Science of the Total Environment 550 (2016) 880–892

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Comparisons of soil nitrogen mass balances for an ombrotrophic bog and a minerotrophic fen in northern Minnesota

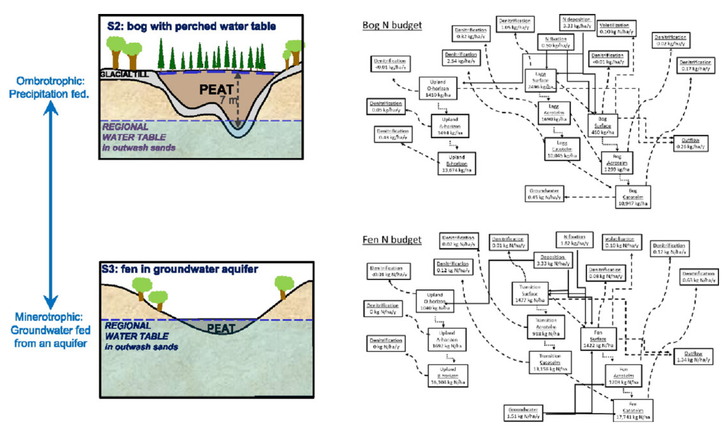
Brian H. Hill [a,*], Terri M. Jicha [a], LaRae L.P. Lehto [a,1], Colleen M. Elonen [a], Stephen D. Sebestyen [b], Randall K. Kolka [b]

[a] US Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Laboratory, Mid-Continent Ecology Division, Duluth, MN 55804, USA
[b] US Forest Service, Northern Research Station, Center for Research on Ecosystem Change, Grand Rapids, MN 55744, USA

HIGHLIGHTS

- We compared N budgets for soils from an ombrotrophic bog and a minerotrophic fen.
- Soil N content depended on location within the bog or fen, and on soil depth.
- We highlight the importance of biogeochemical hotspots within the peatlands.
- We show the importance of organic N storage, as a source of N for denitrification.
- We propose a link between N storage, denitrification and N export from peatlands.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 October 2015
Received in revised form 26 January 2016
Accepted 26 January 2016
Available online xxxx

Editor: D. Barcelo

Keywords:
Bog
Denitrification
Fen
Peat
Soils
Watershed N budget

ABSTRACT

We compared nitrogen (N) storage and flux in soils from an ombrotrophic bog with that of a minerotrophic fen to quantify the differences in N cycling between these two peatlands types in northern Minnesota (USA). Precipitation, atmospheric deposition, and bog and fen outflows were analyzed for nitrogen species. Upland and peatland soil samples were analyzed for N content, and for ambient (DN) and potential (DEA) denitrification rates. Annual atmospheric deposition was: 0.88–3.07 kg NH_4^+ ha^{-1} y^{-1}; 1.37–1.42 kg NO_3^- ha^{-1} y^{-1}; 2.79–4.69 kg TN ha^{-1} y^{-1}. Annual N outflows were: bog—0.01–0.04 kg NH_4^+ ha^{-1} y^{-1}, NO_3^- 0.01–0.06 kg ha^{-1} y^{-1}, and TN 0.11–0.69 kg ha^{-1} y^{-1}; fen—NH_4^+ 0.01–0.16 kg ha^{-1} y^{-1}, NO_3^- 0.29–0.48 kg ha^{-1} y^{-1}, and TN 1.14–1.61 kg ha^{-1} y^{-1}. Soil N content depended on location within the bog or fen, and on soil depth. DN and DEA rates were low throughout the uplands and peatlands, and were correlated with atmospheric N deposition, soil N storage, and N outflow. DEA was significantly greater than DN indicating C or N limitation of the denitrification process. We highlight differences between the bog and fen, between the upland mineral soils and peat, and the importance of biogeochemical hotspots within the peatlands. We point out the importance of organic N storage, as a source of N for denitrification, and propose a plausible link between organic N storage,

* Corresponding author at: USEPA/NHEERL/MED, 6201 Congdon Blvd., Duluth, MN 55804, USA.
E-mail address: hill.brian@epa.gov (B.H. Hill).
[1] Current address: AMI Consulting Engineers, Superior, WI 55792, USA.

http://dx.doi.org/10.1016/j.scitotenv.2016.01.178
0048-9697/Published by Elsevier B.V.

denitrification and N export from peatlands. Finally, we considered the interactions of microbial metabolism with nutrient availability and stoichiometry, and how N dynamics might be affected by climate change in peatland ecosystems.

1. Introduction

The role of wetlands, especially peatlands, in the global carbon budget has been widely discussed (Gorham, 1991; Bridgham et al., 2006; Kayranli et al., 2010), but less attention has been paid to the corresponding role of peatlands in nitrogen cycling (Drewer et al., 2010; Worrall et al., 2012; Loisel et al., 2014). Northern peatlands are particularly sensitive to N additions, owing to their unique hydrological and biogeochemical properties leading to carbon and nutrient limitation (Urban and Eisenreich, 1988; Bragazza et al., 2012; Sheppard et al., 2013; Toberman et al., 2015). Peatlands are divided into two broad classes on the basis of pH and hydrology (Gorham et al., 1985; Bridgham et al., 1996). Minerotrophic fens have ground and surface water inputs, are slightly acidic to neutral pH, while ombrotrophic bogs receive water and nutrients primarily from atmospheric deposition and are acidic. Both fens and bogs generally are dominated by *Sphagnum* mosses, with fens having a much more diverse understory plant community than bogs Gorham et al., 1985. Fens range from open to forested while bogs are generally forested (Bridgham et al., 1996). Both bogs and fens have distinct vertical zonation, with actively photosynthesizing vegetation lying above a transiently aerobic peat zone (the acrotelm), and this underlain by a layer of anaerobic peat (the catotelm; Limpens et al., 2008). Bogs have an added topographic feature called the lagg, which is the interface of the bog with the toe of the upland slope. The lagg has been identified as a biogeochemical “hot spot”, a zone that has particularly high biogeochemical processing rates such as mercury methylation (Mitchell et al., 2008, 2009). A feature unique to some northern temperate peat bogs is the presence of speckled alder (*Alnus rugosa*) in the lagg. The nitrogen-fixing alder is suspected of pumping N into the lagg, further enhancing this biogeochemical hot spot (Compton et al., 2003; Eickenscheidt et al., 2013). Similarly, in both fens and bogs, hummocks (areas of peat raised above the mean peat surface) and hollows (at the mean peat surface) are biogeochemically distinct, with hollows having significantly greater respiration and mercury methylation than the hummocks (Waddington and Roulet, 1996; Branfireum, 2004).

There are four sources of N to peatlands, atmospheric deposition, mineralization, N-fixation, and upwelling from regional groundwater (Verry and Timmons, 1982; Bridgham et al., 1996). For bogs, all N inputs are via atmospheric deposition, watershed mineralization and N-fixation, but for fens some N inputs also occur as groundwater upwelling or surface water inflows (Verry and Timmons, 1982; Bridgham et al., 1998). Peatlands accumulate C as is evident from their global significance in soil C budgets, but they also store large quantities of N and P. Verry and Timmons (1982) reported that 56% of total N (80–90% of inorganic N) and 76% of total P inputs to a bog were retained rather than exported. Bridgham et al. (1998) reported that, while N and P stores in peatlands were large, the available fractions were much smaller and tightly cycled, leading to relative N and P limitations on productivity.

By the above accounting, nearly 50% of N inputs to peatlands are exported, mostly as organic forms (Urban et al., 1988; Seitzinger, 1994; Hayden and Ross, 2005; Keller and Bridgham, 2007; Worrall et al., 2012). Denitrification accounts for less than 5% of nitrate removal from bogs (Urban et al., 1988; Keller and Bridgham, 2007). These authors attribute low denitrification rates to low nitrate availability and low pH (Hayden and Ross, 2005; Keller and Bridgham, 2007).

Our goals for this research were to compare the soil N inputs, storage and outflows of two peatlands, an ombrotrophic bog and a minerotrophic fen, and to compare the relative importance of N gains and losses to the overall soil N budget of peatlands and their watersheds. We also compare N storage and outflows of the upland watersheds to those of the bog/fen and quantify the contribution to the different soil strata to the overall bog and fen N budget.

2. Methods

2.1. Study sites

We studied two peatland watersheds within the US Department of Agriculture's Forest Service Marcell Experimental Forest (MEF; N 47° 30.17′, W 93° 28.97′), located approximately 40 km north of Grand Rapids, Minnesota, USA (Fig. 1). The MEF is within the Laurentian Mixed Forest Province, which is a transitional zone between boreal forests to the north and broadleaf deciduous forests to the south (Verry et al., 2011). The landscape is a typical low-relief, moraine landscape of the Upper Great Lakes Region, and includes uplands, peatlands, and lakes. Peatlands at the MEF range in size from several hectares to several hundreds of hectares and may have forest, shrub, or sedge cover. The MEF has an extensive historical database of hydrology, chemistry, and meteorology that documents ecosystem processes since the early 1960s (Sebestyen et al., 2011). The climate is sub-humid continental, with wide and rapid diurnal and seasonal temperature fluctuations. Over the period of record (1961–2009), the average annual air temperature was 3 °C, with daily mean extremes of −45 °C and 38 °C, and the average annual precipitation was 780 mm, most of which fell as rain from mid-April to early November. Mean annual air temperatures have increased about 0.4 °C per decade over the last 50 y (Sebestyen et al., 2011).

Our two study peatlands include an ombrotrophic bog and a minerotrophic fen. The two peatlands are within 2 km of one another and have similar dominant aspects, watersheds with <20 m of topographic relief, and mixed conifer-deciduous forest covers. The bog watershed, designated as S2, contains a 3.2 ha bog (black spruce, *Picea mariana* on the bog; some areas of dense speckled alder, *Alnus incana*; *Sphagnum* sp. in the lagg) and a 6.5 ha upland (quaking aspen, *Populus tremuloides*; paper birch, *Betula papyrifera*). Mean annual stream pH at the watershed outlet averages 4.1 (Urban et al., 2011). The bog watershed was instrumented for measurement of precipitation volume, surface and sub-surface runoff, bog (perched) water level, and depth to the regional water table starting in the 1960s, and has served as the undisturbed reference watershed for the southern MEF study unit (Sebestyen et al., 2011). Streamflow has been measured at a 120 degree V-notch weir (Sebestyen et al., 2011).

The fen watershed, designated as S3, has an 18.6 ha fen (willow, *Salix* sp.; *A. incana*, *P. mariana*, white cedar, *Thuja occidentalis*) surrounded by a 53.4 ha upland (*P. tremuloides*; *B. papyrifera*; balsam fir, *Abies balsamea*; jack pine, *Pinus banksiana*; red pine, *Pinus resinosa*). Mean annual stream pH at the outlet averages 6.9 (Urban et al., 2011). The fen was instrumented for measurement of precipitation volume, fen water level, and depth to the regional water table starting in the 1960s. Although instantaneous streamflow has occasionally been measured, monthly, annual, and yearly streamflow amount is estimated from a fen water level-discharge relationship (Sebestyen et al., 2011). The entire fen (but not the uplands) was clear-cut and the slash was burned in 1972–1973 (Sebestyen et al., 2011). Water yields did not change after harvesting and nutrient concentrations had returned to pre-harvest levels by 1976 (Sebestyen and Verry, 2011).

