annual flux estimates are similar to those measured by Butterbach-Bahl et al. (2002) and our hourly flux estimates are somewhat lower than those measured by Dannenmann et al. (2008) for forests in Germany using a similar direct flux method.

While our results suggest that N-gas fluxes are linked to patterns of inherent N availability in forests, they do not suggest that these fluxes respond to increases in atmospheric N deposition. Atmospheric N deposition is approximately twice as high at HBEF as at BBWM, but there was no difference in either $N_2O$ or $N_2$ fluxes between these sites. More surprisingly, chronic fertilizer N addition to West Bear Brook (25 kg N $ha^{-1}$ $year^{-1}$ since 1989) did not result in higher denitrification gas fluxes in those sites. In fact, the lowest N-gas fluxes were measured in N-fertilized hardwood plots at BBWM (Table 2). These results are unexpected given the high rates of N input in the fertilized plots and the marked increases in potential net N mineralization and nitrification (Table 1; Jefts et al. 2004), $NO_3^-$ leaching (Fatemi et al. 2012), and stream $NO_3^-$ concentrations (Fernandez et al. 2010) that these inputs have induced at BBWM. It is likely that the aerobic nature of the upland soils at BBWM prevents a denitrification response. It is possible that wet areas downslope, e.g., riparian or hyporheic zones that support anaerobic conditions and that receive input of $NO_3^-$ from uplands, may support very high rates of denitrification at this site.

Soil pH has been found to adversely affect denitrification activity in some environments (Philippot et al. 2011; Van den Heuvel et al. 2011), but substantial denitrification rates have been measured in a variety of ecosystems despite acidic surface soils ranging in pH from 2.9 to 4.0 (see "Introduction"; Kreutzer et al. 2009; Butterbach-Bahl et al. 2002; Morse and Bernhardt 2013; Parkin et al. 1985; Simek and Cooper 2002). We did not determine soil pH in this study, but published soil pH values are low but similar across all study sites [pH 3.0–4.5 (Fernandez et al. 2010; Groffman et al. 2006a; Hazlett et al. 2011)]. Thus, there is not sufficient evidence to suggest that N fertilization at West Bear Brook has lowered soil pH to the extent that denitrification is particularly suppressed in this site.

### N gases, soil moisture and $O_2$ in forests

The most marked pattern observed in N-gas fluxes was the high flux rates in the forested wetlands at TLW. These sites had significant periods of low soil $O_2$, likely receive significant inputs of $NO_3^-$ from surrounding uplands (Spoelstra et al. 2001), have high rates of nitrification (Fig. 1; Foster et al. 2005) and significant pools of labile C (Fig. 1)—all factors that lead to high rates of denitrification (Seitzinger et al. 2006). Indeed, these wetland areas appear to be significant sinks for reactive N in the landscape at TLW (Creed and Beall 2009). More detailed comparison of watershed N exports is still necessary to determine the importance of these N sinks. However, this comparison will be complex because there is significant variation in $O_2$ levels within and between wetlands in the landscape at TLW. The inner wetlands at TLW experienced anoxic conditions for nearly 3 weeks early in the growing season, with $O_2$ levels increasing as soils dried out later in the summer, and the outer wetlands at TLW had much higher levels through the summer. However, these patterns likely vary both across the

landscape and with climate conditions within and between years.

While we expected the wetlands at TLW to have high rates of denitrification, we were curious to determine if upland surface soils in northern forests experience suboxic episodes for biogeochemically significant periods of time as has been shown to occur in upland tropical forests (Liptzin et al. 2011; Silver et al. 1999). We did not find suboxic conditions at 10-cm depth in any of the upland forests during the growing season, with soils remaining at 18–20 % $O_2$ throughout the growing season, despite fluctuations in soil moisture during the same period. Surprisingly, we found no relationship between soil volumetric water content and soil $O_2$ concentrations in the upland sites, which suggests that rainfall-driven hot moments of enhanced denitrification are not important during the growing season in the well-drained sites (Kulkarni et al. 2013). It is likely that soil porosity and drainage are high enough and/or that respiratory consumption of $O_2$ is low enough to prevent the development of anaerobic conditions after rainfall events in these soils.

