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EXPERIMENTAL NITROGEN ADDITION ALTERS STRUCTURE AND FUNCTION OF A BOREAL POOR FEN: IMPLICATIONS FOR CRITICAL LOADS

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Experimental nitrogen addition alters structure and function of a boreal poor fen: Implications for critical loads

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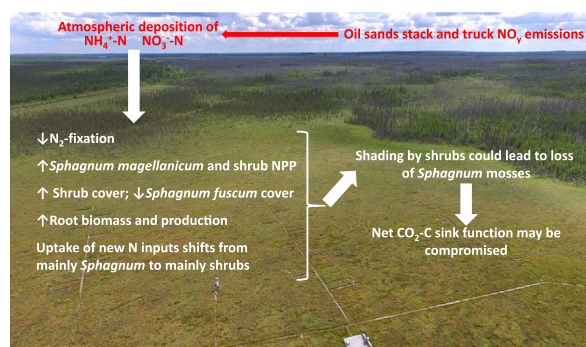
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HIGHLIGHTS

- NO_y emissions from Alberta oil sands development cause increasing N deposition.
- We applied N in simulated rainfall to a poor fen to assess ecosystem responses.
- Increasing N deposition inhibits N₂-fixation, the main source of N to the poor fen.
- Increasing N deposition decreases *Sphagnum* cover and increases shrub cover.
- A critical load of 3 kg N ha⁻¹ yr⁻¹ is recommended for Alberta poor fens.

GRAPHICAL ABSTRACT



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ABSTRACT

Bogs and fens cover 6 and 21%, respectively, of the 140,329 km² Oil Sands Administrative Area in northern Alberta. Regional background atmospheric N deposition is low (<2 kg N ha⁻¹ yr⁻¹), but oil sands development has led to increasing N deposition (as high as 17 kg N ha⁻¹ yr⁻¹). To examine responses to N deposition, over five years, we experimentally applied N (as NH₄NO₃) to a poor fen near Mariana Lake, Alberta, unaffected by oil sands activities, at rates of 0, 5, 10, 15, 20, and 25 kg N ha⁻¹ yr⁻¹, plus controls (no water or N addition). At Mariana Lake Poor Fen (MLPF), increasing N addition: 1) progressively inhibited N₂-fixation; 2) had no effect on net primary production (NPP) of *Sphagnum fuscum* or *S. angustifolium*, while stimulating *S. magellanicum* NPP; 3) led to decreased abundance of *S. fuscum* and increased abundance of *S. angustifolium*, *S. magellanicum*, *Andromeda polifolia*, *Vaccinium oxycoccos*, and of vascular plants in general; 4) led to an increase in stem N concentrations in *S. angustifolium* and *S. magellanicum*, and an increase in leaf N concentrations in *Chamaedaphne calyculata*, *Andromeda polifolia*, and *Vaccinium oxycoccos*; 5) stimulated root biomass and production;

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Nitrogen
N₂-fixation
NPP
Peat
Peatland
Sphagnum

6) stimulated decomposition of cellulose, but not of *Sphagnum* or vascular plant litter; and 7) had no or minimal effects on net N mineralization in surface peat, NH₄⁺-N, NO₃⁻-N or DON concentrations in surface porewater, or peat microbial composition. Increasing N addition led to a switch from new N inputs being taken up primarily by *Sphagnum* to being taken up primarily by shrubs. MLPF responses to increasing N addition did not exhibit threshold triggers, but rather began as soon as N additions increased. Considering all responses to N addition, we recommend a critical load for poor fens in Alberta of 3 kg N ha⁻¹ yr⁻¹.

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1. Introduction

Peatlands, ombrotrophic bogs and minerotrophic fens, cover 365,157 km² of the land surface of northern Alberta, Saskatchewan, and Manitoba, Canada (Vitt et al., 2000). Across much of this region, background atmospheric nitrogen (N) deposition has been and remains low (<2 kg N ha⁻¹ yr⁻¹; Hember, 2018). However, oil sands development north of Fort McMurray, Alberta has led to increasing N deposition, with values as high as 17 kg N ha⁻¹ yr⁻¹ (Fenn et al., 2015; Wieder et al., 2016a). Ecosystems that have persisted under conditions of low atmospheric N deposition have the potential to change, structurally and functionally, when exposed to increasing N deposition (cf. Vitousek et al., 1997).

Bogs and fens cover 6 and 21% of the 140,329 km² oil sands administrative area (Wieder et al., 2016a). Both bogs and poor fens are peat accumulating ecosystems, dominated by *Sphagnum* mosses, with acidic (pH < 5.5) porewaters with low concentrations of base cations, ammonium, nitrate, and phosphate (Vitt, 2006). Unlike bogs, poor fens are hydrologically connected to regional groundwater and/or surface water inputs, and thus, while bogs are ombrogenous and ombrotrophic, fens are geogenous and minerotrophic (Vitt, 2006). Given the susceptibility of bogs and poor fens to increasing N deposition, critical loads of N in Europe have been recommended as 5–10 and 10–15 kg N ha⁻¹ yr⁻¹, respectively (Bobbink and Hettelingh, 2011).

To characterize responses to increasing N deposition, we experimentally applied N at 0, 5, 10, 15, 20, and 25 kg N ha⁻¹ yr⁻¹ (plus controls receiving no water or N) to a bog in the Mariana Lake Peatland Complex, sufficiently far from the oil sands region to be unaffected by oil sands N emissions (Wieder et al., 2019). Based on this study, we recommended a critical load of 3 kg N ha⁻¹ yr⁻¹ for Alberta bogs; at deposition above this level, biological N₂-fixation, the major source of new N to Alberta bogs, is progressively inhibited. In addition, *Sphagnum* moss species composition changes, shrub and *Picea mariana* cover increase, and uptake of atmospherically deposited N switches from being dominated by *Sphagnum* growth to being dominated by shrub growth. Although some of the responses at Mariana Lake Bog were similar to those reported for European bogs, in general, findings did not reflect the triphasic response to increasing N deposition that seems to hold for European bogs (Lamers et al., 2000; Berendse et al., 2001; Heijmans et al., 2002). According to the triphasic response paradigm, at deposition up to 12 kg N ha⁻¹ yr⁻¹, *Sphagnum* mosses take up and retain N through NPP. As N deposition increases to levels in excess of the demand of *Sphagnum* for growth, *Sphagnum* may continue to retain N, exhibiting increases in moss tissue N concentrations. Eventually, as N deposition exceeds 18 kg N ha⁻¹ yr⁻¹, the *Sphagnum* layer becomes saturated and inorganic N moves downward into the peat profile porewater such that inorganic and/or organic N concentrations may increase while vascular plant growth is stimulated, resulting in a shading of the *Sphagnum* mosses and a decrease in moss NPP and cover (Lamers et al., 2000).

Here we report findings from a field N fertilization experiment conducted in a poor fen adjacent to the Mariana Lake Bog site. Our objectives were 1) to assess whether the triphasic response framework applies to the Mariana Lake Poor Fen, 2) to compare structural and

functional responses to increasing N deposition between the Mariana Lake Bog and the Mariana Lake Poor Fen, and 3) to develop a science-based recommendation for poor fen critical loads for northern Alberta.

2. Methods

2.1. Study area

The 164,000 m² Mariana Lake Poor Fen (MLPF) is a part of the larger Mariana Lake Peatland Complex (55°53'49"N, 112°05'32"W, elevation 695 m asl; Graham et al., 2016), located about 100 km SSW of Fort McMurray, Alberta, about 1 km east of Highway 63. The poor fen has a nearly continuous cover of *Sphagnum* mosses (mostly *S. fuscum*, *S. angustifolium*, and *S. magellanicum*, with scattered *S. fallax*, *S. lindbergii*, *S. majus*, *S. riparium*, and *S. russowii*). Vascular plant species include graminoids (mainly *Eriophorum vaginatum*, *Carex aquatilis*, *Carex limosa* and *Carex paupercula*), the forb *Scheuchzeria palustris*, and short-statured ericaceous shrubs (mainly *Andromeda polifolia*, *Kalmia polifolia*, *Chamaedaphne calyculata*, and *Vaccinium oxycoccos*) (Graham et al., 2016). Surface porewater is acidic (pH 4.15), with low reduced conductivity (17 µS cm⁻¹), low concentrations of base cations (Ca²⁺, 1.17 mg L⁻¹; Mg²⁺, 0.38 mg L⁻¹; Na⁺, 1.42 mg L⁻¹; K⁺, 0.53 mg L⁻¹), and low NH₄⁺-N and NO₃⁻-N concentrations (0.15 and 0.01 mg L⁻¹, respectively) (Graham et al., 2016).

The Mariana Lake region has a climate typical of boreal continental Canada. At Wandering River, Alberta, 80 km south of MLPF, long-term (30 year) mean annual temperature averages 0.2 °C, with monthly mean temperatures ranging from −24.5 (January) to 16.0 °C (July); total annual precipitation averages 490 mm, 365 mm as rainfall and 123 mm as snowfall (<http://theweathernetwork.com>). For the duration of the MLPF study, we characterized local meteorological conditions using a WatchDog® weather station (Spectrum Technologies, Aurora, Illinois, USA) located in the adjacent bog (cf. Wieder et al., 2019).

2.2. Experimental design

Site setup and N application protocols mirrored those used in a contemporaneous N application in the bog portion of the Mariana Lake peatland complex (Wieder et al., 2019). In the poor fen, we established 21 7.2 m² experimental plots along three parallel arms, with boardwalks along the plot perimeters. We applied N eight times during each growing season between May and August in seven treatments ($n = 3$): controls (C: no water or N added), and N additions (as soluble NH₄NO₃) equivalent to 0, 5, 10, 15, 20, and 25 kg N ha⁻¹ yr⁻¹. For each application, we sprayed each plot with 200 L of appropriately dosed N, adding 231 mm of water to each plot each year, roughly equivalent to half of the mean annual precipitation of the area. Nitrogen in the water delivered to site had a mean N concentration of 0.5 mg L⁻¹ (NH₄⁺-N, 0.44 mg L⁻¹; NO₃⁻-N, 0.06 mg L⁻¹), adding an additional 1.1 kg N ha⁻¹ yr⁻¹ to each plot that received water. Atmospheric deposition at the site added 2 kg ha⁻¹ yr⁻¹ of dissolved inorganic N (see below). Thus, the annual total N doses for the nominal C, 0, 5, 10, 15, 20, and 25 kg N ha⁻¹ yr⁻¹ treatments were 2.0, 3.1, 8.1, 13.1, 18.1, 23.1, and 28.1 kg N ha⁻¹ yr⁻¹.

2.3. Atmospheric N deposition

We used ion exchange resin tube collectors to quantify ambient atmospheric N deposition (Fenn et al., 2003; Wieder et al., 2016b, 2019). We placed five collectors in the poor fen; two resin tube collectors, sealed at the top and placed in the field within PVC sleeves, served as blank controls. We collected and replaced tubes in mid-May and mid-October, to coordinate with initial and final cranked wire measurements for calculating *Sphagnum* net primary production (NPP) (see below). For the October–May periods, we affixed a 1-m extension tube to each collection funnel to capture snowfall, which upon melt drained through the resin tubes.

We extracted retrieved resins with 1 M KI and analyzed the extract solutions for NH_4^+ -N (phenate method) on a Seal AA3 AutoAnalyzer (Seal Analytical, Madison, Wisconsin, USA) and for NO_3^- -N by ion chromatography (Dionex ICS 1500; Thermo Fisher Scientific, Waltham, Massachusetts, USA). To calculate N deposition, we subtracted volume-weighted concentrations in blank resin tube extracts from concentrations in extracts from exposed resin tubes and applied laboratory-determined extraction efficiencies of 96.9 and 98.2% for NH_4^+ -N and NO_3^- -N, respectively (Wieder et al., 2016b).

2.4. Nitrogen fixation

We measured N_2 -fixation using the acetylene reduction technique (Hardy et al., 1968). At the beginning of each field season, from each plot, we collected one core with a mixture of *Sphagnum angustifolium* and *S. magellanicum*, approximately 8 cm deep and 7 cm in diameter, wrapped each core in mesh screening, and placed cores back into the peat, allowing for repeated measurements over a growing season. We measured N_2 -fixation in these cores 3 times in 2012, 5 times in 2013, 3 times in 2014, and 2 times in 2015, with all measurements made between June and August. For field incubations, we placed each moss core in a gas-tight 500 mL glass screw-top jar, injected 20 mL of acetylene, and reinserted jars into the surface peat with the bottom of the jar facing upward to expose the moss capitula to sunlight. At 20 cm above each jar, we placed a 20-cm by 20-cm window screen mesh providing sufficient shade to prevent extremely high (>40 °C) temperatures within each jar. Using 10 mL syringes, we collected headspace samples at the beginning and end of each 24-h incubation period. After each incubation, we removed the cores from the glass jars and returned them to their original locations in the peat.