The bog peatland is composed of Loxley soils (Dysic, Frigid Typic Haplosaprist) with an Oi horizon from 0 to 20 cm, Oe horizon from

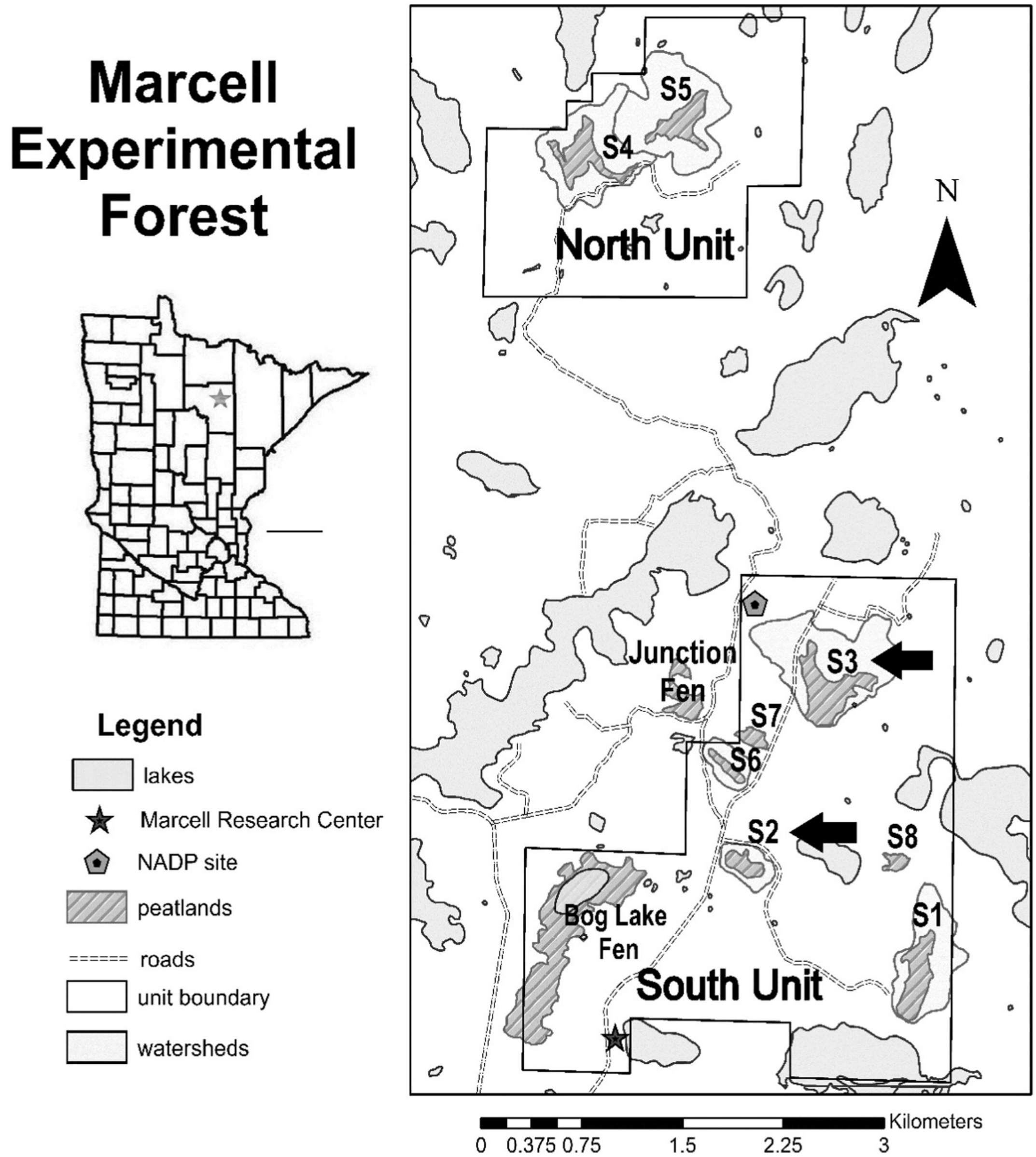


Fig. 1. Map of the Marcell Experimental Forest in northcentral Minnesota (USA). The black arrows indicate the locations of the study bog (S2) and fen (S3) watersheds.

20 to 30 cm, Oa horizon from 30 to 41 cm, Oe horizon from 41 to 43 cm, Oa horizon from 43 to 325 cm, and an Oe horizon from 325 to 362 cm which was the bottom of the peat profile. The fen peatland is composed of Lupton soils (Euic, Frigid Typic Haplosaprist) with an Oa horizon from 0 to 18 cm, Oe horizon from 18 to 56 cm, and an Oa horizon from 56 to 386 cm which was the bottom of the peat profile. Both soil descriptions indicate an abrupt boundary between the bottom of the peat and mineral material below (Soil Classification Database, http://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/survey/class/data/?cid=nrcs142p2_053583).

2.2. Bog and fen area and volume

The areal extent of the bog and fen were estimated from wetland spatial boundary data (Minnesota Geospatial Commons, https://gisdata.mn.gov/). The extent of lagg in the bog and transition zone in the fen was based on field measurements, and these widths were used to calculate the percentage of the bog and fen in these zones, respectively. The percent of the bog and fen supporting speckled alder was estimated by analyzing very high resolution (1.85 m) imagery obtained from the World View 2 satellite (https://www.digitalglobe.com/). The images were analyzed using ArcGIS maximum likelihood classification tools (http://resources.arcgis.com/en/home/).

Bog and fen volumes were calculated as the interpolated product of area and depth of peat. Peat depths for the fen were measured on an approximately 50 m grid by inserting a steel probe into the peat until it met with resistance from the underlying mineral soils. Peat depths for the bog were based on previous measurements (Verry and Janssens, 2011). Average peat depth in the bog lagg and bog proper were 0.75 m and 2.61 m, while those in the transition zone and fen were 1.14 m and 1.46 m, respectively (Fig. 2).

2.3. Atmospheric deposition and outflow water chemistry

Precipitation event samples were analyzed for nitrate (NO_3^-), ammonium (NH_4^+), and total N (TN). These values were multiplied by the precipitation volume for each event or series of events that were sampled, and the event-based estimates were aggregated to annual

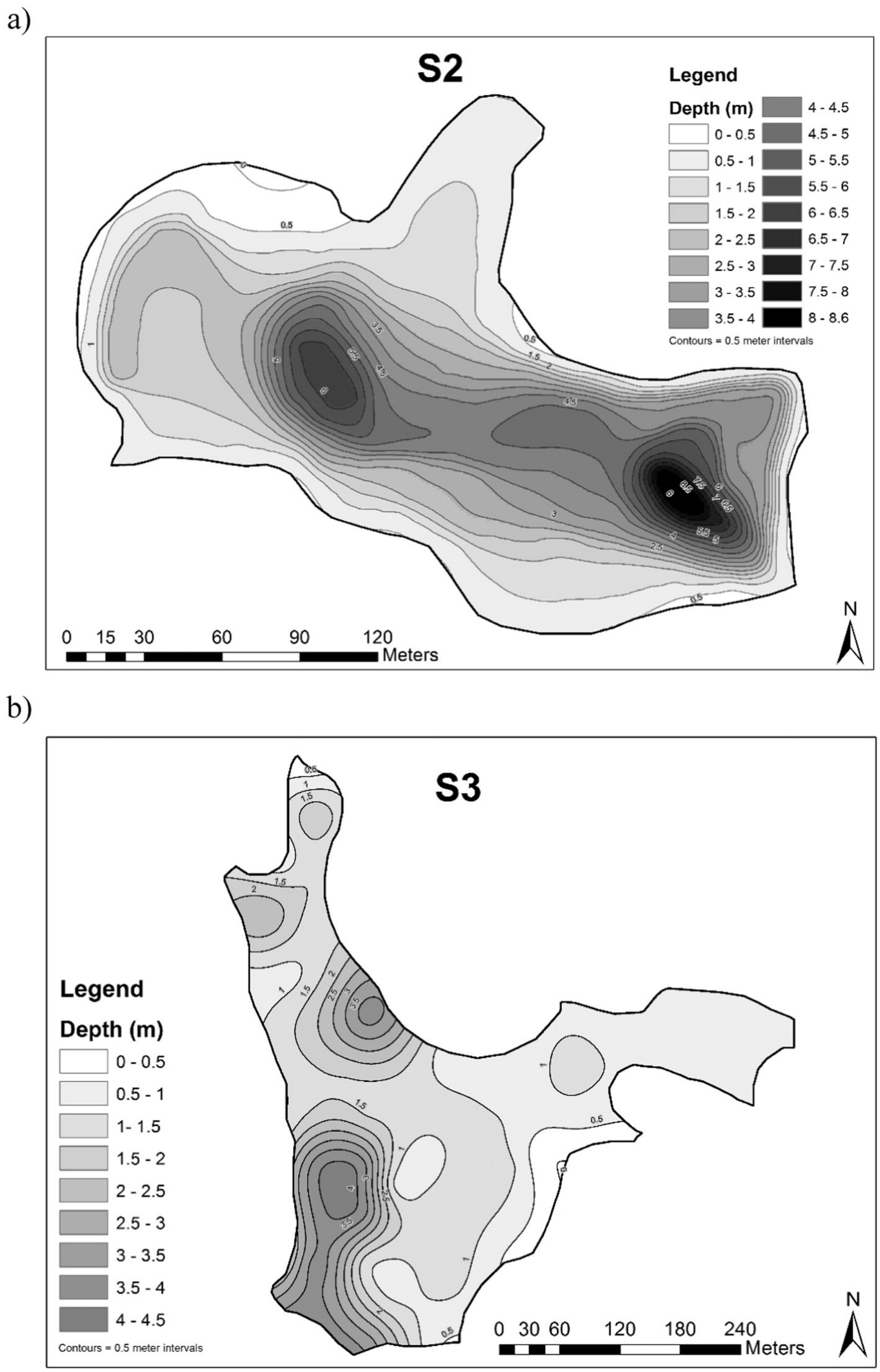


Fig. 2. Peat depth profiles for the a) bog (S2) and b) fen (S3) at the Marcell Experimental Forest. Depth data for the fen were collected by the authors; data from the bog were derived from digitized maps presented in Verry and Janssens, 2011).

deposition for this study. Stream water samples from the bog (weekly and event) and fen (biweekly) were collected as surface (<15 cm) grab samples during the 2010–2013 study. The larger number of samples from bog reflects a focus on stormflow-runoff events as well as weekly sampling for other studies at the bog watershed versus a long-term biweekly sampling scheme for the fen watershed (Sebestyen et al., 2011). We analyzed each sample for NO_3^-, NH_4^+, and TN concentration on unfiltered samples that were refrigerated until analyzed (<7 d). Subsamples for measurement of TN concentrations were digested using the persulfate method, and were analyzed using the cadmium reduction method (QuikChem method 10-107-04-1-P; Lachat Instruments, 2009; APHA, 1998). Dissolved nitrate + nitrite concentrations were measured using the cadmium reduction method (QuikChem 10-107-04-1-C, Lachat Instruments, 2009; APHA, 1998) and ammonium by the salicylate method (QuikChem Method 10-107-06-1-C; Lachat Instruments, 2009).