While soil $O_2$ concentrations at 10-cm depth remained high in upland soils throughout the growing season, denitrification rates measured in the laboratory under 20 % $O_2$ were nonetheless substantial. These results are consistent with the idea that anaerobic microsites are present in "aerated" soils (Sexstone et al. 1985) and that significant amounts of denitrification occur in these microsites. Estimated growing-season N-gas fluxes in upland forest plots ranged from 0.3 to 1.7 kg N $ha^{-1}$. The upper range of these estimates is significant relative to atmospheric input, suggesting that N-gas fluxes can return up to 24 % of atmospheric N inputs to the atmosphere under oxic soil conditions and over 84 % of inputs under the poorly oxygenated conditions that occur at the upland/wetland interface, e.g., the outer wetland at TLW (Table 2). If we consider the redistribution of atmospheric N deposition along lateral and vertical flowpaths, likely lower $O_2$ concentrations at depth, the significant denitrification potential of deeper soils (Morse et al. 2014), seasonal dynamics of denitrification (Morse et al. accepted), and the high rates of denitrification in wetlands, then this return of N to the atmosphere could be much higher.

## The importance of $N_2O$ yield

We measured greater N fluxes as $N_2$ than as $N_2O$, with a maximum $N_2O$ yield of 50 % in low-elevation plots at HBEF. Perhaps most importantly, the sites with the highest total N-gas fluxes (TLW) had the lowest $N_2O$ yield, suggesting that these fluxes are not converting reactive $NO_3^-$ into $N_2O$, an important greenhouse gas that also depletes stratospheric $O_3$ (Ravishankara et al. 2009; Schlesinger 2009). Further, it is important to note that our soil core incubation system likely overestimates $N_2O$ yield as the residence time of $N_2O$ in the system is shorter than in the field, decreasing opportunities for further reduction of $N_2O$ to $N_2$. Our $N_2O$ yields were higher than those reported in recent studies using the N-FARM or similar methodology (Butterbach-Bahl et al. 2002; Dannenmann et al. 2008; Burgin and Groffman 2012; Kulkarni et al. 2013). This was likely due to the low pH of the soils in our study; low pH frequently leads to higher $N_2O$ yields (Firestone and Davidson 1989).

## Implications for critical loads

There is great interest in setting critical loads for atmospheric N deposition to protect multiple aspects of forest structure, function and services (Pardo et al. 2011; Porter et al. 2013). These loads could be set higher for sites with an inherently high potential for N-gas fluxes that return reactive N to the atmosphere. Our results suggest that these fluxes are returning a significant portion of atmospheric N deposition to the atmosphere, and that this return varies with inherent site N richness, i.e., TLW had higher total N-gas fluxes than HBEF or BBWM. However, our results from the fertilized plots at BBWM, and the lack of difference between HBEF and BBWM, strongly suggest that N-gas fluxes will not offset atmospheric inputs by potentially returning a significant portion of increased anthropogenic inputs to the atmosphere. The marked variation in N-gas fluxes across the landscape at TLW suggests that any assessment of the importance of these fluxes to critical loads must be spatially explicit and must have sufficiently high resolution to account for this variation (Weathers et al. 2006).

**Acknowledgments** We thank Don Buso, Tammy Wooster, Nick Grant, Alexandra Rodríguez, Neil Bettez, Lisa Martel, Kate Shepard, Erica Morgan, Robin Schmidt, and Madeleine Mineau for excellent field, laboratory, and data analysis work. This research was supported by the Northeastern States Research Cooperative and the National Science Foundation (DEB no. 0949664 and DEB no. 0919047). This research was conducted in part at the Hubbard Brook Experimental Forest, which is operated by the Northeastern Research Station, USDA Forest Service, Newtown Square, Pennsylvania. This paper is a contribution to the Hubbard Brook Ecosystem Study.

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