We analyzed headspace samples for ethylene concentrations on a Shimadzu 2014 gas chromatograph (Shimadzu Scientific US, Columbia, Maryland, USA) with a flame ionization detector and HaySep® T column (Shimadzu Scientific US) using He as the carrier gas and with the following temperature settings: detector 175 °C, injector 125 °C, and column 100 °C. Blank incubations (–moss, + C_2H_2) yielded consistently low C_2H_4 concentrations (<5 ppm); there was no background C_2H_4 production in control incubations (+moss, – C_2H_2). To convert ethylene production rates to N_2 -fixation rates, we used a conversion factor of 0.46 mol of ethylene produced per mole of N_2 fixed, based on previous $^{15}\text{N}_2$ -fixation measurements in *S. angustifolium* moss cores in Alberta bogs (Vile et al., 2014).

2.5. *Sphagnum* growth and N assimilation

We measured net primary production (NPP) of *Sphagnum* using the cranked wire method (Clymo, 1970). We calculated NPP as the product of stem linear growth and stem mass density (SMD), defined as the mass of 1-cm length of *Sphagnum* stems immediately below the moss capitula (top 1-cm of *Sphagnum* plants) per m^2 . In May 2011, 30 wires were set in each of the 21 study plots. The poor fen plots had a mixture of *Sphagnum fuscum*, *S. magellanicum*, and *S. angustifolium*; the 30 wires were placed according to species representation within each plot, with wires divided between one, two, or all three species. Plot-based species

representation was: one plot with 30 wires in *S. fuscum*, one plot with 15 wires in both *S. fuscum* and *S. angustifolium*, three plots with 15 wires in both *S. fuscum* and *S. magellanicum*, nine plots with 15 wires in both *S. angustifolium* and *S. magellanicum*, and seven plots with 10 wires in each of the three species. Each year in May, wires were set such that 6.5 cm of each wire extended upwardly from the peat surface. In late September or early October when temperatures approached freezing and growth was assumed to have ceased, we remeasured length above the peat surface of each wire and calculated vertical growth.

To measure SMD, we used a 6.5-cm diameter sharpened steel cylinder to extract cores of target *Sphagnum* species between July 7th and 10th of each year. Cores were harvested from a monoculture of the target species in each plot. Because monocultures were often difficult to find, we quantified the average area that an individual of the three study species occupied so that by counting the number of “foreign” species in a core, a corrected value could be attained by subtracting the area of the core occupied by nontarget species. After several years of coring, monocultures of moss became scarce and collection of SMD cores for each species in each plot was not possible.

From each SMD core, all capitula were removed and counted, from which we estimated plant density of each *Sphagnum* species. From each core, we cut 70 stems of *S. fuscum*, and 40 stems of *S. angustifolium*, and 40 stems of *S. magellanicum* to a length of 2 cm and dried the stems at 55 °C for 5 days before weighing. These values were then divided by 2 and again divided by either 70 or 40, depending on the species, to obtain the average mass of 1-cm length of a *Sphagnum* individual. Stem mass density was calculated by multiplying the average mass of 1-cm *Sphagnum* stems by the density (number of individuals m^{-2}) of the appropriate species. Because SMD cores could not be collected for each *Sphagnum* species in each plot in each year, and given that SMD was not affected by N addition in any year (see Results), we used mean SMD values in each year for each species in calculating NPP.

We measured N concentrations in the 0–2 cm stem sections of each *Sphagnum* species from each core (Flash EA 1112 CN Analyzer; Thermo Fisher Scientific). We calculated N assimilation attributable to annual *Sphagnum* growth by multiplying NPP by N concentrations in the *Sphagnum* stems.

2.6. Vascular plant tissue and *Sphagnum* capitulum nitrogen concentrations

Between July 10 and July 17 of each year, we collected samples of new vascular plant growth (current year growth leaves of *Chamaedaphne calyculata*, *Andromeda polifolia*, *Eriophorum vaginatum*, and *Scheuchzeria palustris* in 2011–2015; current year leaves of *Vaccinium oxycoccos* in 2013–2015; capitula (top 1-cm of plants) of *Sphagnum fuscum*, *Sphagnum angustifolium*, and *Sphagnum magellanicum* in 2011–2015). To avoid re-sampling of individual plants in subsequent years, we marked each vascular plant from which leaves were collected. For N analysis, we removed debris, dried samples for a minimum of 6 days at 55 °C, and homogenized samples in a Thomas Wiley Mini-Mill prior to analysis on a Flash EA 1112 CN Analyzer.

2.7. Root biomass and production

We measured root biomass in the top 30 cm of peat in 2014. On 31 May 2014, we collected two cores (7.6 cm diam., 30 cm deep) from each plot using a PVC pipe with a sharpened metal cylindrical tip. We manually extracted roots from cores and dried them at 55 °C for four days prior to weighing.

We quantified root production using the ingrowth bag approach (Finér and Laine, 2000). On 1 June 2014, we inserted two tulle bags (7.6 cm diam.; 30 cm tall) filled with root-free Premier organic peatmoss (Premier Tech, Rivière-du-Loup, Québec, Canada) vertically into the peat in each plot. To calculate 1- and 2-year root production,

we removed one bag on 5 October 2014 and the other on 23 September 2015. We cut the peat in each of the retrieved bags in half (2 15-cm sections) prior to freezing. Due to the high density of fine roots throughout the cores, we quantified ingrown root biomass in 6.5–8.5 cm and 16.5–18.5 subsamples of each core, manually separating roots from the peat, and drying the roots for 4 days at 55 °C prior to weighing them. Although root biomass in 3-cm depth increments is variable in Alberta bog peat, root biomass in the midsections of the 0–15 and 15–30 cm depth increments reasonably represents root biomass across the 15-cm depth increments (cf. Xu, 2004).

2.8. Plant community composition

We used the point intercept method (Levy and Madden, 1933; Jonasson, 1988) to assess plant cover. Using a 1.2-m long point frame anchored permanently in each plot, we characterized bog plant community composition between July 17 and 19 of each year. We recorded all species touched by a 1-mm rod inserted vertically through the frame from 30 points placed 3.5 cm apart.

2.9. Decomposition

We measured decomposition of filter paper (2012–2014), and later native plant material taken from each plot (2014–2015), using litter bags placed 10 cm below the peat surface. We placed five filter papers (Whatman® 41; 70 mm diam.) inside nylon liner fabric bags and sealed the bags. In May 2012, we placed 10 litter bags into the peat in each plot. We retrieved five of these bags in October 2012 and the remaining five in October 2013. Each subsequent May until 2014, we buried 10 bags in each plot and in each October we removed 10 bags, 5 deployed in the previous year and five deployed in May of the current year.

In 2014 and 2015, we quantified decomposition of plant litter material collected from each plot. In late May, we collected vascular plant litter from the surface of each plot, along with *Sphagnum* peat from roughly 5–15 cm below the capitula. We placed nylon mesh litter bags with mixed vascular plant litter (approx. 1.7 g dry mass; comprised of leaves from *Andromeda polifolia* and *Eriophorum vaginatum*, along with remnants of the flowering components of *E. vaginatum* and *Scheuchzeria palustris*) or peat approximately 10 cm below the peat surface in early June (in 2014, 3 bags per plot of vascular litter and 3 bags of *Sphagnum* peat; in 2015, 4 bags of each material). In October of each year, we manually removed debris from each retrieved bag before drying at 55 °C for a minimum of 5 days and weighing.

2.10. Net nitrogen mineralization in surface peat

From 2011 through 2014, we quantified net N mineralization in each plot using the *in situ* buried polyethylene bag technique (Verhoeven et al., 1990; Robertson et al., 1999; Bayley et al., 2005). In the last week of June of each year, we collected a peat core from each plot (6.5 cm diam., 10 cm deep) and divided it in half longitudinally, with one half placed into a Whirl Pak® bag (Nasco, Fort Atkinson, Wisconsin, USA) and inserted back into the peat for field incubation (approximately 30 days); the other half was returned to the laboratory for initial extraction. We extracted initial and field-incubated cores with 0.2 M KCl. For 2011–2012 samples, we measured extractable NH_4^+ -N (phenate method) and NO_3^- -N (hydrazine reduction method) concentrations on a Seal AA3 Autoanalyzer; for 2013–2014 samples, we measured extractable NH_4^+ -N (alkaline phenol method) and NO_3^- -N concentrations (cadmium reduction method) on an OI Analytical Flow Solution IV instrument (OI Analytical, College Station, Texas, USA). We calculated net ammonification from the difference between final and initial extractable NH_4^+ -N contents, net nitrification from the difference between final and initial extractable NO_3^- -N contents, and net DIN production (net N mineralization) from the difference between final and initial extractable NH_4^+ -N plus NO_3^- -N contents (Robertson et al., 1999).

2.11. Porewater chemistry

We constructed samplers for porewater collection by depth using 2.5-cm diameter slotted PVC pipe, segmented into 20 cm depth increments, with Tygon tubing (Saint Gobain North America, Malvern, Pennsylvania, USA) extending from the bottom of each section to the peat surface. Here, we report only data from the top of the poor fen water table. On each sampling date, we collected water from the topmost segment of the PVC sampler and field-filtered each sample (Whatman 41 filter) into sample bottles. In 2011 and 2012, there were 11 and 10 sampling events, respectively, including 8 events each year on the day following N addition. Sampling was reduced to 3 events (June, July, and August) per year in 2013, 2014, and 2015. We froze samples prior to shipping to Villanova University for analysis of NH_4^+ -N (phenate method, Seal AA3 AutoAnalyzer), NO_3^- -N (hydrazine reduction method, Seal AA3 AutoAnalyzer), and total dissolved N (Shimadzu TOC-V_{CSH}/TNM-1 analyzer, with prefiltration through 0.45 µm filters). Dissolved organic nitrogen concentration (DON) was calculated as total dissolved N minus NH_4^+ -N and NO_3^- -N concentrations.

2.12. Microbial biomass and community composition

In July 2015, we collected a surface peat core (10 cm diam.) from each plot and divided it into 0–5 and 5–10 cm depth increments. Samples were frozen and shipped to Ward Laboratories, Kearney, Nebraska, for quantification of phospholipid fatty acid (PLFA) profiles (cf. Andersen et al., 2010). Bacterial PLFA biomarkers (i14:0, i15:0, a15:0, 15:0, i16:0, 16:1ω7c, 16:1ω9c, i17:0, a17:0, 17:0, 17:0cy, 18:1ω7c, 19:0cy) followed Frostegård and Bååth (1996), Högborg et al. (2007), and Stromberger et al. (2012). Gram-negative bacterial biomarkers (16:1ω7c, 16:1ω9c, 18:1ω7c, 17:0cy, 19:0cy) and gram-positive biomarkers (i14:0, i15:0, a15:0, i16:0, i17:0, a17:0) followed Stromberger et al. (2012). Actinomycete biomarkers (10Me16:0, 10Me17:0, 10Me18:0) followed Frostegård et al. (1993). Fungal biomarkers (18:2ω6c, 18:2ω9c, 18:1ω9c) followed Högborg et al. (2007) and Cardinali et al. (2015).

2.13. Statistical analyses

We characterized the responses to N addition using linear regression or two-segment piecewise regression, the latter providing an objective assessment of whether a particular parameter exhibited a threshold response to N addition. We assessed the effect of water addition to the experimental plots using analyses of variance to examine responses across all nominal N addition treatments, focusing on *a posteriori* comparisons (Tukey's Honestly Significant Difference) between control (no N or water added) and the 0 kg N ha⁻¹ yr⁻¹ treatments. When measurements were made over multiple years, we used a repeated measures approach, with each plot as the experimental unit.

Where appropriate, we tested for differences in responses to N addition between years using analysis of covariance (ANCOVA) for homogeneity of slopes, with N addition as a continuous variable and year as a categorical variable; the significance of the N addition by year interaction term indicated whether an N response, if significant, was consistent across all years. *A posteriori* comparisons of slopes and/or elevations for years where the regression was significant were carried out following Zar (2010). In these regression analyses, N addition values were 2.0, 3.1, 8.1, 13.1, 18.1, 23.1 and 28.1 kg N ha⁻¹ yr⁻¹, including N in source water used in the fertilizations, N in background atmospheric deposition, and experimentally applied N. All analyses were conducted using SAS, version 9.3.