2.4. Soil collection and chemistry

Soil samples were collected along downslope transects from the uplands, through the lagg/transition zone, and into the bog/fen center. Upland soil samples were collected with either a Plexiglas tube corer or a shovel, and peat samples were collected using a Russian-style peat corer (Rickly Hydrological Company, Columbus, OH). Soil samples from the bog and uplands were collected once each in May, July and October 2010; May, June, August and October 2011; and in May, July and October 2012. Similar core samples from the fen and uplands were collected in May, August and October 2011; and in June, August and October 2012 and 2013. On each sampling date, triplicate soil samples (corer: 3.8 cm diameter, 30 cm depth; shovel: 100 cm^2, 30 cm depth) from the upland sites were divided, based on soil texture and color, into O, A, and B soil horizons. Triplicate cores (5 cm diameter, 60 cm depth [10 cm surface; 50 cm core]) were collected each from the bog (lagg, hummocks, and hollows) and fen (transitional zone, hummocks, and hollows). These cores were divided into the actively growing surface moss; the lighter colored, more fibric, predominantly aerobic acrotelm; and the darker colored, more hemic and sapric, predominantly anaerobic catotelm horizons (Clymo, 1984). Given the fibric nature of *Sphagnum* peat, there is some potential for compression of the surface and acrotelm horizons. To minimize this potential sampling error, we removed the top 10 cm of *Sphagnum* prior to coring the peat.

We analyzed soil samples for NH_4^+, NO_3^-, and TN. All N extracts/digests were analyzed using a Lachat flow-injection analyzer. Field moist subsamples were extracted with 2 M potassium chloride (KCl) for available NO_3^- and NH_4^+ (Keeney and Nelson, 1986); extracts were analyzed using the cadmium reduction and the salicylate methods (QuikChem Methods 12-107-04-1E and 10-107-06-2-C; Lachat Instruments, 2009), respectively. Soil water content was determined by gravimetric methods using a drying oven at 60 °C for 24 h to a constant weight. The percent solids were used to calculate available N content on a dry weight basis. Soil samples were dried and ground (mortar and pestle) for TN analysis and were analyzed by combustion using a Model 1112 EA Carla Erba elemental analyzer. Soil bulk density (BD, g cm^{-3}) was calculated as the dry mass of the sample divided by the volume of the sample (Arshad et al., 1996).

2.5. Ambient and potential denitrification

Fresh soil samples (30 cm^3; collected the previous day and stored at 4 °C) from each soil horizon were analyzed for ambient and potential denitrification rates by mixing soils with 50 mL of site water, 1 g L^{-1} final concentration of chloramphenicol, and glucose and NO_3^- amendments, as needed, in 125 mL bottles (Tiedje, 1982). Samples were capped and purged with N_2 gas for 5 min to achieve anaerobic conditions within the sample bottles. Acetylene (C_2H_2; 10 mL) was added to each sample bottle to inhibit N_2O reduction to N_2, marking the start of the assay. We measured both ambient and potential denitrification rates on soil samples from uplands and peatlands in the two study watersheds. Ambient denitrification rates (DN) were estimated from soil samples that had not been amended with glucose or NO_3^-. Potential denitrification rates (DEA) were estimated from soil samples amended with both glucose and NO_3^-. Amendments were done by adding KNO_3 and glucose, with final concentrations 1 mM L^{-1} above background. Completion of the denitrification process was also determined for both DN and DEA rates following the same method above, but without the addition of C_2H_2. Headspace gas (10 mL) was sampled after 1, 2, 3, and 4 h of incubation. Gas samples were stored in purged and evacuated headspace vial for N_2O analysis within 24 h of collection by gas chromatography using micron electron capture detector and HP PLOTQ column. To maintain pressure within media bottle, 10 mL of 1:5 C_2H_2:N_2 gas mix or 10 mL of N_2 was added back after each sample point.

2.6. N mass balance

Nitrogen mass balances for the bog and fen watersheds at the Marcell Experimental Forest were based on mean annual atmospheric deposition and outflow volume and chemistry; soil and peat standing stocks of N, and mean annual estimates of denitrification. All estimates are based on the weighted contributions of the uplands, lagg or transition zone, and bog or fen hollows and hummocks, and accounting for the weighted differences between soil horizons. The N mass balance for the bog and fen was:

$$N_{dep} + N_{fix} + RO + GW_I = \Delta N + DN + N_{vol} + GW_E + N_{exp} \quad (1)$$

where N_{dep} is atmospheric N deposition directly on the bog or fen, N_{fix} is N fixation by bog/fen vegetation, RO is the combined surface and subsurface N runoff from the uplands to the bog or fen, GW_I is groundwater N upwelling into the fen (this term is zero for the bog), GW_E is deep seepage from the bog and fen to groundwater, ΔN is the change in N storage within the bog or fen between years, N_{vol} is N losses through volatization (primarily NH3), DN is denitrification losses from the bog or fen, and N_{exp} is N leaving the bog or fen at the weir. All fluxes are kg N ha^{-1} y^{-1}.

The N mass balance for the upland that drains to the bog and fen was:

$$N_{dep} = \Delta N + DN + GW_E + RO \quad (2)$$

These mass balance calculations were run for the bog and fen, their uplands, and combined for bog and fen watersheds.

2.7. Statistical analyses

We computed monthly, seasonal, and annual means for atmospheric N deposition, watershed N storage and outflow, and for microbial N processing. We tested for differences within the bog (lagg, hummocks, hollows) and fen (transitional, hummocks, hollows), and for differences between the bog versus the fen watersheds, uplands versus the bog and fen, years, seasons, locations, soil horizons, and their interactions, using a Type III General Linear Model analysis of variance. The Type III model is appropriate for unbalanced, nested sampling designs in which there is significant interaction among the main effects. We evaluated the relationships between atmospheric N deposition, watershed N storage, outflow N and soil microbial activity using Spearman rank correlation (r) to avoid problems associated with non-normal data distribution. All analyses were done using SAS for Windows, release 9.2 (SAS Institute, Inc., Cary, NC, USA).

3. Results

3.1. Bog and fen area and volume

The 3.2 ha bog is partitioned into a central bog (3.04 ha) and a fringing lagg (0.16 ha). The volume of peat in the acrotelm (ca. 0–30 cm) and catotelm (ca. >30 cm) was: center bog acrotelm 9120 m^3, center bog catotelm 71,280 m^3; lagg acrotelm 480 m^3, lagg catotelm 720 m^3 (Table 1a). The 18.6 ha fen consists of a 16.8 ha central fen surrounded by a 1.81 ha transition zone (geographically similar to, but functionally different from a lagg). The volume of peat in the fen acrotelm (ca. 0–30 cm) and catotelm (ca. >30 cm) was: center fen acrotelm 34,976 m^3, center fen catotelm 137,018 m^3; transition acrotelm 5086 m^3, transition catotelm 15,553 m^3 (Table 1a).

3.2. Atmospheric deposition and outflow water chemistry

Annual precipitation (PPT) ranged from 710 to 831 mm y^{-1} (Table 1b), with the bulk of PPT delivered during June–August (Appendix A). Annual deposition of N species was: NH_4^+ 0.85–1.57 kg N ha^{-1} y^{-1}; NO_3^- 0.85–1.76 kg N ha^{-1} y^{-1}; TN 1.01–3.15 kg N ha^{-1} y^{-1} (Table 1b). NH_4^+ deposition on the bog and fen was highest in July and lowest in the winter months; NO_3^- deposition was highest in the spring and early summer and lower in the autumn

Table 1
a) Bog and fen upland, peatland, and total watershed area and the percentage of the bog cover by speckled alder (% *Alnus incana*).; mean (2010–2013) depth to water table; and estimated bog/fen volume in the lagg/transition zone, and by peat layers (acrotelm and catotelm); b) mean annual (2010–2013) precipitation (PPT) and atmospheric deposition of NH_4^+, NO_3^-, and TN to the watersheds; c) mean watershed outflow chemistry during the May–October study periods; and d) mean annual soil bulk density and watershed storage of NH_4^+, NO_3^-, and TN for the bog and the fen watersheds at the Marcell Experimental Forest.

a) Physical properties		
	Bog	Fen
Watershed area		
Peatland, ha	3.2	18.6
Bog/fen	3.04	16.8
Lagg/transition	0.16	1.81
% *Alnus*	3.00	10.9
Upland, ha	6.5	53.4
Total, ha	9.7	72.0
Depth to water table		
Bog/Fen hollow, cm	15.6	16.8
Peatland volume		
Acrotelm, m^3		
Bog/fen	9120	34,976
Lagg/transition	480	5086
Catotelm, m^3		
Bog/fen	71,280	137,018
Lagg	720	15,553

b) Atmospheric deposition (measured on the bog watershed)				
	2010	2011	2012	2013
PPT, mm y^{-1}	831	710	764	741
NH_4, kg ha^{-1} y^{-1}	1.57	1.08	0.85	1.24
NO_3, kg ha^{-1} y^{-1}	1.76	0.85	0.98	1.37
TN, kg ha^{-1} y^{-1}	3.05	2.05	1.01	3.15

	Bog watershed				Fen watershed			
	2010	2011	2012	2013	2010	2011	2012	2013
c) Annual outflows								
Water, m^3 ha^{-1} y^{-1}	1176	1788	1269	1321	4828	7266	10,450	11,091
NH_4^+, kg ha^{-1} y^{-1}	0.01	0.05	0.03	0.34	0.13	0.36	0.01	0.02
NO_3^-, kg ha^{-1} y^{-1}	0.05	0.03	0.01	0.06	0.25	1.27	0.31	0.30
TN, kg ha^{-1} y^{-1}	0.09	0.10	0.11	0.74	1.45	2.20	0.48	1.36
d) Watershed storage								
Upland soils								
Bulk density, g cm^{-3}	1.15	1.29	0.80	–	–	0.97	0.42	0.85
NH_4^+, kg m^{-3}	0.003	0.002	0.002	–	–	0.001	<0.001	0.001
NO_3^-, kg m^{-3}	0.001	<0.001	<0.001	–	–	<0.001	<0.001	<0.001
TN, kg m^{-3}	2.31	2.57	1.27	–	–	5.61	0.49	1.05
Peat								
Bulk density, g cm^{-3}	0.12	0.11	0.07	–	–	0.55	0.21	0.24
NH_4^+, kg m^{-3}	0.005	0.007	0.005	–	–	0.003	0.001	0.002
NO_3^-, kg m^{-3}	<0.001	<0.001	<0.001	–	–	<0.001	<0.001	<0.001
TN, kg m^{-3}	1.61	1.93	1.22	–	–	5.94	0.98	1.57

and winter; and TN deposition was highest in late summer and lowest in the autumn and winter (Appendix A).

Annual outflows of N from the bog were: NH_4^+ 0.01–0.34 kg ha^{-1} y^{-1}, NO_3^- 0.01–0.06 kg ha^{-1} y^{-1}, and TN 0.09–0.74 kg ha^{-1} y^{-1}. Outflows from the fen were: NH_4^+ 0.01–0.36 kg ha^{-1} y^{-1}, NO_3^- 0.25–1.27 kg ha^{-1} y^{-1}, and TN 0.48–2.20 kg ha^{-1} y^{-} (Table 1c). NH_4^+ outflows exhibited significant watershed, seasonal and interaction effects, being generally higher in fen than in the bog and higher in summer than for the remainder of the year; NO_3^- and TN outflows revealed significant watershed, year, season, and interaction effects, with higher outflows from the fen than from the bog; NO_3^- generally higher in the spring and summer than in the autumn and winter with TN yields higher in the spring compared to the rest of the year (Table 2, Appendix A). Outflow total N was positively correlated with atmospheric N deposition (Table 3a).

3.3. Soil physical and chemical properties

Mean bulk density, NH_4^+ and NO_3^- content of the upland soils were similar between the bog and fen (Table 1d). Bulk density of bog peat was less than that of the fen peat, and bog peat contained more NH_4^+ than did fen peat. Otherwise, the N content of bog and fen peat was similar (Table 1d).

Soil bulk density, NO_3^-, and TN exhibited significant watershed, year, season, location, depth, and interaction effects; NH_4^+ revealed significant location, depth and interaction effects (Table 2, Appendix B). In general, soil N concentrations were higher in the bog watershed than in the fen watershed and were variable across the years of the study (Appendix B). Within the bog, hollow and hummock, NH_4^+, NO_3^-, and TN were similar, but lower than in the lagg. Lagg with alder had greater N stores than did the lagg without alder (Table 4). These trends did not hold for the fen, where the hollows had greater NH_4^+ content; the hummocks had greater TN content; and there were no differences in NO_3^- content of the peat (Table 4). Unlike the lagg, which had greater N stores

Table 2
Summary statistics of the Type III General Linear Model analysis of an unbalanced, nested sampling design for outflow water and soil chemistry for the bog and the fen at the Marcell Experimental Forest. Samples were collected May–October 2010–2013. Significant watershed, season, soil horizon and interaction effects are indicated by bold *P* values.

Variable	Effects	df	*F*	*P*
Outflow water chemistry				
NH_4^+	Watershed	1	54.3	**<0.0001**
	Year	3	50.4	**<0.0001**
	Season	2	6.43	**<0.0001**
	Season × (watershed × year)	11	7.99	**<0.0001**
NO_3^-	Watershed	1	340	**<0.0001**
	Year	3	89.5	**<0.0001**
	Season	2	7.40	**0.0007**
	Season × (watershed × year)	11	14.2	**<0.0001**
TN	Watershed	1	97.0	**<0.0001**
	Year	3	18.5	**<0.0001**
	Season	2	13.0	**<0.0001**
	Season × (watershed × year)	11	6.32	**<0.0001**
Soil density and chemistry				
BD	Watershed	1	30.31	**<0.0001**
	Year	3	37.8	**<0.0001**
	Season	2	3.29	**0.0380**
	Soil horizon	5	140	**<0.0001**
	Soil horizon × (year × season)	58	2.55	**<0.0001**
NH_4^+	Watershed	1	75.5	**<0.0001**
	Year	3	1.73	0.1605
	Season	2	0.06	0.9408
	Soil horizon	5	17.8	**<0.0001**
	Soil horizon × (year × season)	58	0.84	0.7952
NO_3^-	Watershed	1	0.34	0.5616
	Year	3	15.1	**<0.0001**
	Season	2	16.2	**0.0010**
	Soil horizon	5	20.4	**<0.0001**
	Soil horizon × (year × season)	58	4.17	**<0.0001**
TN	Watershed	1	11.7	**0.0007**
	Year	3	4.59	**0.0035**
	Season	2	3.73	**0.0246**
	Soil horizon	5	8.03	**<0.0001**
	Soil horizon × (year × season)	58	1.27	0.0942

Table 3
Spearman correlations (r_S) of a) atmospheric N deposition with outflow N and soil N stores; and biological N processes (DN, DEA; μmol g^{-1} DW d^{-1}) with b) NH_4^+, NO_3^-, and TN in atmospheric N deposition; c) watershed N storage; and d) outflow N for the bog and fen watersheds at the Marcell Experimental Forest during the 2010–2013 sampling seasons. The r_S for significant ($P < 0.05$) correlations is reported; non-significant correlations are indicated by dashes (–).

a) N deposition vs. N outflow and storage						
Deposition (kg ha^{-1} y^{-1})	Bog watershed			Fen watershed		
	NH_4^+	NO_3^-	TN	NH_4^+	NO_3^-	TN
Outflow N						
NH_4^+, kg ha^{-1} y^{-1}	−0.11	0.58	−0.13	0.68	0.60	–
NO_3^-, kg ha^{-1} y^{-1}	0.81	0.46	0.83	0.70	0.34	0.30
TN, kg ha^{-1} y^{-1}	0.86	0.48	0.87	0.89	0.21	0.48
Soil N storage						
NH_4^+, kg ha^{-1}	–	–	–	–	–	–
NO_3^-, kg ha^{-1}	0.44	0.30	0.49	–	–	–
TN, kg ha^{-1}	−0.12	–	–	–	–	–

	Bog watershed		Fen watershed	
	DN	DEA	DN	DEA
b) Microbial N processes vs. N deposition				
Upland soils				
NH_4, kg ha^{-1} y^{-1}	–	–	–	–
NO_3, kg ha^{-1} y^{-1}	−0.45	–	–	–
TN, kg ha^{-1} y^{-1}	–	–	–	–
Peat				
NH_4, kg ha^{-1} y^{-1}	–	−0.22	0.30	−0.22
NO_3, kg ha^{-1} y^{-1}	–	0.19	−0.19	–
TN, kg ha^{-1} y^{-1}	–	−0.18	–	−0.16
c) Microbial N processes vs. soil N stores				
Upland soils				
Bulk density, g cm^{-3}	0.35	−0.50	–	0.65
NH_4^+, kg m^{-3}	–	0.81	–	0.63
NO_3^-, kg m^{-3}	–	–	–	–
TN, kg m^{-3}	–	0.76	–	0.52
Peat				
Bulk density, g cm^{-3}	0.13	0.18	–	−0.33
NH_4^+, kg m^{-3}	0.31	0.42	0.14	0.50
NO_3^-, kg m^{-3}	–	−0.13	0.16	–
TN, kg m^{-3}	0.21	0.43	0.15	0.51
d) Microbial N processes vs. outflow N				
NH_4^+, μg L^{-1}	−0.16	0.25	0.26	–
NO_3^-, μg L^{-1}	–	−0.12	0.24	−0.13
TN, μg L^{-1}	–	−0.13	0.29	–

than the bog or the upland, the fen transition zone appears to be a gradient from the lower upland N stores to the greater N content of peat in the central fen (Table 4). There were significant depth differences in soil bulk density and N stores in both the upland and the peatland soils. Regardless of location, soil bulk density increased with increasing depth. Upland soil N stores decreased with increasing depth, but the opposite was generally true for bog and fen peat, with the exception of TN which either increased with depth or exhibited no depth response (Table 4). With the exception of NO_3^-, soil N stores were not correlated with atmospheric N deposition (Table 3a).

3.4. Ambient and potential denitrification

We report ambient and potential denitrification rates as both μmol g^{-1} dry weight (DW) d^{-1} and μmol m^{-3} d^{-1}. The former value is a standard expression of microbial N processing rates and the latter value adjusts the N processing rates to available soil C pools (bulk density × soil C content) which facilitates the estimation of N flux (kg N ha^{-1} y^{-1}) and watershed N balances (kg N ha^{-1}). Ambient denitrification (DN) was low across most of the bog and fen study locations, with upland soil DN generally lower than peat DN, especially DN in the

Table 4
Mean soil horizon depth (Z, cm), bulk density (BD, g cm^{-3}) and soil N chemistry (mg kg^{-1}) for upland and bog/fen soil horizons from the Marcell Experimental Forest. Samples were collected May–October 2010–2013.

Location	Horizon	Z	BD	NH_4^+	NO_3^-	TN
Bog watershed						
Upland	O-horizon	0–5	0.61	4.80	0.70	5178
	A-horizon	6–14	1.37	2.35	0.42	1383
	B-horizon	>14	1.41	0.98	0.12	1021
Lagg w/alder	Surface	0–13	0.10	58.1	2.40	19,649
	Acrotelm	14–32	0.13	96.9	1.05	19,330
	Catotelm	>32	0.18	130	0.79	18,416
Lagg w/o alder	Surface	0–18	0.07	26.0	1.97	16,300
	Acrotelm	19–27	0.11	54.2	0.76	18,710
	Catotelm	>27	0.14	84.9	0.52	18,781
Bog hollow	Surface	0–11	0.06	14.2	7.04	9445
	Acrotelm	12–31	0.08	35.1	7.30	13,945
	Catotelm	>31	0.10	40.1	3.00	17,555
Bog Hummock	Surface	0–20	0.06	17.5	7.02	9314
	Acrotelm	21–46	0.07	29.7	5.33	13,229
	Catotelm	>46	0.10	43.7	4.24	16,504
Fen watershed						
Upland	O-horizon	0–5	0.37	4.58	0.74	8257
	A-horizon	6–12	0.91	0.87	0.16	2086
	B-horizon	>12	1.03	0.64	0.16	2544
Transitional	Surface	0–7	0.14	12.7	2.04	18,882
	Acrotelm	8–14	0.53	8.70	0.71	6675
	Catotelm	>14	1.24	4.41	0.32	2210
Fen hollow	Surface	0–14	0.08	31.6	3.57	28,794
	Acrotelm	15–25	0.14	32.9	2.82	21,163
	Catotelm	>25	0.73	17.2	0.82	12,760
Fen hummock	Surface	0–14	0.05	9.37	4.46	33,650
	Acrotelm	15–24	0.10	8.73	4.74	34,322
	Catotelm	>24	0.22	24.3	2.05	30,303

two lagg categories (Tables 4 and 5). Changes in DN with soil depth were variable and inconclusive (Tables 4 and 5). DN was highest in the spring and lowest in the summer in both the bog and fen watersheds (Appendix C). DN was correlated with bulk density of upland and bog soils, with bog peat NH_4^+ and TN, and with fen peat NH_4^+, NO_3^-, and TN (Table 6c). With a few exceptions, DN was not correlated with atmospheric N inputs, but was correlated with NH_4^+ outflows from the bog and with each N form in outflows from the fen (Table 3b and d).