We used non-metric multi-dimensional scaling (NMDS) to analyze changes in plant community composition over the course of the study with Primer 6 (Bray-Curtis similarity, Kruskal fit 1 scheme and no pre-treatment of the data; Clarke and Gorley, 2006). We correlated environmental vectors (shrub frequency, change in shrub

frequency between 2011 and each subsequent year, year, N treatment, and total *Sphagnum* species frequency) with the ordination; ordination distance values were calculated using Euclidean distance. We used a two-way permutational analysis of variance (PERMANOVA) to assess differences in N treatment, year (fixed effects), and the N treatment by year interaction, making *a posteriori* pairwise comparisons using PERMANOVA (Clarke and Gorley, 2006). We performed the analysis on absolute frequency data, the number of point-intercept wire contacts for each species, from every plot for each year. The environmental variables shrub frequency and change in shrub frequency between 2011 and each subsequent year included *Andromeda polifolia*, *Vaccinium oxycoccos*, *Kalmia polifolia*, and *Chamaedaphne calyculata* combined.

3. Results

3.1. Atmospheric N deposition

Over all ion exchange resin tube collection dates, deposition of NH_4^+ -N, NO_3^- -N, and dissolved inorganic N (DIN; NH_4^+ -N + NO_3^- -N) averaged 240 ± 29 , 318 ± 27 , and $557 \pm 41 \mu\text{g m}^{-2} \text{da}^{-1}$ (Fig. 1), values that are equivalent to 0.87 ± 0.10 , 1.16 ± 0.10 , and $2.03 \pm 0.15 \text{ kg N ha}^{-1} \text{yr}^{-1}$. While NH_4^+ -N deposition was higher in the growing season than in non-growing season ($p = 0.0006$), NO_3^- -N deposition was higher in non-growing season than during the growing season ($p = 0.0052$), with the net result being no seasonal difference in DIN deposition ($p = 0.6428$) (Fig. 1).

3.2. Acetylene reduction and N_2 -fixation

Averaged across all N treatments, acetylene reduction/ N_2 -fixation rates were variable within and between measurement dates (Fig. 2A). Averaged across all measurement dates, whether described by a linear or segmented regression, N_2 -fixation rates decreased with increasing N addition (Fig. 2B). On an annual (140 da growing season) basis, N_2 -fixation was highest in the control and $0 \text{ kg N ha}^{-1} \text{yr}^{-1}$ treatments ($12.6 \pm 1.5 \text{ kg N ha}^{-1} \text{yr}^{-1}$), decreasing with increasing N addition to $1.9 \pm 0.3 \text{ kg N ha}^{-1} \text{yr}^{-1}$ in the highest N treatment. N_2 -fixation rates were not different between the water addition only treatments and the control treatments ($p = 0.44$).

3.3. *Sphagnum* growth and N responses to N addition

Over the 5 years of measurement, across all N treatments, linear growth of *S. fuscum*, *S. angustifolium*, and *S. magellanicum* averaged 3.0 ± 0.1 , 3.9 ± 0.1 , and $3.6 \pm 0.2 \text{ cm yr}^{-1}$, respectively, and NPP averaged 319 ± 18 , 260 ± 16 , and $243 \pm 11 \text{ g m}^{-2} \text{yr}^{-1}$, respectively. Linear growth and NPP of *S. fuscum* were unaffected by N addition and were consistent across the 5 years (Fig. 3A, D). Linear growth and NPP of *S. angustifolium* differed between years, but were unaffected by N addition (Fig. 3B, E). In contrast, linear growth and NPP of *S. magellanicum* increased with increasing N addition in three of the five years of measurement (Fig. 3C, F). Water addition alone had no significant effect on linear growth or NPP of any of the three *Sphagnum* species ($p \geq 0.97$).

Both *Sphagnum* stem mass density (g m^{-2} per 1-cm stem length) and plant density (# of capitula in pure stands of a species) enter into the calculation of *Sphagnum* NPP. Stem mass density of *S. fuscum*, *S. angustifolium*, and *S. magellanicum* and plant density of *S. fuscum* and *S. angustifolium* were unaffected by N addition (Fig. S1). Across all five years, *S. magellanicum* plant density increased significantly with N addition, contributing to the observed NPP response.

Stem N concentrations differed between years, but not as a function of N addition for *S. fuscum*. Concentrations increased with increasing N addition in two of the five years for *S. angustifolium*, and increased with increasing N addition at a rate that was consistent across all

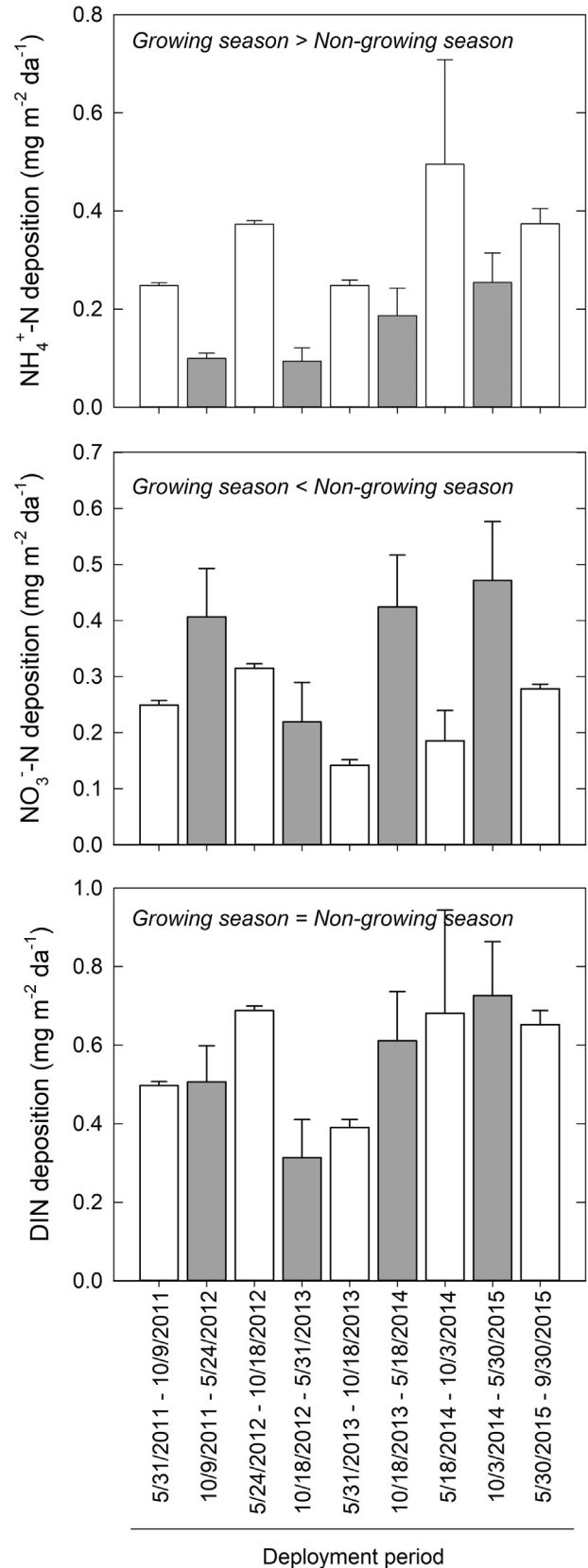


Fig. 1. Atmospheric deposition of NH_4^+ -N, NO_3^- -N, and DIN (NH_4^+ -N plus NO_3^- -N) at Mariana Lake Poor Fen, quantified using ion exchange resin collectors. Resins were deployed during growing season (May through October; white bars) and during non-growing season periods (October through May; dark grey bars). Values are means $\pm$ standard errors ($n = 5$).

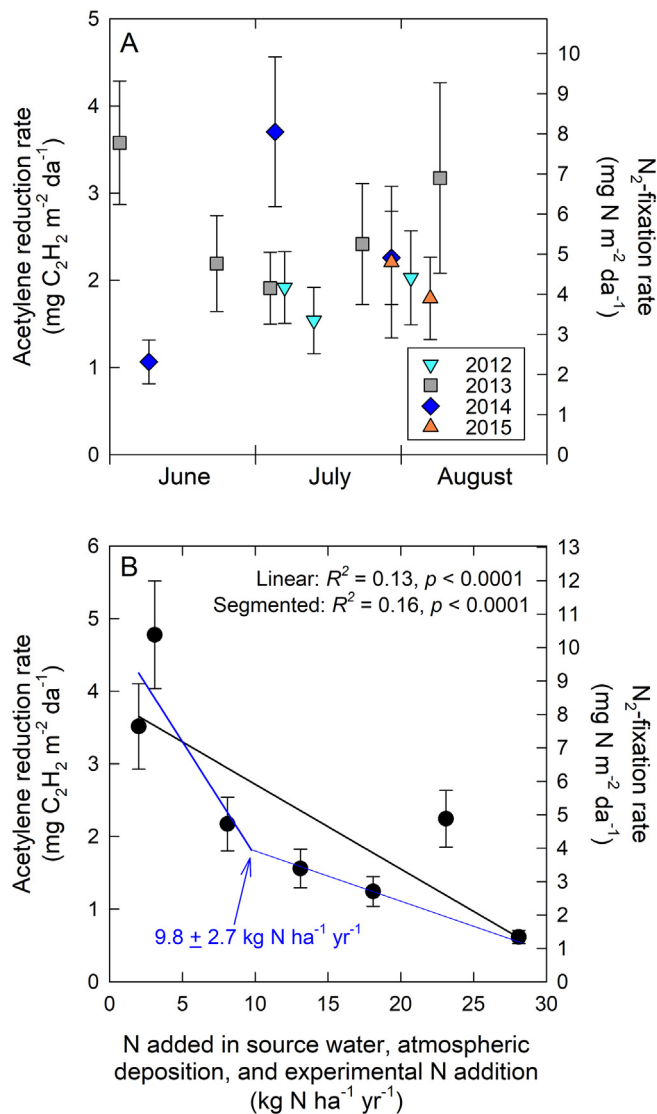


Fig. 2. A: Rates of acetylene reduction (left axis), converted to N₂-fixation (right axis) throughout the growing season (2012–2015) (A; values are means $\pm$ standard errors, averaged across all N treatments, $n = 21$), and as a function of N addition (B; values are means $\pm$ standard errors, averaged across all measurement dates, $n = 45$). In B, results from both a linear regression and a 2-segment piecewise linear regression are shown, the latter highlighting the N deposition value at which the slope changes.

years for *S. magellanicum* (Fig. 4A–C). Nitrogen assimilation attributable to annual growth differed between years, but not as a function of increasing N addition for *S. fuscum* and *S. angustifolium*, and increased with increasing N addition at a rate that was consistent across all years for *S. magellanicum* (Fig. 4D–F). For *S. fuscum* and *S. angustifolium*, water addition alone had no significant effect on stem N concentrations ($p = 0.18$ and $p = 1.00$, respectively) or on N assimilation ($p = 0.99$ and $p = 1.00$, respectively). Water only effects could not be assessed for *S. magellanicum* as samples of this species were not collected in control treatment plots because of the low abundance of this species.

Capitulum N concentrations for *S. fuscum* and *S. magellanicum* were not significantly affected by N addition ($p = 0.8506$ and $p = 0.0520$, respectively) and did not differ between years ($p = 0.1991$ and $p = 0.0885$, respectively) (Table S1). In contrast, capitulum N concentrations for *S. angustifolium* increased with increasing N addition ($p = 0.0033$) at a very small rate that was consistent among the five years (Table S1).

3.4. Vascular plant N responses to N addition

Leaf N concentrations in *C. calyculata*, *A. polifolia*, and *V. oxycoccus* increased significantly with increasing N addition (Fig. 5). For *C. calyculata* and *A. polifolia*, there were differences in N concentrations between years, but the regression slopes describing the response to N addition were consistent across all years. Leaf N concentrations were unaffected by N addition for *E. vaginatum* and *S. palustris*, with the latter exhibiting interannual differences in leaf N concentrations. Water addition alone had no significant effect on N concentrations for any of the vascular plant species ($p \geq 0.67$).

3.5. Plant species abundances and community composition

Regression analyses indicated that *S. fuscum* absolute frequency from point frame measurements decreased with N addition, while *S. angustifolium*, *S. magellanicum*, *A. polifolia*, and *V. oxycoccus* frequencies increased with N addition, with slopes being consistent across the five years (Fig. 6A, B, D, E, G). Neither *Scheuchzeria palustris* nor *Eriophorum vaginatum* frequency was affected by N addition; *E. vaginatum* frequencies differed between years (Fig. 6C, H). For dominant shrubs and all vascular species combined, N addition had no effect in the first two years, but frequencies increased with increasing N addition in 2013–2015 (Fig. 6F, I). With the exception of *Scheuchzeria palustris*, which had on average a higher frequency in control treatment plots (0.10) than in $0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ plots (0.04), water addition alone had no significant effect on absolute frequencies individual species, dominant shrubs, or all vascular plants ($p > 0.08$).

Plant community composition, examined through PERMANOVA and NMDS ordination, was affected by N treatment ($p = 0.001$) and year ($p = 0.001$), with no interaction ($p = 1.00$). Pairwise year comparisons (Table S2) indicated a gradual change in plant species composition as the experiment progressed. Of the 21 pairwise comparisons between nominal N treatments, 18 were significant (Table S3), indicating a clear influence of N addition on plant community composition. Graphically (Fig. 7), two clusters are evident on the ordination – to the left a scattered cluster composed mostly of controls and the $0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ treatment, and to the right, a tight cluster composed mostly of 10 through $25 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ treatment plots. Vectors indicate that *Sphagnum* abundance, total shrub density, and overall change in shrub frequency relative to 2011 increase downward and to the right, while N treatment effects are greater from left to right.

3.6. Root responses to N addition

Root biomass, measured in 2014, increased with increasing N addition in the 0–15 and 15–30 cm depth increments (Fig. 8A), and hence in the in the top 30 cm of peat (Fig. 8B). Root production, measured using the ingrowth bag method, increased with increasing N addition in the 0–15 cm, but not the 15–30 cm depth increment; annual root production in the 0–15 cm depth increment was higher when ingrowth bags remained in the peat for two growing seasons, compared to first-year root production (Fig. 8C). As a result, over the top 30 cm, annual root production was greater when ingrowth bags were in the peat for two growing seasons, although the root production response to increasing N addition (regression slopes) was not significantly different after one- and two-year placement of the ingrowth bags (Fig. 8D). Root biomass over the top 30 cm of peat was significantly, albeit slightly, higher in the control treatment than in the water only treatment. Root production in the water only treatment was significantly higher than in the control treatment after one and after two growing seasons.

3.7. Decomposition

Decomposition of cellulose filter paper increased with increasing N addition; the slopes of the responses did not differ between years,

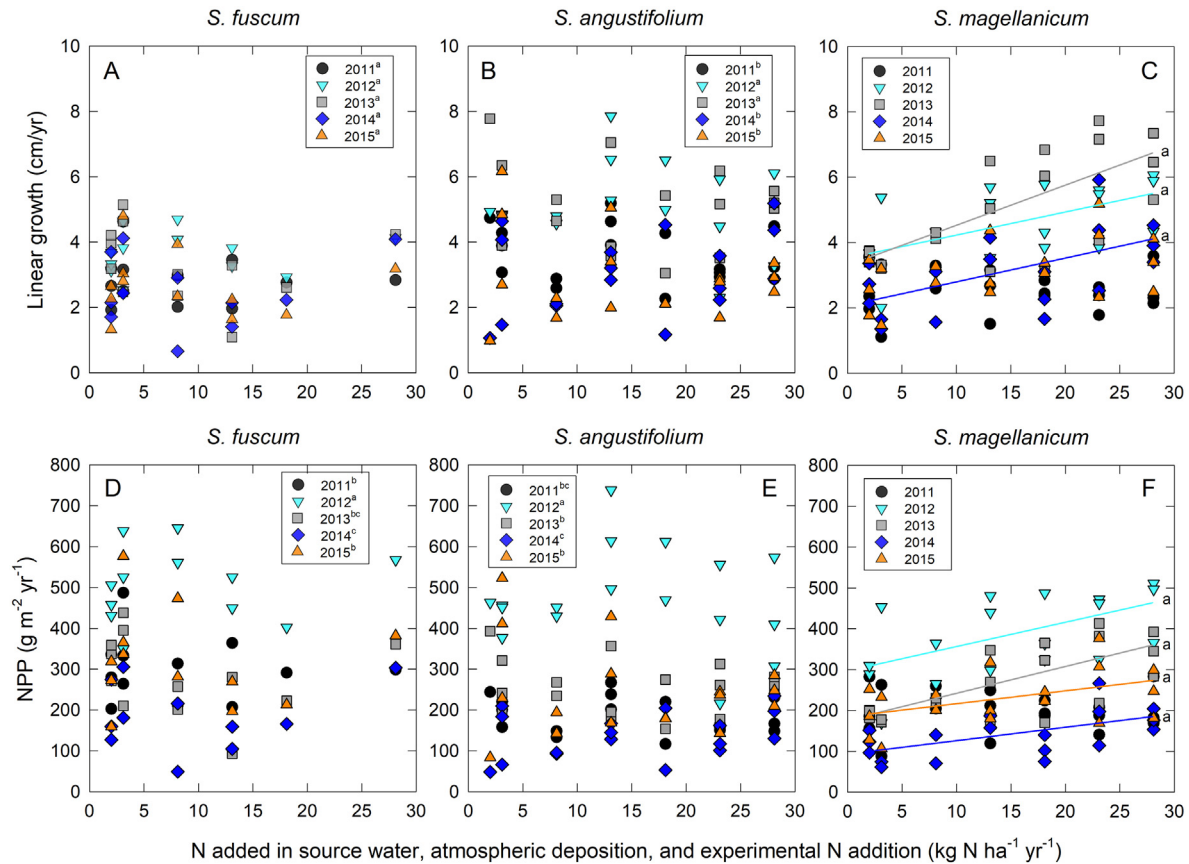


Fig. 3. *Sphagnum fuscum*, *Sphagnum angustifolium*, and *Sphagnum magellanicum* linear growth (A-C) and annual NPP (D-F). Significant linear regressions are shown. When there were differences between years, but no significant effect of N addition, years with the same letter superscript do not differ significantly (ANCOVA, *a posteriori* Tukey's Honestly Significant Difference test). Slopes with the same lower case letter to the right of regression lines plotted for individual years do not differ significantly (ANCOVA).

although decomposition was faster for filter paper placed in the field in 2014 than in either 2012 or 2013 (Fig. 9A). In contrast, decomposition of *Sphagnum* moss was unaffected by N addition (Fig. 9C). Vascular plant litter decomposition decreased with N addition in 2015, but not 2014. Water addition alone had no significant effect on cellulose decomposition k values in any of the three years ($p \geq 0.26$) or on vascular plant litter mass in either year ($p = 0.81$). However, *Sphagnum* mass loss was significantly higher in the control treatment than in 0 kg N ha⁻¹ yr⁻¹ treatment ($p = 0.0046$) averaged over the two years of decomposition.

3.8. Net N mineralization

Concentrations of initial KCl-extractable NH₄⁺-N, NO₃⁻-N, and DIN differed between years, and were either minimally affected (NH₄⁺-N) or not affected (NO₃⁻-N and DIN) by increasing N addition (Fig. 10A-C). Water addition alone had no significant effect on initial KCl-extractable NH₄⁺-N, NO₃⁻-N, and DIN concentrations in any of the four years of measurement ($p \geq 0.06$).

Net nitrification rates increased significantly with increasing N addition in 2012, decreased with increasing N addition in 2013, and were unaffected by N addition in 2011 and 2014 (Fig. 10E). Overall, net nitrification rates were much lower than net ammonification rates. Net ammonification and net DIN production rates were unaffected by N addition and did not differ between years (Fig. 10D, F). Water addition alone had no significant effect on net ammonification or net DIN production rates in any of the four years ($p \geq 0.92$), and no significant effect on net nitrification rates ($p \geq 0.39$), except in 2013 when net

nitrification rates were significantly lower in the control treatment than in the 0 kg N ha⁻¹ yr⁻¹ treatment.

3.9. Porewater N concentrations

Over the 5 years of the study, porewater NH₄⁺-N, NO₃⁻-N, and DON concentrations at the top of the poor fen water table were unaffected by N addition ($p = 0.06, 0.30, 0.16$, respectively). However, porewater NH₄⁺-N, NO₃⁻-N, and DON concentrations were substantially higher in 2011 than in 2012–2015 (Fig. 11). Water addition alone had no significant effect on porewater concentrations of NH₄⁺-N, NO₃⁻-N, or DON ($p \geq 0.99$).

3.10. Microbial community composition

For total microbial, total bacterial, fungal, and actinomycete abundances, a segmented regression described the response to N addition substantially better than a linear regression for 0–5 cm peat; while for 5–10 cm peat, linear and segmented regressions were either nonsignificant or regression convergence was not obtained (Fig. S2A, B, E, F). In each instance, the slope of the segmented regression was negative and nonsignificant up to an N addition of 15.8–17.1 kg N ha⁻¹ yr⁻¹, with a positive and significant slope at higher N addition. A similar pattern was obtained for Gram-negative bacterial abundance (Fig. S2D). Gram-positive bacterial abundance increased linearly with increasing N addition, with the response being statistically similar for 0–5 and 5–10 cm peat (Fig. S2E). Neither the fungal:bacterial abundance ratio nor the Gram-positive:Gram negative bacterial abundance ratio was affected by N addition; the Gram-positive:Gram negative bacterial

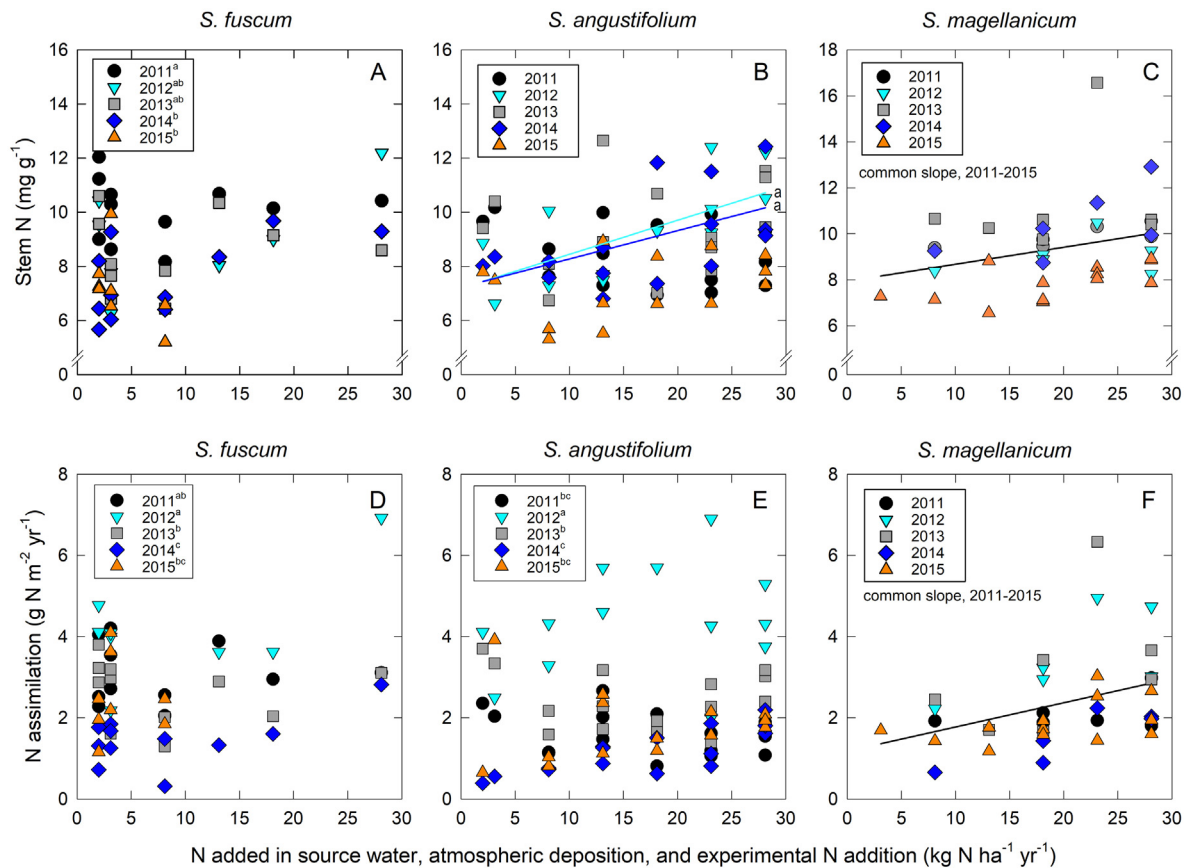


Fig. 4. *Sphagnum fuscum*, *Sphagnum angustifolium*, and *Sphagnum magellanicum* stem N concentrations (A–C) and N assimilation associated with annual NPP (D–F). Significant linear regressions are shown. When there were differences between years, but no significant effect of N addition, years with the same letter superscript do not differ significantly (ANCOVA, *a posteriori*Tukey's Honestly Significant Difference test). Slopes with the same lower case letter to the right of regression lines plotted for individual years do not differ significantly (ANCOVA).

abundance ratio was higher in 5–10 cm peat than in 0–5 cm peat (Fig. S2G, H).