Potential denitrification (DEA) in the bog and fen watersheds followed an upland, hummock, hollow, transition zone or lagg gradient, with the highest DEA values measured in the lagg containing the N-fixing speckled alder (Table 5). DEA exhibited significant inter-annual, location and interaction differences, but lacked seasonal or depth effects (Tables 5, 6, Appendix C). DEA was significantly greater than DN indicating C or N limitation of the denitrification process. Given the abundance of C in upland and peatland soils, and the paucity of NO_3^- in these soils, it can be concluded that DN is N limited in both the bog and fen watersheds, as is confirmed by our denitrification rates based on N-amended samples (TM Jicha, manuscript in preparation). There were no correlations with atmospheric N inputs for DEA in upland soils of the bog or fen watersheds, but DEA was correlated with atmospheric inputs of NH_4^+, NO_3^- and TN for bog peat and with NH_4^+ and TN for fen peat (Table 3b). DEA was correlated with bulk density and with N content of upland soils and peat in both the bog and fen watersheds (Table 3c); and DEA was correlated with NH_4^+, NO_3^- and TN outflows from the bog, and with NO_3^- outflows from the fen (Table 3d).

3.5. Annual soil N mass balance

We constructed simplified soil N budgets for the bog and fen watersheds at the Marcell Experimental Forest based on area- and depth-weighted annual N flux and storage, and assuming that microbial N processing rates were zero during the time of year when surface soil horizons were frozen (generally December–April). N inputs to the bog

Table 5
Mean ambient denitrification (DN), and potential DN (DEA) presented as activity per unit mass (μmol g^{-1} DW d^{-1}) and as activity per unit bog volume (μmol m^{-3} d^{-1}) for upland and bog/fen soil horizons from the Marcell Experimental Forest. Samples were collected May–October 2010–2013.

		μmol g^{-1} DW d^{-1}		μmol m^{-3} d^{-1}			
Location/Horizon		DN	DEA	DN	DEA		
Bog watershed							
Upland	O-horizon	<0.01	0.52	1.20	279		
	A-horizon	0.01	0.06	17.1	71.4		
	B-horizon	0.01	0.01	9.82	17.6		
Lagg w/alder	Surface	0.36	17.2	37.0	1362		
	Acrotelm	0.05	17.5	6.27	1397		
	Catotelm	0.02	5.22	2.81	507		
Lagg w/o alder	Surface	1.58	3.65	115	322		
	Acrotelm	0.68	9.66	80.0	935		
	Catotelm	0.91	3.14	135	334		
Bog hollow	Surface	<0.01	0.34	<0.01	16.3		
	Acrotelm	0.01	0.11	0.29	11.3		
	Catotelm	0.02	0.12	1.28	8.79		
Bog hummock	Surface	<0.01	0.15	0.03	3.88		
	Acrotelm	0.03	0.62	1.44	30.4		
	Catotelm	0.04	0.70	2.64	40.5		
Fen watershed							
Upland	O-horizon	0.01	0.12	3.81	27.8		
	A-horizon	0	<0.01	0	4.40		
	B-horizon	0	<0.01	0	0.72		
Transitional	Surface	0.01	3.83	1.61	424		
	Acrotelm	0.02	0.65	7.26	231		
	Catotelm	<0.01	0.07	2.77	46.6		
Fen hollow	Surface	0.05	7.87	2.98	416		
	Acrotelm	0.28	1.55	34.2	126		
	Catotelm	0.01	0.69	2.71	70.2		
Fen hummock	Surface	0.02	0.46	0.62	5.35	1.40	14.7
	Acrotelm	0.19	9.07	7.74	18.1	14.5	466
	Catotelm	0.09	7.10	8.50	23.2	11.4	553

watershed were primarily from atmospheric inputs (3.3 kg N ha^{-1} y^{-1}) with a smaller fraction attributed to N fixation by watershed vegetation (0.5 kg N ha^{-1} y^{-1}; Urban and Eisenreich, 1988; Table 7, Fig.3). These

Table 6
Summary statistics of the Type III General Linear Model analysis of an unbalanced, nested sampling design for ambient (DN) and potential (DEA) denitrification for the bog and the fen at the Marcell Experimental Forest. Samples were collected May–October 2010–2013. Activities are reported per unit peat mass (μmol g^{-1} DW d^{-1}) and per unit peat volume (μmol m^{-3} d^{-1}). Significant watershed, season, soil horizon and interaction effects are indicate by bold *P* values.

Variable	Effects	df	*F*	*P*
Activity per unit peat mass				
DN	Year	3	0.94	0.4202
	Season	2	5.94	**0.0028**
	Location	7	4.83	**<0.0001**
	Soil horizon	4	0.31	0.8684
	Soil horizon × (season × location)	66	1.75	**0.0005**
DEA	Year	3	15.6	**<0.0001**
	Season	2	0.18	0.8368
	Location	7	8.59	**<0.0001**
	Soil horizon	4	1.27	0.2814
	Soil horizon × (season × location)	66	1.32	0.0541
Activity per unit peat volume				
DN	Year	3	2.25	0.0821
	Season	2	7.02	**0.0010**
	Location	7	4.65	**0.0001**
	Soil horizon	4	0.04	0.9969
	Soil horizon × (season × location)	66	1.54	**0.0061**
DEA	Year	3	13.3	**<0.0001**
	Season	2	2.33	0.0985
	Location	7	15.0	**<0.0001**
	Soil horizon	4	1.93	0.1050
	Soil horizon × (season × location)	66	1.99	**<0.0001**

inputs were countered by DN losses and stream outflows from the watershed. We assumed that N gases released during DN, regardless of the soil depth at which DN was taking place, were lost from the watershed. DN losses from upland soils of the bog watershed were greatest in the B-horizon, and the sum of upland soil DN was equivalent to 12% of N inputs (Table 7, Fig. 3). DN was greater in the lagg than in the bog center, and DN increased with increasing peat depth in both locations. DN in the lagg ranged from 0.32–2.54 kg N ha^{-1} y^{-1}, equivalent to 97% of N inputs to the bog watershed. DN in the bog center ranged from <0.01–0.17 kg N ha^{-1} y^{-1}, representing 5% of the N inputs to the bog. N seepage to the regional water table was previously estimated at 0.45 kg N ha^{-1} y^{-1} (Boelter and Verry, 1977; Verry and Timmons, 1982). This loss was the equivalent of 12% of N inputs to the bog watershed. Stream export averaged 0.26 kg N ha^{-1} y^{-1} (7% of N inputs). Overall, the bog watershed was a net source of N to the atmosphere or the downstream environment, exporting more than twice as much N as it receives (Table 7, Fig. 3).

In addition to atmospheric N deposition and N fixation, N inputs to the fen watershed included 1.51 kg N ha^{-1} y^{-1} from the regional water table (Boelter and Verry, 1977; Verry and Timmons, 1982). Unlike the bog watershed, there were negligible denitrification losses from upland soils, the fen transition zone, or the central fen, with the greatest denitrification loss from the catotelm layer of peat (0.63 kg N ha^{-1} y^{-1}), representing the equivalent of 9% of the N inputs to the fen watershed. Deep seepage from the fen, which is a discharge zone for the regional water table, was assumed to be negligible and with no N loss via this pathway. Mean annual stream outflow was 1.34 kg N ha^{-1} y^{-1}, or 20% of annual N inputs. Overall. The fen is a net sink for N, exporting less than half of its N inputs (Table 7, Fig. 3). In both the bog and fen watersheds, the magnitude of N flux is dwarfed by N storage in watershed soils, which did not appear to vary between years (Tables 1d and 7).

4. Discussion

A soil nitrogen budget of our study bog was previously reported based on data collected across several studies spanning the 1970s and 1980s (Urban and Eisenreich, 1988). What has changed since that time is a nearly 40% reduction in atmospheric N deposition, from 5.4 kg N ha^{-1} y^{-1} to the present mean of 3.3 kg N ha^{-1} y^{-1} for our study watersheds. Both the earlier and current atmospheric N inputs are in the middle of the range (2.2–7.5 kg N ha^{-1} y^{-1}) of N deposition reported for peatlands in North America and Europe (Rosswall and Granhall, 1980; Hemond, 1983; Limpens et al., 2006). N fixation by bog vegetation and microbes has been reported as ranging from 0.5 to 10 kg N ha^{-1} y^{-1} (Rosswall and Granhall, 1980; Hemond, 1983; Urban and Eisenreich, 1988; Limpens et al., 2006). Since we did not measure nitrogen fixation in our study, we used the value of 0.5 kg N ha^{-1} y^{-1} reported for laboratory and field incubations from the same Marcell bog as in our study (Urban and Eisenreich, 1988), for the N budgets of both the bog and fen.

The remaining N input to the study watersheds comes from groundwater seepage. We did not measure groundwater seepage into either the bog or fen, instead we relied on previous research on the hydrology of these peatlands (Boelter and Verry, 1977; Verry and Timmons, 1982). Groundwater seepage into the bog is zero, but groundwater inflow to the fen has been estimated at 1.51 kg N ha^{-1} y^{-1} when normalized to the area of the fen watershed, though the contributing area for this groundwater upwelling is much larger than the watershed itself (Boelter and Verry, 1977; Verry and Timmons, 1982). Total N inputs to the bog are 3.83 kg N ha^{-1} y^{-1} compared to 5.34 kg N ha^{-1} y^{-1} to the fen, both of which are similar to N inputs reported from other North American and European peatlands (Rosswall and Granhall, 1980; Hemond, 1983; Limpens et al., 2006; Worrall et al., 2012).

Nitrogen inputs to the bog and fen are identical, with the exception of groundwater upwelling in the fen. Nitrogen outputs, however, differ markedly between the bog and fen. Nitrogen losses from the study

Table 7

Comparison of total nitrogen storage and flux (2010–2013) for soils from an ombrotrophic bog and a minerotrophic fen watershed at the Marcell Experimental Forest. N storage is reported as kg N ha^{-1}; N inputs and outputs as kg N ha^{-1} y^{-1}; (%) is the fraction of N inputs that are lost through denitrification, GW export, volatile N losses, and stream outflow.