4. Discussion

Peatland classification has a long history (cf. Wheeler and Proctor, 2000; Vitt, 2006). Distinctions between bogs, poor fens, and rich fens have long been recognized (DuRietz, 1949) with the term poor fen being used broadly to include acidic, minerotrophic peatlands dominated by *Sphagnum*; sedges and ericaceous shrubs are sometimes abundant. Less research has focused on poor fens than on bogs, in Europe (Bobbink and Hettelingh, 2011) and North America. Intercontinental comparisons are hampered by floristic differences in key plant indicator species (Vitt and Chee, 1990). For example, *Rhododendron groenlandicum* is not present in Eurasian bogs (Hultén and Fries, 1986), while *Erica tetralix* and *Calluna vulgaris* are not present in North American bogs (although *C. vulgaris* does occur in Newfoundland; Vitt, 2006). *Sphagnum tenellum*, *Sphagnum papillosum*, *Molinia caerulea*, and *Narthecium ossifragum* are found in European poor fens (Sjörs, 1983), but not in the poor fens of Alberta. These indicator species can be abundant and often form an important component of the plant community.

In this discussion, we strive to interpret findings from Mariana Lake Poor Fen in light of literature on poor fens that have a nearly continuous cover of *Sphagnum* mosses, have acidic porewater, are treeless, and whose vascular plant cover is contributed predominantly by ericaceous shrub and graminoid species. Further, we focus on comparisons between Mariana Lake Poor Fen and Mariana Lake Bog with respect to parameters measured in control plots (Table 1) and responses to N addition (Table 2).

4.1. Inputs of new nitrogen at Mariana Lake Poor Fen

Annual DIN deposition at Mariana Lake Poor Fen over the course of the study, averaging $2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, was similar to DIN deposition across northern Alberta at sites unaffected by N emissions from oil sands development (e.g., Wieder et al., 2010, 2016a, 2016b, 2019, Fenn et al., 2015). Hember (2018) determined that from 1990 to 2013, N deposition (wet plus dry deposition) across the Canadian boreal plains ecozone has averaged $2.93 \pm 0.45 \text{ kg ha}^{-1} \text{ yr}^{-1}$, having increased by 23.2% over that time period. Although Mariana Lake Poor Fen is not likely to be affected by oil sands N emissions from operations north of Fort McMurray, within a 50 km radius of the fen, there are 21 point sources of N_2O , mainly related to oil sands and oil/gas pipelines and storage, that collectively released 2194 metric tonnes of N_2O to the atmosphere in 2017 (National Pollutant Release Inventory; <https://www.canada.ca/en/services/environment/pollution-waste-management/national-pollutant-release-inventory.html>). As a result, it is possible that the current N deposition at Mariana Lake Poor Fen is higher than in pre-industrial times.

We are aware of no reported measurements of N_2 -fixation in boreal poor fens dominated by *Sphagnum fuscum*, *S. magellanicum*, and *S. angustifolium*, in Canada or elsewhere. However, in Alberta bogs, N_2 -fixation is much more important than atmospheric deposition as a source of new N input (Vile et al., 2014; Wieder et al., 2019). This is also the case at Mariana Lake Poor Fen, although N_2 -fixation rates in control plots were about 70% of rates in the adjacent bog (Table 1). Why N_2 -fixation is so much higher at Mariana Lake Bog than at Mariana Lake Poor Fen is unclear, although it may be the case that the shade provided by the *Picea mariana* canopy and relatively taller more abundant ericaceous shrubs (*Chamaedaphne calyculata* and *Rhododendron*

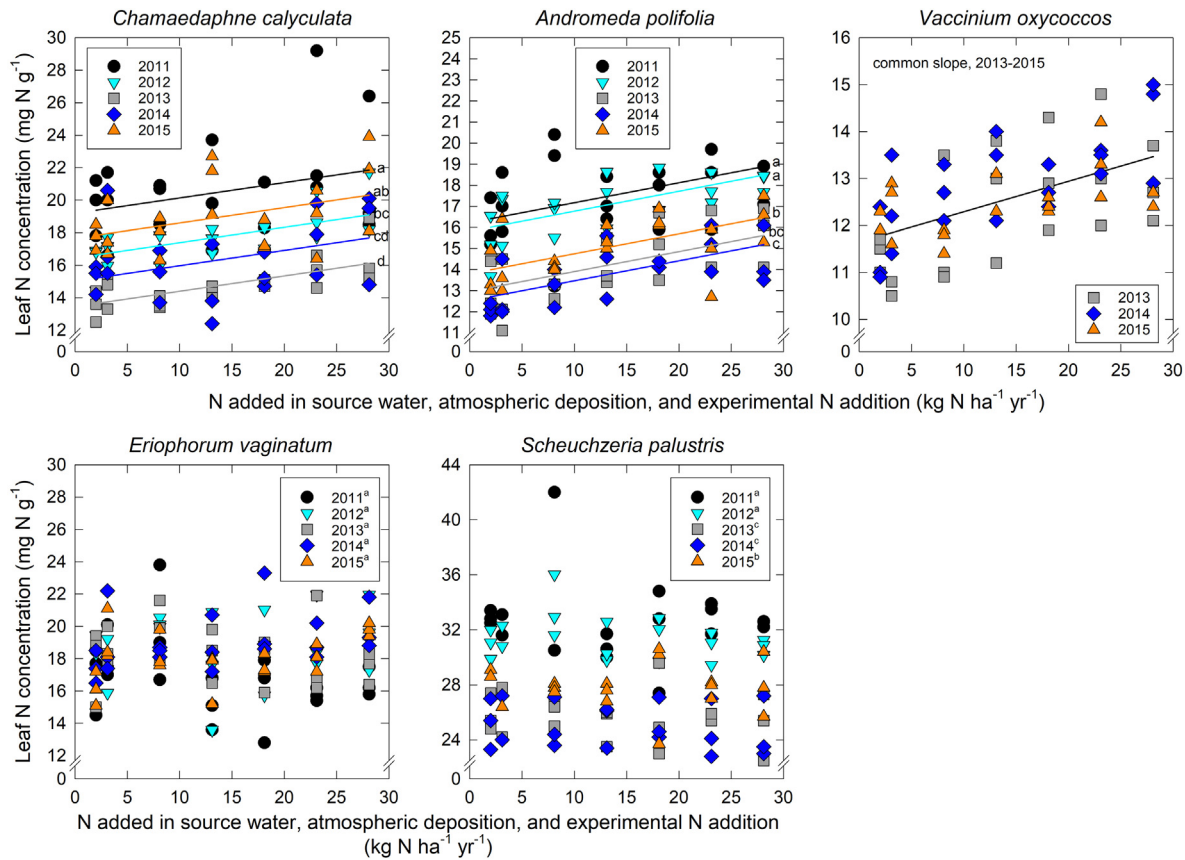


Fig. 5. Leaf N concentrations for five vascular plant species as a function of N addition. For *C. calyculata* and *polifolia*, slopes of the regressions were consistent across the five years, but elevations of the regressions differed; elevations of the regressions with the same lower case do not differ significantly. When there were differences between years, but no significant effect of N addition, years with the same letter superscript do not differ significantly (ANCOVA, *a posteriori* Tukey's Honestly Significant Difference test).

groenlandicum) in the bog moderate temperatures in surface peat, favoring the N_2 -fixing process. Further, N_2 -fixation requires a combination of anaerobic microsites and air-filled pores to allow for the diffusion of N_2 to these microsites. The generally wetter surface peat conditions in the poor fen than in the bog (cf. Graham et al., 2016) may be less favorable for N_2 -fixation.

In the Mariana Lake Poor Fen, N_2 -fixation rates decreased with increasing N deposition, in contrast to the pattern in the bog where N_2 -fixation rates increased at N addition up to $3.1 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ and subsequently declined (Wieder et al., 2019). In the adjacent bog, as N addition increased from 3.1 to $27.6 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, N_2 -fixation decreased from 31.1 to $11.3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$; as a result, total inputs of new N from N_2 -fixation plus N additions remained between 35 and $45 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ across all N addition levels. In contrast, in the poor fen, as N addition increased from 2.0 to $28.1 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, N_2 -fixation decreased from 12.9 to $3.4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ such that total N inputs increased from 14.9 to $31.5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. At the bog and poor fen, as N addition increased, new N inputs shifted from predominantly organic N, produced by N_2 -fixers, to inorganic N in deposition; this shift occurred at N addition levels of 20.9 and $7.6 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, in the bog and poor fen, respectively. The lower N_2 fixation rates and the shift to DIN deposition exceeding N_2 -fixation inputs at lower N addition levels suggests that the poor fen may be more sensitive to increasing atmospheric N deposition than the bog.

4.2. Sphagnum responses

Few studies have quantified growth of *S. fuscum*, *S. magellanicum*, and *S. angustifolium* in boreal acidic poor fens. At Bleak Lake Poor Fen, Alberta, *S. angustifolium* linear growth and NPP (Szumigalski, 1995) were similar to rates in control plots at Mariana Lake Poor Fen. Linear

growth and NPP of *S. fuscum* in control plots were similar at Mariana Lake Poor Fen and the adjacent bog (Table 1).

In bogs, moss growth has been thought to be supported primarily by atmospherically deposited N (Malmer et al., 1994). The triphasic response framework suggests that at low ambient N deposition, *Sphagnum* growth is N-limited (Lamers et al., 2000). Therefore, we initially hypothesized that at Mariana Lake Poor Fen, for all three *Sphagnum* species, growth would be stimulated by increasing N addition. While we found a modest increase in linear growth and net primary production of *S. magellanicum* with increasing N addition in three of the five years (Fig. 3C, F), *S. fuscum* and *S. angustifolium* growth were unaffected by N addition (Fig. 3A, B, D, E). At Mariana Lake Bog, *S. fuscum* linear growth was unaffected by N addition, although there was evidence for inhibition of NPP (Table 2). Over four years, Rochefort et al. (1990) added NO_3^- -N (and SO_4^{2-} -S) to a wooded poor fen at the Experimental Lakes Area, Ontario, Canada. In the oligotrophic region, fertilization stimulated *S. fuscum* linear growth and NPP. Linear growth and NPP of *S. magellanicum* were stimulated by fertilization in years 3 and 4 of the study, but not in years 1 and 2. Further, *S. angustifolium* linear growth and NPP were stimulated by fertilization in the first year of fertilization, but by the fourth year fertilization had no effect. Given the paucity of data on *Sphagnum* growth in boreal acidic poor fens, with or without experimental N addition, it is difficult to make generalizations, although it appears that *S. fuscum*, *S. magellanicum*, and *S. angustifolium* responses to increasing N deposition may be species-specific and may differ between bogs and acidic poor fens (cf. Loisel et al., 2012).

It is generally acknowledged that air temperature and precipitation regimes have strong influences on *Sphagnum* growth (Gunnarsson, 2005). At Mariana Lake Poor Fen, linear growth and NPP of all three *Sphagnum* species were positively correlated with growing season degree days and growing season total precipitation, negatively correlated

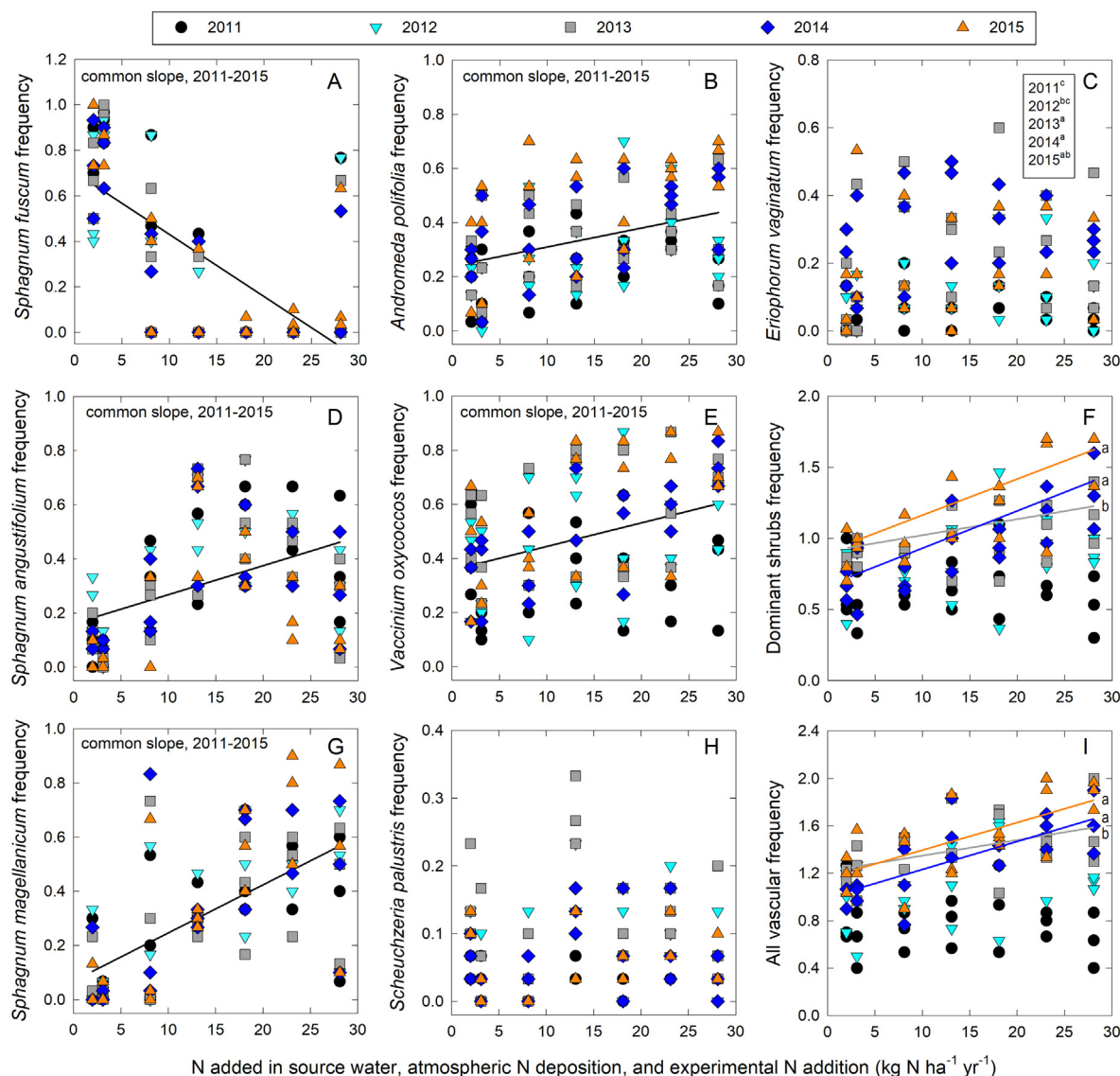


Fig. 6. Frequency of occurrence from point frame measurements for the three most frequently occurring *Sphagnum* species (*Sphagnum fuscum*, *Sphagnum angustifolium*, *Sphagnum magellanicum*), the two most frequently occurring shrub species (*Andromeda polifolia*, *Vaccinium oxycoccos*), the most frequently occurring graminoid species (*Eriophorum vaginatum*), the most frequently occurring forb species (*Scheuchzeria palustris*), dominant shrubs combined (*Andromeda polifolia*, *Vaccinium oxycoccos*, *Chamaedaphne calyculata*, *Kalmia polifolia*), and all vascular plant species combined (*Scheuchzeria palustris*, *Eriophorum vaginatum*, *Andromeda polifolia*, *Vaccinium oxycoccos*, *Chamaedaphne calyculata*, *Kalmia polifolia*, *Drosera rotundifolia*, *Rubus chamaemorus*, *Maianthemum trifolium*, *Carex aquatilis*, *Carex limosa*, *Picea mariana* < 1-m tall) as a function of N addition. When there were differences between years, but no significant effect of N addition, years with the same letter superscript do not differ significantly (ANCOVA, *a posteriori* Tukey's Honestly Significant Difference test). Slopes with the same lower case letter to the right of regression lines plotted for individual years do not differ significantly (ANCOVA).

with growing season aridity index (growing season potential evapotranspiration/growing season precipitation), and often positively correlated with monthly mean temperatures or precipitation (Table S4). Similar correlations between weather variables and *Sphagnum fuscum* growth or NPP have been reported for bogs across northern Alberta, including Mariana Lake Bog (Wieder et al., 2016b, 2019). At Mariana Lake Poor Fen, precipitation, and to a lesser extent temperature, variables also were correlated with stem mass density and plant density for *S. angustifolium* and *S. fuscum*, but generally not for *S. magellanicum* (S4).

Initially we hypothesized that N concentrations in *Sphagnum* capitula of each species would increase with increasing N addition in a sigmoidal manner following the triphasic N response framework (Lamers et al., 2000). *Sphagnum* capitulum N concentrations were unaffected by N addition (Table S1), as was observed for *S. fuscum* in Mariana Lake Bog (Table 2). Indeed, for all three *Sphagnum* species in both the Mariana Lake Poor Fen and Bog, capitulum N concentrations in control plots (Table 1) were greater than or equal to the 12 mg/g value that

Lamers et al. (2000) observed for mosses receiving 18 kg N ha⁻¹ yr⁻¹ deposition, where the moss layer was deemed to be saturated. In Alberta poor fens, the high capitulum N concentrations are probably maintained through N₂-fixation inputs.

4.3. Aboveground vascular plant responses

We initially hypothesized that at Mariana Lake Poor Fen, *Sphagnum* species covering the peat surface would take up atmospherically deposited N to fuel growth at low levels of N addition; once N additions exceeded the capacity for *Sphagnum* uptake, DIN would move downward into the peat profile thereby stimulating shrub/forb growth and increasing shrub/forb cover. As N additions increase to a level exceeding the uptake capacity of mosses and shrubs/forbs combined, N concentrations in vascular plant tissues should increase. These hypotheses followed from the triphasic response framework (Lamers et al., 2000) and the concept that mosses rely primarily on atmospherically

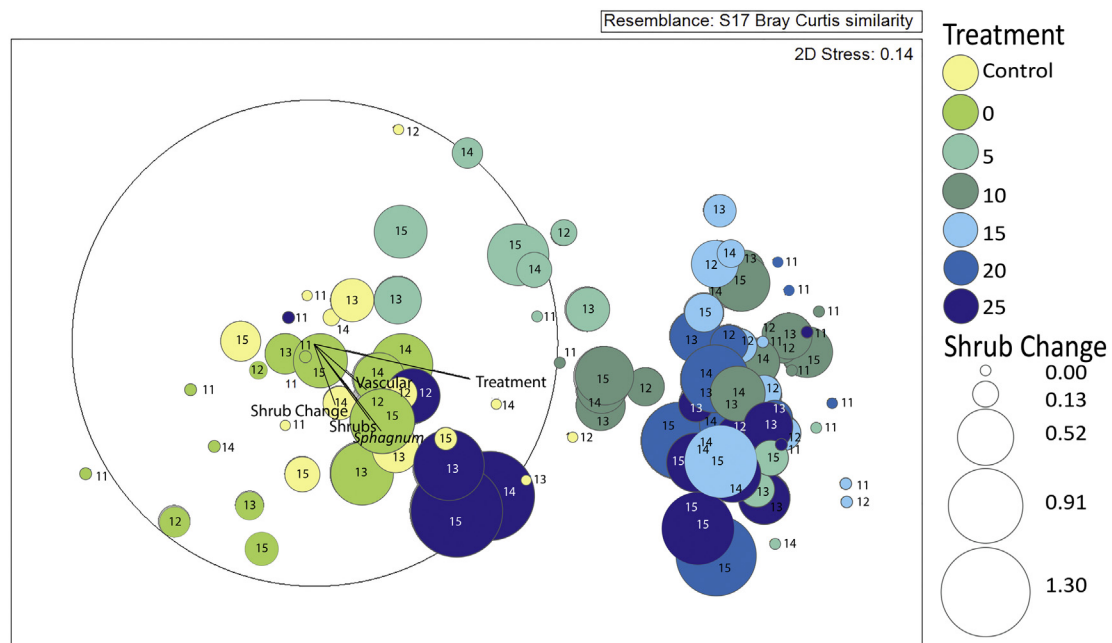


Fig. 7. Results of nonmetric multidimensional scaling, differentiating the N treatments and years, with vectors for increasing shrub frequency, increasing vascular plant frequency, increasing change in shrub frequency between 2011 and 2015, and increasing *Sphagnum* frequency. Shrub change is the absolute difference in shrub frequency (*Andromeda polifolia* + *Vaccinium oxycoccos* + *Kalmia polifolia* + *Chamaedaphne calyculata*) between 2011 and each successive year 2015. Each circle represents a replicate plot, color coded by N treatment and sized according to change in shrub frequency (shrub change) between 2011 and each successive year. Year of sampling for each plot is indicated adjacent to each circle.

deposited N for growth, while vascular plants rely on inorganic N made available through mineralization processes (cf. Malmer et al., 1994).

Few studies have quantified shrub production or shrub cover and their responses to N addition in boreal, open, *Sphagnum*-dominated poor fens. Nitrogen fertilization at Alborn Fen, Minnesota, stimulated NPP of *Andromeda polifolia* and of *Scheuchzeria palustris*; responses were less clear for other vascular species (Chapin et al., 2004). At Degerö Stormyr, Sweden, N fertilization of a poor fen, with and without experimental warming, led to an increase in vascular plant cover (*Andromeda polifolia*, *Vaccinium oxycoccos*, *Eriophorum vaginatum*); however, these changes in cover in response to N addition exhibited a time lag, not being evident over the first 4 years of N addition (Wiedermann et al., 2007). Similarly, at Mariana Lake Poor Fen, the increases in dominant shrub and total vascular plant frequency were not evident in the first two years of N addition (Fig. 6F, I). At Degerö Stormyr, after 8 years, leaf N concentrations were higher across all N treatments than in control treatments for *A. polifolia*, *V. oxycoccos*, but not *E. vaginatum*, similar to the leaf N responses to N addition at Mariana Lake Poor Fen (Fig. 6).

At Mariana Lakes Poor Fen, frequency of *A. polifolia* and *V. oxycoccos* increased with increasing N addition (Figs. 6B, E, 8B, E; *Chamaedaphne calyculata* frequency was low, averaging 0.06 ± 0.01 , and did not increase with N addition). Leaf N concentrations in *A. polifolia* and *V. oxycoccos* increased with increasing N addition (Fig. 5B, C), such that N assimilation associated with NPP of these species likely increased, as well.

It is not surprising that at Mariana Lake Poor Fen, neither *Eriophorum vaginatum* nor *Scheuchzeria palustris* leaf N concentrations or frequencies responded to increasing N addition. Although *E. vaginatum* produces fibrous shallow, non-mycorrhizal roots, it also can produce deeper aerenchymatous roots (Wallén, 1992; Innes, 2014). In arctic tundra, *E. vaginatum* is capable of taking up dissolved organic N, and may do so preferentially over NH_4^+ -N (Chapin III et al., 1993). *S. palustris* taproots are thicker, with more well developed aerenchyma than roots of *E. vaginatum*, and may extend deeper into the peat profile (Dorodnikov et al., 2011). Because of efficient uptake of N by *Sphagnum*, deep-rooted plants growing in *Sphagnum* peat may not have direct

access to atmospherically deposited N (cf. Malmer et al., 1994; Berendse et al., 2001; Nordbakken et al., 2003).

Several studies have noted that a consequence of increasing vascular plant abundance (assessed by cover or frequency) in response to increasing N addition is a reduction of *Sphagnum* NPP, most likely caused by shading of the moss (e.g. Clymo, 1973; Hayward and Clymo, 1983; Heijmans et al., 2001). This concept was supported by the synthesis of NPP data that revealed that photosynthetically active radiation integrated over the growing season was the best predictor of NPP for *S. fuscum* and *S. magellanicum* globally (Loisel et al., 2012). At Mariana Lake Bog, we reported that *S. fuscum* NPP declined exponentially with increasing NPP or frequency of dominant shrubs, in part because *S. fuscum* plant density decreased with increasing vascular plant frequency (Wieder et al., 2019). None of these relationships was significant at Mariana Lake Poor Fen. Mariana Lake Poor Fen is treeless, with no *Rhododendron groenlandicum* and a lower abundance of *Chamaedaphne calyculata* than the adjacent bog. Although increasing N addition led to increasing shrub frequency at Mariana Lake Poor Fen, across all N treatments the moss surface was considerably less shaded than at Mariana Lake Bog. Nonetheless, after five years of treatment, the increase in shrub cover in response to N addition was visually quite evident (Fig. 12).