			Bog	(%)	Fen	(%)
Storage						
	Upland	O-horizon	1410		1040	
		A-horizon	1494		1692	
		B-horizon	13,674		16,104	
	Bog/fen					
	Lagg/transition	Surface	2496		1477	
		Acrotelm	1690		918	
		Catotelm	10,045		13,158	
	Central peat	Surface	450		1422	
		Acrotelm	1299		1203	
		Catotelm	10,947		17,741	
Inputs						
	Deposition		3.33		3.33	
	N fixation[a]		0.50		1.82	
	GW import[b]		0		1.51	
	Total inputs		3.83		6.66	
Outputs						
	Denitrification					
	Upland	O-horizon	<0.01	(<1)	<0.01	(<1)
		A-horizon	0.05	(1)	0	(0)
		B-horizon	0.43	(11)	0	(0)
	Bog/fen					
	Lagg/transition	Surface	1.05	(27)	0.01	(<1)
		Acrotelm	0.32	(8)	0.02	(<1)
		Catotelm	2.54	(66)	0.12	(2)
	Central peat	Surface	<0.01	(<1)	0.08	(1)
		Acrotelm	0.02	(1)	0.12	(2)
		Catotelm	0.17	(4)	0.63	(9)
GW export[b]			0.45	(12)	0	
N volatilization			0.10	(3)	0.10	(2)
Outflow			0.26	(7)	1.34	(20)
Total outputs			5.30	(138)	2.42	(36)
Net ΔN			−1.47		4.24	

[a] Data from Urban and Eisenreich (1988); N fixation in the fen was assumed to be higher than in the bog because of 3.63× more alder in the fen (see methods).
[b] Data from Boelter and Verry (1977); Verry and Timmons (1982).

watersheds included denitrification, seepage to groundwater, volatile N losses other than denitrification, and surface outflows from the watersheds. Our estimates of denitrification were based on laboratory incubations of soil slurries using acetylene to block the final step of denitrification, the conversion of produced N_2O to N_2, and employing chloramphenicol to halt microbial synthesis of new enzymes used in denitrification (Tiedje, 1982; Groffman et al., 2006). This method has been criticized because the acetylene also inhibits nitrification thereby limiting the actual rate of denitrification via a coupled nitrification–denitrification process (Groffman et al., 2006) and because soil slurries disturb the physical structure and natural mixing dynamics of undisturbed soils. However, Bernot et al. (2003) reported no differences between sediment cores and sediment slurries when both used chloramphenicol. Despite these shortcomings, Groffman et al. (2006) found that this approach is valid for "comparisons of sites and experimental treatments." For the bog watershed, denitrification from the upland soils ranged from <0.01 to 0.43 kg N ha^{-1} y^{-1}, and increased with soil depth.

These values are similar to those reported for denitrification in well-drained, sandy soil forest soils, but an order of magnitude or more lower that rates from loam and clay loam forest soils (Muller et al., 1980; Groffman and Tiedje, 1986; Westbrook and Devito, 2004). Upland denitrification rates were equivalent to <1 to 11% of the nitrogen inputs to the watershed. Moving downslope from the upland to the bog, denitrification in the lagg zone ranged from 0.32 to 2.54 kg N ha^{-1} y^{-1}, equivalent to 8–66% of the N input to the bog watershed. These values are similar to those previously reported from this bog (Urban et al., 1988), and to those reported using similar methods from North American and European peatlands (0–4 kg N ha^{-1} y^{-1}; Rosswall and Granhall, 1980; Hemond, 1983; Hayden and Ross, 2005; Limpens et al., 2006).

None of these other studies of denitrification in peatlands considered the lagg and bog center, or hollow and hummock, locations separately. Urban and Eisenreich (1988) reported similar denitrification rates for the lagg and bog center, but these were based on an estimated maximum potential rate of denitrification. Mitchell et al. (2008, 2009) reported significantly greater rates of mercury methylation in the lagg zone of a bog located 1 km from our study bog. Similarly, we found significantly greater rates of denitrification in the lagg compared to either hollows or hummocks in the bog interior, supporting the idea that the lagg is a biogeochemical hot spot in the watershed (McClain et al., 2003; Mitchell et al., 2008, 2009).

These upland and transition zone differences were not as pronounced in the fen watershed, where upland soil denitrification ranged from 0–<0.1 kg N ha^{-1} y^{-1} and denitrification in the upland-bog transition zone was lower (0.01–0.12 kg N ha^{-1} y^{-1}) than in the fen center (0.08–0.63 kg N ha^{-1} y^{-1}). Denitrification in the fen watershed was on the lower end of the range of rates reported for European fens (Limpens et al., 2006), and was equivalent to <1 to 9% of the N inputs to the fen watershed.

The remaining N losses from the bog and fen watersheds are apportioned among surface water outflows, seepage into the regional groundwater pool, and volatile N losses that are not represented by denitrification. Measured surface water N outflows were equivalent to 7% and 20% of the N inputs to the bog and fen, respectively. We used previously measured seepage of waters from the bog and fen into the regional groundwater pool to estimate that groundwater seepage (Nichols and Verry, 2001). Groundwater seepage represented N losses equal to 12% and 7% of annual N inputs to the bog and fen, respectively. We did not measure volatile N losses in our study, but other researchers suggest that N volatilization, primarily as ammonia, ranges from 0 to

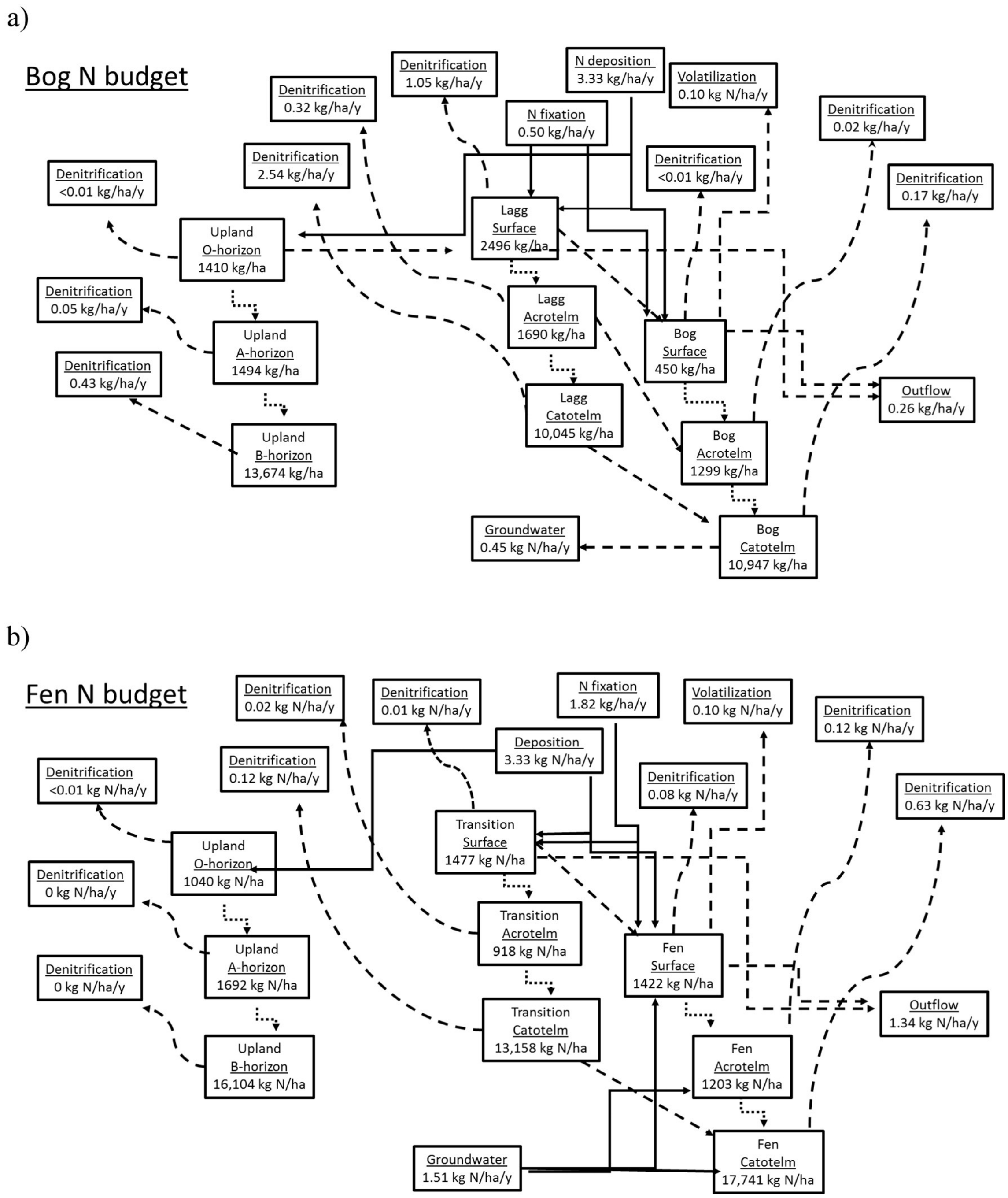


Fig. 3. Nitrogen budgets for an a) ombrotrophic bog and a b) minerotrophic fen in the Marcell Experimental Forest in northcentral Minnesota (USA). Boxes represent storage (kg ha^{-1}) and fluxes (kg ha^{-1} y^{-1}). Input pathways are depicted by solid lines (———); outputs are illustrated with dashed lines (– – – –); and internal flows are represented by dotted lines (···).

1 kg N ha^{-1} y^{-1} (Rosswall and Granhall, 1980; Hemond, 1983; Limpens et al. 2006). Hemond (1983) concluded that volatile ammonia losses occurred only from the well-oxygenated, unsaturated strata of a bog, and parallels the seasonal patterns of N fixation, and we used his reported rate of N volatilization (0.1 kg N ha^{-1} y^{-1}) for our bog and fen N budgets.

Total N losses from the bog watershed were 138% of N inputs, suggesting that current biogeochemical cycling in the bog is substantially supported by N stores within the bog. Peatland N losses exceeding N inputs, while unusual, are not unprecedented. Both Rosswall and Granhall (1980) and Worrall et al. (2012) report similarly large losses of N from northern latitude peatlands, which is attributed to N saturation of peatlands (Worrall et al., 2012). N losses from the fen watershed were 43% of N inputs, indicating that atmospheric N deposition and N inputs from groundwater upwelling are more than adequate to support the N demands of the fen.

The largest components of both the bog and fen watershed N budgets are the standing stocks of N of the soils. Whether for the uplands or for the bog/fen, soil N content increased with depth with the lowest soil horizons having N contents 5–10 times greater than that of the upper soil horizons. This pattern has been reported for temperate forests (Huntington et al., 1988) and for peatlands (Damman, 1988; Urban and Eisenreich, 1988; Limpens et al., 2006). While N is abundant in all of the watershed compartments, the available NO_3^- and NH_4^+ are quite small with the bulk of the N pool assumed to be bound in organic matter (Table 2).