4.4. Root responses

Consistent with the hypothesis that vascular plant growth in poor fens is N-limited, we anticipated that root biomass and production would increase with increasing N addition at Mariana Lake Poor Fen. We are aware of no studies that have quantified root biomass and/or production at open, acidic poor fens. Responses of root biomass and production to increasing N addition at Mariana Lake Poor Fen (Fig. 8) were similar to responses at the adjacent bog (Table 2), although in control plots, both root biomass and production were lower in the fen than in the bog (Table 1). According to the triphasic response framework, at low N addition levels, vascular plant production should remain low, but as N addition increases to a level above the capacity of *Sphagnum* mosses to take it up, dissolved inorganic N will move downward into

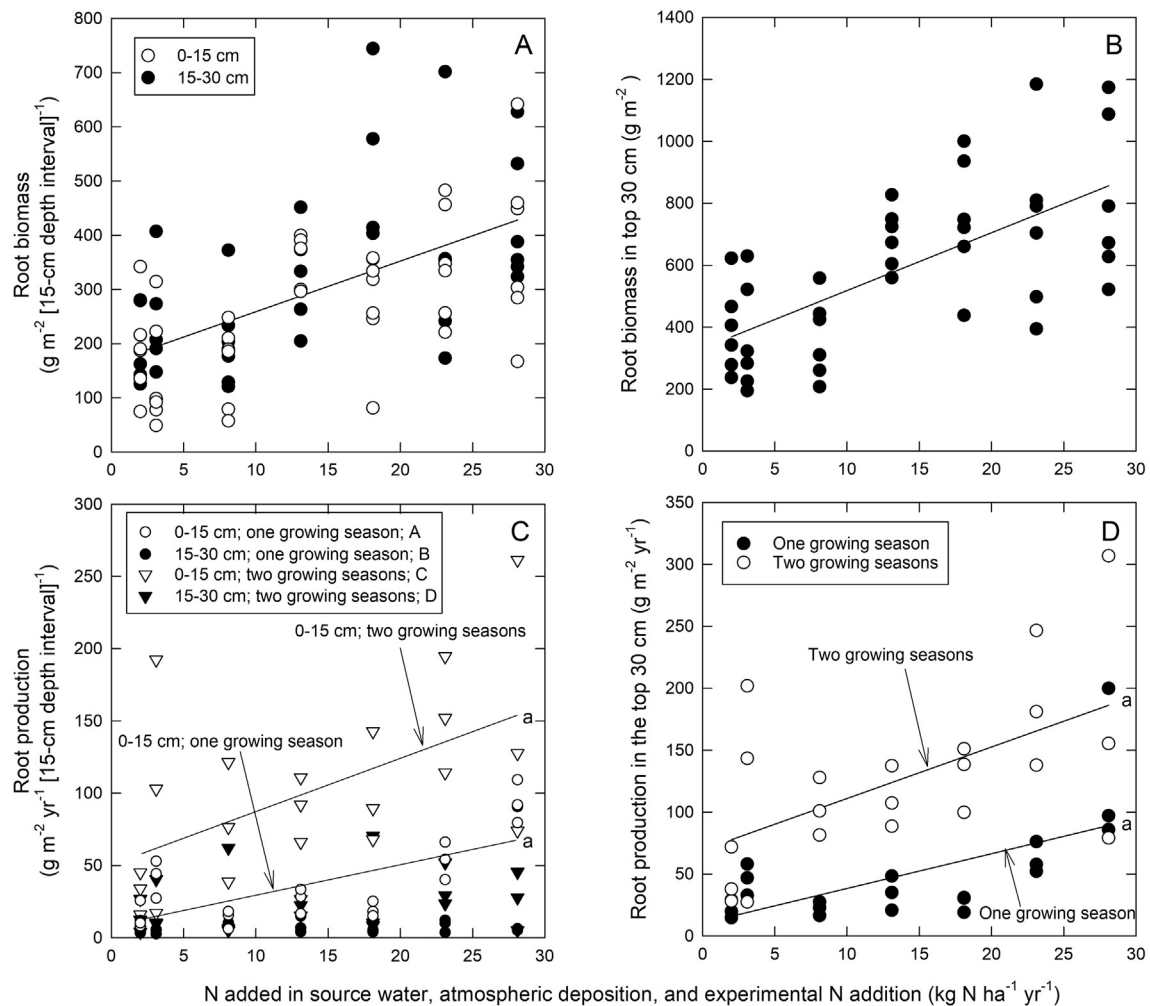


Fig. 8. Root biomass in the 0–15 and 15–30 cm depth increments (A), root biomass in the top 30 cm of peat (B), root production in the 0–15 and 15–30 cm depth increments over one and two growing seasons (C), and root production in the top 30 cm of peat over one and two growing seasons (D). Significant regressions are shown as solid lines. Slopes with the same lower case letter to the right of regression lines do not differ significantly (ANCOVA).

the peat where it will stimulate vascular plant production (Lamers et al., 2000). Thus, there should be a threshold N addition level associated with stimulation of root production. We found no evidence of such a threshold at Mariana Lake Poor Fen (Fig. 8C, D), or at the adjacent bog (Wieder et al., 2019).

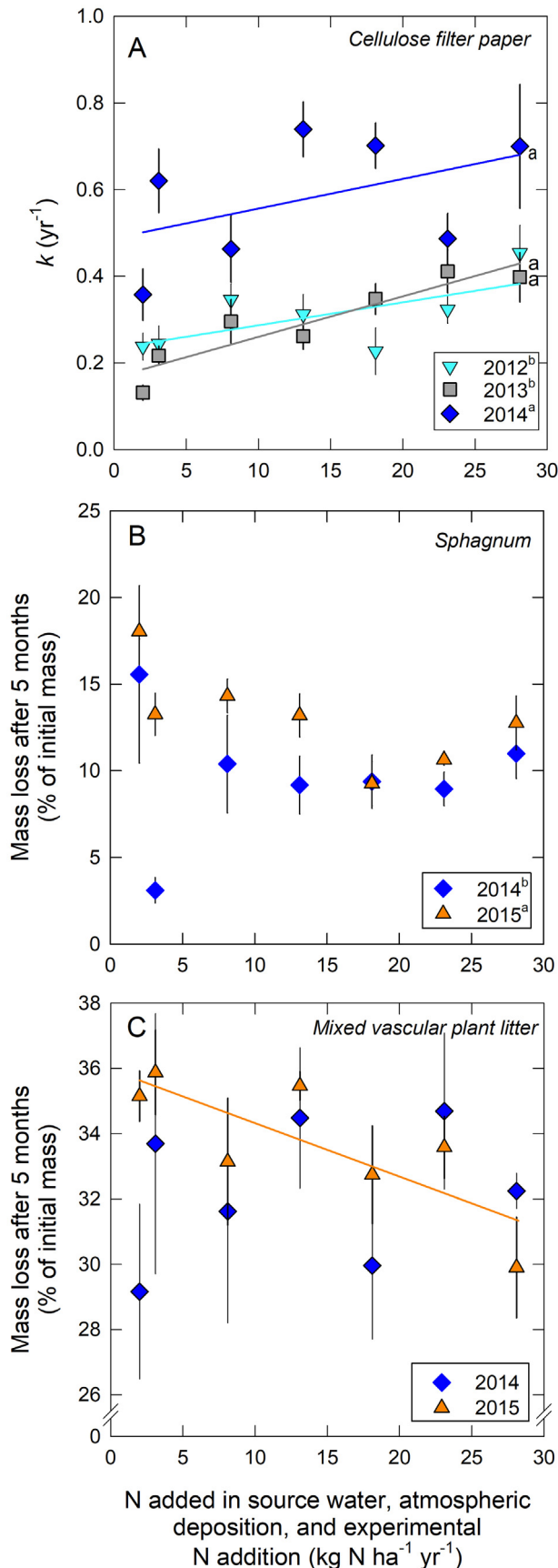
4.5. Plant community composition responses

We had hypothesized that increasing N addition would drive changes in plant community composition, increasing shrub abundance and decreasing overall *Sphagnum* abundance at least in part because of shading (e.g. Clymo, 1973; Hayward and Clymo, 1983; Lamers et al., 2000; Heijmans et al., 2001). We did not anticipate species-specific differences in *Sphagnum* abundance, however. With increasing N addition, absolute frequencies of *S. fuscum* decreased, while absolute frequencies of *S. angustifolium* and *S. magellanicum* increased (Fig. 6A, D, G). The nature of these responses was consistent across all five years of the study, suggesting that there may have been preexisting differences between N treatments and/or N addition early in the first year may have affected the relative abundances of *Sphagnum* species, changing *Sphagnum* community structure before the first measurements in August of year one.

At Mariana Lake Bog, increasing N addition led to a decrease in *S. fuscum* frequency and an increase in *S. magellanicum* frequency, with no effect on *S. angustifolium* (Table 2). While at both sites, increasing N addition resulted in an increase in vascular plant frequency in

years 3–5 of the study, vascular plant frequency was higher (Table 1) and the response to N addition were more pronounced in the bog. In the fifth year of the study, with increasing N addition, vascular plant frequency had increased by 50% in the poor fen (Fig. 6), and nearly doubled in the bog (Wieder et al., 2019). While we reported negative relationships between vascular plant frequency and *Sphagnum fuscum* NPP and plant density at Mariana Lake Bog (Wieder et al., 2019), these relationships were not evident at the poor fen. Moreover, multivariate analysis indicated that increasing N addition was positively associated with increasing shrub frequency and negatively associated with *Sphagnum* frequency in the bog. In the poor fen plant community changes in response to N addition were positively associated with both increasing shrub frequency and combined *Sphagnum* frequency (Fig. 7).

It is evident that within the Mariana Lake peatland complex, plant community composition is affected by increasing N addition in both the bog and the poor fen, progressively favoring vascular plants over *Sphagnum*. While increased shading from stimulated vascular plants may play a role in what appear to be species-specific *Sphagnum* responses, in general the peat surface in treed bogs is more shaded than the peat surface in open poor fens. Beyond the effects of shading on light availability for photosynthesis in *Sphagnum*, shading also may affect ambient air temperatures at the moss surface, and may attenuate exposure to UV radiation. All of these factors may affect *Sphagnum* photosynthesis, growth, and competitive ability (Murray et al., 1993; Gunnarsson, 2005; Bengtsson et al., 2016; Hyyryläinen et al., 2018),



contributing to differences in plant community responses between the Mariana Lake Poor Fen and Bog. Given that *Sphagnum* mosses are major contributors to the net CO₂-C sink in Alberta bogs (Wieder et al., 2009) and most likely poor fens, a consequence of increasing N deposition would be a weakening of the C sink function.

4.6. Decomposition responses

We hypothesized that decomposition of *Sphagnum*, vascular plant litter, and a uniform substrate (cellulose filter paper) would exhibit a threshold response to increasing N addition; decomposition would be low at low N addition and would increase only when N addition exceeded the N uptake capacity of the *Sphagnum* layer. Few studies have quantified cellulose, *Sphagnum*, or vascular plant litter decomposition in acidic, open poor fens, and we are aware of none examining N addition effects.

At Mariana Lake Poor Fen, while cellulose decomposition increased with increasing N addition, there was no indication of a threshold response (Fig. 9A). This general pattern was observed for the adjacent bog, as well (Wieder et al., 2019). In control plots, cellulose decomposition was similar in the Mariana Lake Poor Fen and in the adjacent bog (Table 1). However, the stimulation of cellulose decomposition by N addition was substantially stronger in the bog than in the poor fen (Table 2). The generally wetter conditions of near-surface peat in the poor fen than in the bog (cf. Graham et al., 2016) may have modulated the response to N addition.

At Mariana Lake Poor Fen, *Sphagnum* decomposition was unaffected by N addition ($p = 0.0517$), while vascular plant litter decomposition actually decreased with increasing N addition in 2015, but not in 2014 (Fig. 9B, C). Exponential decay constants for *Sphagnum* species decomposing in poor fens have ranged from 0.015 to 0.228 yr⁻¹ (Bartsch and Moore, 1985; Moore et al., 2007; Turetsky et al., 2008). In poor fens in Québec, Ontario, and New Hampshire, decomposition of various graminoids, shrubs, and *Typha latifolia* leaves have produced estimates of k values ranging from 0.131 to 0.371 yr⁻¹ (Bartsch and Moore, 1985; Moore et al., 2007). Decomposition of mixed *Sphagnum* and mixed vascular plant material in control plots at Mariana Lake Poor Fen was measured over a 5-month period. Assuming that no decomposition occurs outside of this time period, these percent loss values correspond to k values of 0.08 and 0.17 yr⁻¹, respectively.