Most reports on peatland denitrification and soil nitrogen budgets, including our study, present a conundrum: how is measured denitrification supported by the low levels of available NO_3^-, especially in anoxic soil strata? Several researchers have reported low measured or estimated denitrification rates for peat and attribute these low rates to low NO_3^- availability and low pH (Rosswall and Granhall, 1980; Hemond, 1983; Urban and Eisenreich, 1988; Urban et al., 1988; Hayden and Ross, 2005; Limpens et al., 2006). Urban and Eisenreich (1988) went so far as to estimate the maximum possible annual rate of denitrification based on NO_3^- availability. Contrary to these results, we report relatively high levels of denitrification in our deepest soil horizons (B horizon and catotelm), environs that are characterized as having minimal O_2 content, low pH, and poor organic C quality (Hill et al., 2014), supported by our lowest measures of NO_3^- availability. Hayden and Ross (2005) considered a coupled nitrification–denitrification model for a bog, but concluded that nitrification rates were too low to support even the low denitrification rates they were reporting. We also have found nitrification rates that are too low to supply the necessary amounts of NO_3^- to support our measured rates of denitrification in the bog, and as further evidenced by the lack of correlation between nitrification and denitrification rates in our soil strata (TM Jicha, manuscript in preparation).

The general conclusion of our denitrification studies on the bog is that some mechanisms other than denitrification must be in play to account for the levels of N_2O and N_2 gases that we are attributing to denitrification. Burgin and Hamilton (2007) and Thamdrup (2012) reviewed alternative pathways of NO_3^- removal from ecosystems, including anaerobic ammonium oxidation (anammox). Anammox is the microbially mediated reaction between NH_4^+ and nitrite (NO_2^-) that yields N_2 (Burgin and Hamilton, 2007; Thamdrup, 2012). Anammox has been reported to account for 0–80% of N_2 evolution from marine, estuary and freshwater ecosystems (Burgin and Hamilton, 2007), but since it requires relatively high levels of available NO_3^- and NH_4^+, both in short supply in our study bog and fen, it is likely insignificant for our N budgets.

An alternative pathway, co-denitrification, which derives some of its N from organic compounds, has recently been recognized. Co-denitrification, involves a microbial enzyme mediated reaction of NO_2^- with organic N compounds (e.g., amino compounds and azides; Spott et al., 2011). Co-denitrification requires an anoxic environment and is favored by abundant respirable C and organic N pools (Spott et al., 2011). The resulting “hybrid” N_2O or N_2, which draws heavily on organic N pools for the gaseous end products, may account for the majority of gaseous N releases from aquatic and terrestrial ecosystems (Spott et al., 2011). Such a pathway may explain why our bog denitrification estimates are greater than those values previously reported for bogs (Rosswall and Granhall, 1980; Hemond, 1983; Hayden and Ross, 2005; Limpens et al., 2006).

While most ecosystem N-budgets focus on the bioavailable N species (NO_3^-, NH_4^+), some researchers have demonstrated the importance of organic N pools. Hedin et al. (1995) reported that organic N accounted for greater than 90% of the N exported by streams draining old-growth forests, despite the apparent high biotic N demand in these watershed that were not impacted by elevated atmospheric N deposition. They attributed these organic N losses to the long-term accumulation and humification of soil organic matter with subsequent leaching. The apparent N leak from N-limited watersheds was discussed by Neff et al. (2003), who hypothesized that refractory organic N was beyond microbial control and leaked from watersheds as a consequence of water movement through watershed soils and flowpaths. Given the size of our bog and fen organic N pools, and the complex hydrology of bogs and fens, it is not hard to imagine large amounts of organic N leaking from these watersheds. These losses are especially noticeable in our bog watershed where 0.26 kg N ha^{-1} y^{-1} is exported, despite an overall N budget deficit of 1.47 kg N ha^{-1} y^{-1}.

In addition to being a source of N for the various N removal pathways discussed above, the large organic N pools in both the bog and fen watersheds may also play a role in regulating the biogeochemistry of these peatlands. Alewell et al. (2008) investigated the role of organic matter pools on the sequential reduction of organic matter via alternative electron acceptors, including NO_3^- via denitrification. They reported that in C-limiting environments, thermodynamics, as governed by redox potential, created a competition among organisms employing the various alternative electron acceptors (NO_3^-, Mn^{2+}, Fe^{2+}, SO_4^{2-}, and CO_2) resulting in a limitation of organic matter decomposition. This competition and metabolic limitation was not evident under conditions of high organic C availability (Alewell et al., 2008). Under these conditions, we would expect multiple, concurrent electron acceptors to be functioning in our study watersheds, resulting in higher than expected rates of denitrification and organic matter processing, even under the anaerobic, acidic conditions of our peat catotelm layers (Hill et al., 2014; Tfaily et al., 2014).

The large organic N pools of peatlands also have implication for the responses of peatlands to climate change. Most researchers have focused on the impact of climate change on C in peatlands, with reports of increased C sequestration by aboveground primary producers being balanced by increased respiratory C losses related to microbial growth and organic matter decomposition, the latter of which also results in increased DOC concentrations in waters exported from watersheds (Rosswall and Granhall, 1980; Limpens et al., 2006; Urban et al., 2011; Weedon et al., 2012, 2014). Carbon lost as evolved CO_2 or CH_4 or exported as DOC during peat decomposition, or stored as microbial biomass, represents the interface of microbial metabolism and the ecosystem pools of C, N and P (Hill et al., 2014; Weedon et al., 2014; Toberman et al., 2015). These peatland organic matter pools are C rich and N and P poor relative to microbial biomass, and ecological stoichiometry theory dictates that the microbes striving to meet their stoichiometric balance will constrain the flow of C and nutrients in the ecosystem (Sterner and Elser, 2002; Hill et al., 2014). As such, respiratory C losses (including denitrification) during peat decomposition should result in losses of N, as evolved N_2, N_2O, and NH_3, or as exported DON from the watershed, that is stoichiometrically related to organic matter production, storage and decomposition. However, the microbial processing of organic N under a warming climate is not simply a function of increased metabolism. Weedon et al. (2014) demonstrated that N cycling in a sub-Arctic peat bog was driven more by the seasonality of N availability than by the metabolic demands for N by the microbial assemblage. However, the availability of N is strongly influenced by microbial enzyme activity and that increased available N leads to increased rates of peat decomposition, resulting in C release from the peat (Rosswall and Granhall, 1980; Limpens et al., 2006; Weedon et al., 2012, 2014). All of which complicates the understanding of peatland biogeochemical responses to climate change.

5. Conclusions

The goals of our project were to compare the soil N budget of an ombrotrophic bog with that of a minerotrophic fen with a focus on the relative importance of denitrification to the overall N budget. We demonstrated significant differences in denitrification between the bog and the fen, despite the paucity of nitrate in the bog. Denitrification in both the bog and fen, and their uplands, was significant relative to N inputs. Our results highlighted differences between the bog and fen, between the upland watersheds and the downslope peatlands, and the importance of biogeochemical hotspots within the peatlands. Our results also point out the importance of stored organic N as a source of N for denitrification, and also as a source of N to support microbial biomass production. We hypothesize that there is a plausible link between organic N storage, denitrification and N export from peatland watersheds. Finally, we considered the interactions of microbial metabolism with nutrient availability and stoichiometry, and how N dynamics might be affected by climate change in peatland ecosystems.

Acknowledgments

We thank Jonathan Launspach (SRA, Inc.), Carrie Dorrance (US Forest Service) and Sandy Verry (US Forest Service, Retired) for their assistance with the site map and bog/fen depth profiles and peat volume calculations. The views expressed in this paper are those of the authors and do not necessarily reflect the views or policies of the US Environmental Protection Agency or the US Forest Service. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.01.178.

References

Alewell, C., Paul, S., Lischeid, G., Strock, F.R., 2008. Co-regulation of redox processes in freshwater wetlands as a function of organic matter availability. Sci. Total Environ. 404, 335–342.

American Public Health Association (APHA), 1998. In: LS, Clesceri, AE, Greenberg, AD, Eaton (Eds.), Standard Methods for the Examination of Water and Wastewater, 20th ed. American Public Health Association, Washington, DC.

Arshad, M.A., Lowery, B., Grossman, B., 1996. Physical tests for monitoring soil quality. In: Doran, J.W., Jones, A.J. (Eds.), Methods for Assessing Soil Quality, Special Publication 49. Madison, Soil Science Society of America, pp. 123–141.

Bernot, M.J., Dodds, W.K., Gardner, W.S., McCarthy, M.J., Sobolev, D., Tank, J.L., 2003. Comparing dentrification estimates for a Texas estuary by using acetylene inhibition and membrane inlet mass spectrometry. Appl. Environ. Microbiol. 69, 5950–5956.

Boelter, D.H., Verry, E.S., 1977. Peatland and water in the northern Lake States. Forest Service General Technical Report NC-31. North Central Forest Experiment Station, US Department of Agriculture, St. Paul, MN (22 pp.).

Bragazza, L., Buttler, A., Habermacher, J., Brancaleoni, L., Gerdol, R., Fritze, H., Hanajik, P., Laiho, R., Johnson, D., 2012. High nitrogen deposition alters the decomposition of bog plant litter and reduces carbon accumulation. Glob. Chang. Biol. 18, 1163–1172.

Branfireum, B.A., 2004. Does microtopography influence subsurface pore-water chemistry? Implications for the study of methylmercury in peatlands. Wetlands 24, 207–211.

Bridgham, S.D., Megonigal, J.P., Keller, J.K., Bliss, N.B., Trettin, C., 2006. The carbon balance of North American wetlands. Wetlands 26, 889–916.

Bridgham, S.D., Pastor, J., Janssens, J.A., Chapin, C., Malterer, T.J., 1996. Multiple limiting gradients in peatlands: a call for a new paradigm. Wetlands 16, 45–65.

Bridgham, S.D., Updegraff, K., Pastor, J., 1998. Carbon, nitrogen, and phosphorus mineralization in northern wetlands. Ecology 79, 1545–1561.

Burgin, A.J., Hamilton, S.K., 2007. Have we overemphasized the role of dentrification in aquatic ecosystems? A review of nitrate removal pathways. Front. Ecol. Environ. 5, 89–96.

Clymo, R.S., 1984. The limits to peat bog growth. Philos. Trans. R. Soc. Lond. B 303, 605–654.

Compton, J.E., Church, M.R., Larned, S.T., Hogsett, W.E., 2003. Nitrogen export from forested watersheds in the Oregon coast range: the role of N_2-fixing red alder. Ecosystems 6, 773–785.

Damman, A.W.H., 1988. Regulation of nitrogen removal and retention in Sphagnum bogs and other peatlands. Oikos 51, 291–305.

Drewer, J., Lohila, A., Aurela, M., Laurila, T., Minkinen, K., Penttila, T., Dinsmore, K.J., McKenzie, R.M.M., Helfter, C., Flechard, C., Sutton, M.A., Skiba, U.M., 2010. Comparison of greenhouse gas fluxes and nitrogen budgets from an ombrotrophic bog in Scotland and a minerotrophic sedge fen in Finland. Eur. J. Soil Sci. 61, 640–650.

Eickenscheidt, T., Heinichen, J., Augustin, J., Freibauer, A., Drosler, M., 2013. Nitrogen mineralization and gaseous nitrogen losses from waterlogged and drained organic soils in a black alder (*Alnus glutinosa* (L.) Gaertn.) forest. Biogeosciences 11, 2961–2976.

Gorham, E., 1991. Northern peatland: role in the carbon cycle and probable responses to climatic warming. Ecol. Appl. 1, 182–195.

Gorham, E., Eisenreich, S.J., Ford, J., Santelmann, M.V., 1985. The chemistry of bog waters. In: Stumm, W. (Ed.), Chemical processes in lakes. New York, John Wiley and Sons, pp. 339–363.

Groffman, P.M., Tiedje, J.M., 1986. Denitrification in north temperate forest soils: spatial and temporal patterns at the landscape and seasonal scale. Soil Biol. Biochem. 21, 613–620.

Groffman, P.M., Altabet, M.A., Bohlke, J.K., Buterbach-Bahl, K., David, M.B., Firestone, M.K., Giblin, A.E., Kana, T.M., Nielsen, L.P., Voytek, M.A., 2006. Methods for measuring denitrification: diverse approaches to a difficult problem. Ecol. Appl. 16, 2091–2122.

Hayden, M.J., Ross, D.S., 2005. Denitrification as a nitrogen removal mechanism in a Vermont peatland. J. Environ. Qual. 34, 2052–2061.

Hedin, L.O., Armesto, J.J., Johnson, A.H., 1995. Patterns of nutrient loss from unpolluted, old-growth temperate forests: evaluation of biogeochemical theory. Ecology 76, 493–509.

Hemond, H.F., 1983. The nitrogen budget of Thoreau's bog. Ecology 64, 99–109.

Hill, B.H., Elonen, C.M., Jicha, T.M., Kolka, R.K., Lehto, L.L.P., Sebestyen, S.D., Seifert-Monson, L.R., 2014. Ecoenzymatic stoichiometry and microbial processing of organic matter in northern bogs and fens reveals a common P-limitation between peatland types. Biogeochemistry 120, 203–224.

Huntington, T.G., Ryan, D.F., Hamburg, S.P., 1988. 1988. Estimating soil nitrogen and carbon pools in a northern hardwood forest ecosystem. Soil Sci. Soc. Am. J. 52, 1162–1167.

Lachat Instruments, 2009. Methods List for Automated Ion Analyzers: Flow Injection, Ion Chromatography. Lachat Instruments, Loveland, CO.

Kayranli, B., Scholz, M., Mustafa, A., Hedmark, A., 2010. Carbon storage and fluxes within freshwater wetlands: a critical review. Wetlands 30, 111–124.

Keeney, D.R., Nelson, D.W., 1986. Nitrogen: inorganic forms. In: AL, Page (Ed.), Methods in Soil Analysis. Part 2. Chemical and Microbiological Properties, second ed. Agronomy monograph 9. American Society of Agronomy and Soil Science Society of America, Madison, pp. 643–649.

Keller, J.K., Bridgham, S.D., 2007. Pathways of anaerobic carbon cycling across an ombrotrophic-minerotrophic peatland gradient. Limnol. Oceanogr. 52, 96–107.

Limpens, J., Berendse, F., Blodau, C., Canadell, J.G., Freeman, C., Holden, J., Roulet, N., Rydin, H., Schaepman-Strub, G., 2008. Peatlands and the carbon cycle: from local processes to global implications—a synthesis. Biogeosciences 5, 1475–1491.

Limpens, J., Heijmans, M., Berendse, F., 2006. The nitrogen cycle of peatlands. In: RK, Wieder, DH, Vitt (Eds.), Boreal Peatland EcosystemsEcological Studies vol. 188. Springer-Verlag, Berlin, pp. 195–230.

Loisel, J., Yu, Z., DW, Beilman, et al., 2014. A database and synthesis of northern peatland soil properties and Holocene carbon and nitrogen accumulation. Holocene 24, 1028–1042.

McClain, M.E., Boyer, E.W., Dent, C.L., Gergel, S.E., Grimm, N.B., Groffman, P.M., Hart, S.C., Harvey, J.W., Johnston, C.A., Mayorga, E., McDowell, W.H., Pinay, G., 2003. Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems 6, 301–312.

Mitchell, P.J., Branfireum, B.A., Kolka, R.K., 2008. Spatial characteristics of net methylmercury production hot spots in peatlands. Environ. Sci. Technol. 42, 1010–1016.

Mitchell, P.J., Branfireum, B.A., Kolka, R.K., 2009. Methylmercury dynamics at the upland-peatland interface: topographic and hydrogeochemical controls. Water Resour. Res. 45, W02406.

Muller, M.M., Sundman, V., Skujins, J., 1980. Denitrification in low pH spodosols and peats determined with the acetylene inhibition method. Appl. Environ. Microbiol. 40, 235–239.

Neff, J.C., Chappi, F.S., Vitousek, P.M., 2003. Breaks in the cycle: dissolve organic nitrogen in terrestrial ecosystems. Front. Ecol. Environ. 1, 205–211.

Nichols, D.S., Verry, E.S., 2001. Stream flow and ground water recharge from small forested watershed in north central Minnesota. J. Hydrol. 245, 89–103.

Rosswall, T., Granhall, T., 1980. Nitrogen cycling in a subartctic ombrotrophic mire. Ecol. Bull. 30, 209–234.

Sebestyen, S.D., Verry, E.S., 2011. Effects of watershed experiments on water chemistry at the marcell experimental forest. In: Kolka, R.K., Sebestyen, S.D., Verry, E.S., Brooks, K.N. (Eds.), Peatland Biogeochemistry and Watershed Hydrology at the Marcell Experimental Forest. Boca Raton, CRC Press, pp. 433–458.

Sebestyen, S.D., Dorrance, C., Olson, D.M., Verry, E.S., Kolka, R.K., Elling, A.E., Kyllander, R., 2011. Long-term monitoring sites and trends at the Marcell Experimental Forest. In: Kolka, R.K., Sebestyen, S.D., Verry, E.S., Brooks, K.N. (Eds.), Peatland Biogeochemistry and Watershed Hydrology at the Marcell Experimental Forest. Boca Raton, CRC Press, pp. 15–71.

Seitzinger, S.P., 1994. Linkages between organic matter mineralization and denitrification in eight riparian wetland. Biogeochemistry 25, 19–39.

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: immolbilisation in the vegetation and peat, leakage into pore water and losses as N2O depend on the form of N. Biogeosciences 10, 149–160.

Spott, O., Russow, R., Strange, C.F., 2011. Formation of hybrid N2O and hybrid N2 due to codenitrification: first review of a barely considered process of microbially mediated N-nitrosation. Soil Biol. Biochem. 43, 1995–2011.

Sterner, R.W., Elser, J.J., 2002. Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere. Princeton, Princeton University Press p.439.

Tfaily, M.M., Cooper, W.T., Koska, J.E., Chanton, P.R., Schadr, C.W., Hanson, P.J., Iversen, C.M., Chanton, J.P., 2014. Organic matter transformation in the peat column at Marcell Experimental Forest: humification and vertical stratification. J. Geophys. Res. Biogeosci. 119, 661–675.

Thamdrup, B., 2012. New pathways and processes in the global nitrogen cycle. Annu. Rev. Ecol. Evol. Syst. 43, 407–428.

Tiedje, J.M., 1982. Denitrification. In: AL, Page (Ed.)Methods of soil analyses: agronomy monographs vol. 9. American Society of Agronomy, Madison, WI, pp. 1011–1026.

Toberman, H., Tipping, E., Boyle, J.F., Helliwell, R.C., Lilly, A., Henrys, P.A., 2015. Dependence of ombrotrophic peat nitrogen on phosphorus and climate. Biogeochemistry 125, 11–20.

Urban, N.R., Eisenreich, S.J., 1988. 1988. Nitrogen cycling in a forested Minnesota bog. Can. J. Bot. 66, 435–449.

Urban, N.R., Eisenreich, S.J., Bayley, S.E., 1988. 1988. The relative importance of denitrification and nitrate assimilation in midcontinental bogs. Limnol. Oceanogr. 33, 1611–1617.

Urban, N.R., Verry, E.S., Eisenreich, S.J., Grigal, D.F., Sebestyen, S.D., 2011. Nutrient cycling in upland/peatland watersheds. In: RK, Kolka, SD, Sebestyen, ES, Verry, KN, Brooks (Eds.), Peatland Biogeochemistry and Watershed Hydrology at the Marcell Experimental Forest. CRC Press, Boca Raton, pp. 213–241.

Verry, E.S., Janssens, J., 2011. Geology, vegetation, and hydrology of the S2 bog at MEF: 12,000 years in northern Minnesota. In: Kolka, R.K., Sebestyen, S.D., Verry, E.S., Brooks, K.N. (Eds.), Peatland Biogeochemistry and Watershed Hydrology at the Marcell Experimental Forest. Boca Raton, CRC Press, pp. 93–134.

Verry, E.S., Timmons, D.R., 1982. Waterbourne nutrient flow through an upland-peatland watershed in Minnesota. Ecology 63, 1456–1467.

Verry, E.S., Brooks, K.N., Nichols, D.S., Ferris, D.R., Sebestyen, S.D., 2011. Watershed hydrology. In: Kolka, R.K., Sebestyen, S.D., Verry, E.S., Brooks, K.N. (Eds.), Peatland Biogeochemistry and Watershed Hydrology at the Marcell Experimental Forest. Boca Raton, CRC Press, pp. 193–212.

Waddington, J.M., Roulet, N.T., 1996. Atmosphere-wetland carbon exchanges: scale dependency of CO_2 and CH_4 exchange on the developmental topography of a peatland. Glob. Biogeochem. Cycles 10, 233–245.

Weedon, J.T., Aerts, R., Kowalchuk, G.A., van Bodegom, P.M., 2014. No effects of experimental warming but contrasting seasonal patterns for soil peptidase and glycosidase enzymes in a sub-arctic peat bog. Biogeochemistry 117, 55–66.

Weedon, J.T., Kowalchuk, G.A., Aerts, R., van Hal, J., van Logtestijn, R., Roling, W.F.M., van Bodegom, P.M., 2012. Summer warming accelerates sub-arctic peatland nitrogen cycling without changing enzyme pools or microbial community structure. Glob. Chang. Biol. 18, 138–150.

Westbrook, C.J., Devito, K.J., 2004. Gross nitrogen transformations in soils from uncut and cut boreal upland and peatland coniferous stands. Biogeochemistry 68, 33–50.

Worrall, F., Clay, G.D., Burt, T.P., Rose, B., 2012. The multi-annual nitrogen budget of a peat-covered catchment—changing from sink to source? Sci. Total Environ. 433, 178–188.