Vascular plant litter decomposition is thought to occur more rapidly in poor fens than in bogs (Aerts et al., 1999; Verhoeven et al., 1990; Glime, 2017), and in general vascular plant litter decomposes more rapidly than most *Sphagnum* species (Aerts et al., 1999; Heal et al., 1978; Hobbie, 1996; Moore and Basiliko, 2006). Within the Mariana Lake peatland complex, in control plots, *Sphagnum*, but not vascular plant material, decomposed more rapidly in the poor fen than in the bog, while in the poor fen and the bog, vascular plant material decomposed more rapidly than *Sphagnum* (Table 1). We offer no mechanistic explanation for the apparent decrease in decomposition rates of mixed vascular plant litter with increasing N addition in one of the two years of measurement at Mariana Lake Poor Fen. It is clear, however, that our initial hypothesis that increasing N addition should stimulate decomposition was not supported.

Fig. 9. Decomposition of cellulose filter paper (A; exponential decay k values, yr⁻¹ ± standard errors) placed into the peat in May of 2012, 2013, and 2014, and collected 5 and 17 months after initial placement, as a function of N addition. Decomposition of *Sphagnum* (B) and of mixed vascular plant litter (C) placed in the peat in May of 2014 and 2015 and retrieved in October of the same year; values are means ± standard errors ($n = 3$ in 2014; $n = 4$ in 2015). Significant regressions are shown. Slopes with the same lower case letter to the right of regression lines do not differ significantly (ANCOVA). When there were differences between years, but no significant N addition by year interaction, years with the same letter superscript do not differ significantly (ANCOVA, *a posteriori* Tukey's Honestly Significant Difference test).

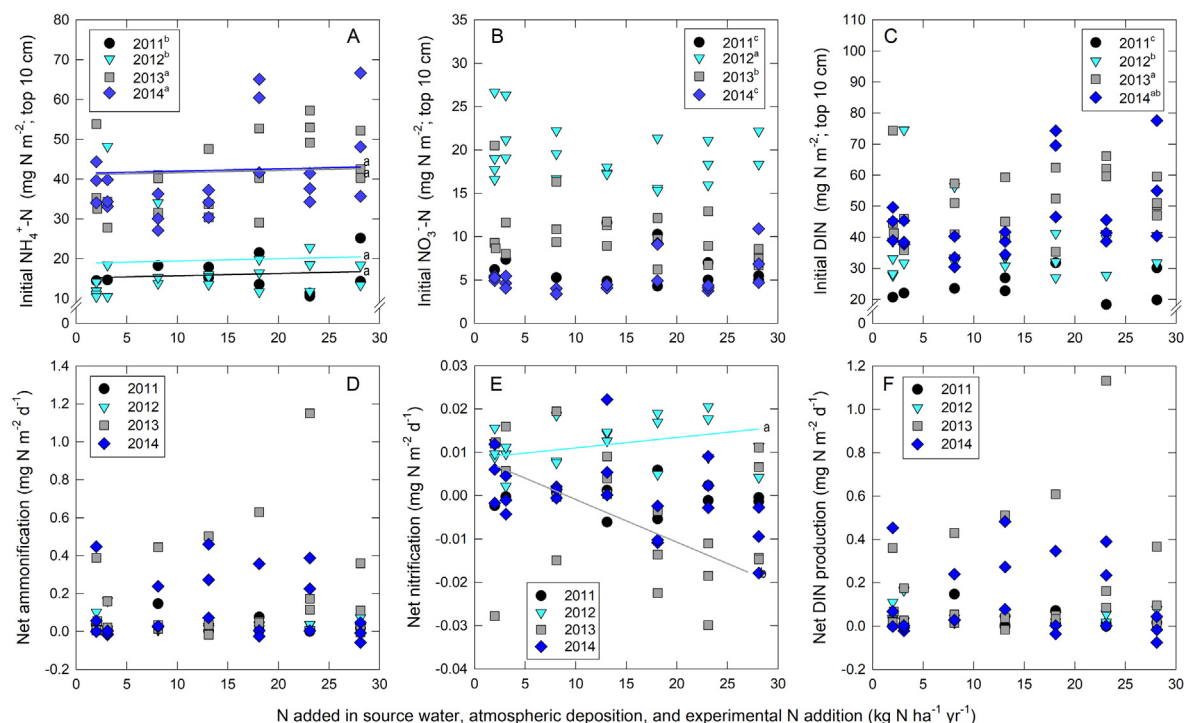


Fig. 10. Initial KCl-extractable $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and DIN concentrations in 0–10 cm deep peat (A–C), net ammonification (D), net nitrification (E), and net DIN production (F) in the top 10 cm of peat as a function of N addition. Slopes with the same lower case letter to the right of regression lines do not differ significantly (ANCOVA). When there were differences between years, but no significant N addition by year interaction, years with the same letter superscript do not differ significantly (ANCOVA, *a posteriori* Tukey's Honestly Significant Difference test).

4.7. Net N mineralization and porewater DIN concentration responses

We had hypothesized that as N deposition increases to a level that exceeds the capacity for DIN uptake by *Sphagnum* and vascular plant uptake, atmospherically deposited N will move downward into the peat profile, increasing porewater DIN concentrations, thereby inhibiting net ammonification and stimulating nitrification (because of higher porewater $\text{NH}_4^+\text{-N}$ concentrations) (cf. McGill and Cole, 1981, Robertson and Groffman, 2015, Bragazza and Limpens, 2004). At Mariana Lake Poor Fen, as in the adjacent bog (Wieder et al., 2019), none of these hypotheses was supported.

Initial KCl-extractable concentrations of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and DIN at Mariana Lake Poor Fen were similar to those obtained at the adjacent bog (Table 1) and were at the low end of the range of values reported for poor fens in Alberta (Bayley et al., 2005; Hartsock et al., 2019). Net ammonification, net nitrification, and net DIN production rates at Mariana Lake Poor Fen were generally lower than rates at the

adjacent bog (Table 1); rates at both the poor fen and bog were lower than those reported for other Alberta poor fens (Bayley et al., 2005; Hartsock et al., 2019). Nitrogen mineralization generally increases with the degree of minerotrophy in peatlands (cf. Updegraff et al., 1995; Bridgman et al., 1998; Bayley et al., 2005; Hartsock et al., 2019). The low rates that we obtained in both the Mariana Lake Poor Fen and Bog, along with low porewater N concentrations (Table 1) suggest extremely oligotrophic conditions. Under such conditions, we might have expected mineralization processes to be stimulated with increasing N addition, which generally was not the case (Fig. 10, Table 2). In both the Mariana Lake Poor Fen and Bog, as N addition increased, we did not observe significant increase in porewater $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ or DON concentrations (Table 2), suggesting that experimentally increased N inputs were used to support enhanced growth of *Sphagnum* (at least *S. magellanicum*) and of vascular plant species, thereby precluding any effects on N mineralization processes.

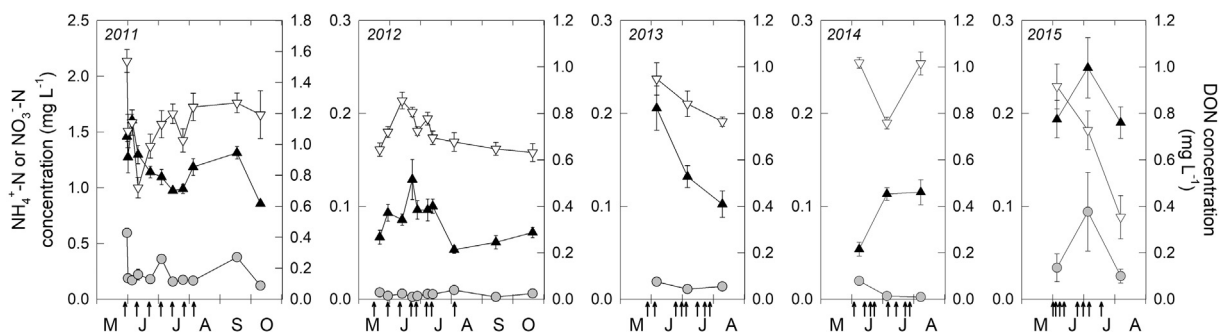


Fig. 11. Concentrations of $\text{NH}_4^+\text{-N}$ (black triangles), $\text{NO}_3^-\text{-N}$ (grey circles), and DON (white inverted triangles) at the top of the poor fen water table. Values are means $\pm$ standard errors ($n = 3$). Arrows along the x-axes indicate N addition dates. Note the different y-axis scales for N concentrations for 2011 than for 2012–2015.

Table 1

Comparisons between ecosystem structural or functional parameters between Mariana Lake Poor Fen and Mariana Lake Bog for control plots (receiving no added N or water). Values are means $\pm$ standard errors averaged across all years of measurement.

Ecosystem structural or functional parameter	Values in control treatments averaged over all years	
	Mariana Lake Poor Fen	Mariana Lake Bog
N ₂ -fixation (kg N ha ⁻¹ yr ⁻¹)	10.6 $\pm$ 0.2	36 $\pm$ 5
<i>Sphagnum</i> linear growth (cm yr ⁻¹)	<i>S. fuscum</i> : 2.8 $\pm$ 0.2 <i>S. angustifolium</i> : 3.9 $\pm$ 1.3 <i>S. magellanicum</i> : 3.0 $\pm$ 0.2	<i>S. fuscum</i> : 2.3 $\pm$ 0.2
<i>Sphagnum</i> NPP in monospecific stands (g m ⁻² yr ⁻¹)	<i>S. fuscum</i> : 299 $\pm$ 29 <i>S. angustifolium</i> : 246 $\pm$ 82 <i>S. magellanicum</i> : 203 $\pm$ 18	<i>S. fuscum</i> : 281 $\pm$ 29
<i>Sphagnum</i> stem N (mg N g ⁻¹)	<i>S. fuscum</i> : 8.5 $\pm$ 0.3 <i>S. angustifolium</i> : 8.7 $\pm$ 0.2 <i>S. magellanicum</i> : 9.4 $\pm$ 0.3	<i>S. fuscum</i> : 9.9 $\pm$ 0.3
<i>Sphagnum</i> N assimilation in monospecific stands (g N m ⁻² yr ⁻¹)	<i>S. fuscum</i> : 2.7 $\pm$ 0.2 <i>S. angustifolium</i> : 2.2 $\pm$ 0.2 <i>S. magellanicum</i> : 2.4 $\pm$ 0.2	<i>S. fuscum</i> : 2.7 $\pm$ 0.3
Midsummer <i>Sphagnum</i> capitulum N (mg N g ⁻¹)	<i>S. fuscum</i> : 12.6 $\pm$ 0.3 <i>S. angustifolium</i> : 11.8 $\pm$ 0.2 <i>S. magellanicum</i> : 12.4 $\pm$ 0.4	<i>S. fuscum</i> : 15.9 $\pm$ 0.6
<i>Sphagnum</i> plant density in monospecific stands (# of plants m ⁻²)	<i>S. fuscum</i> : 60896 $\pm$ 1668 <i>S. angustifolium</i> : 29029 $\pm$ 932 <i>S. magellanicum</i> : 19658 $\pm$ 1107	<i>S. fuscum</i> : 51811 $\pm$ 2828
<i>Sphagnum</i> frequency	<i>S. fuscum</i> : 0.62 $\pm$ 0.09 <i>S. angustifolium</i> : 0.28 $\pm$ 0.07 <i>S. magellanicum</i> : 0.07 $\pm$ 0.02	<i>S. fuscum</i> : 0.73 $\pm$ 0.05 <i>S. angustifolium</i> : 0.12 $\pm$ 0.02 <i>S. magellanicum</i> : 0.09 $\pm$ 0.03
Dominant shrub frequency	0.30 $\pm$ 0.04	0.74 $\pm$ 0.05
All vascular plant frequency	1.04 $\pm$ 0.06	1.46 $\pm$ 0.09
Root biomass – top 30 cm of peat (g m ⁻²)	392 $\pm$ 57	569 $\pm$ 130
Root production (g m ⁻² yr ⁻¹)	33.6 $\pm$ 8.3	76.9 $\pm$ 33.6
Cellulose decomposition <i>k</i> values (yr ⁻¹)	0.37 $\pm$ 0.02	0.39 $\pm$ 0.02
<i>Sphagnum</i> decomposition, mass loss over 5 months (% of initial mass)	17.1 $\pm$ 2.0	10.6 $\pm$ 1.0
Vascular plant decomposition, mass loss over 5 months (% of initial mass)	32.6 $\pm$ 1.4	35.4 $\pm$ 2.4
Peat extractable NH ₄ ⁺ -N (mg N m ⁻² ; top 10 cm)	31.6 $\pm$ 4.9	41.9 $\pm$ 7.7
Peat extractable NO ₃ ⁻ -N (mg N m ⁻² ; top 10 cm)	12.7 $\pm$ 2.7	16.2 $\pm$ 2.5
Peat extractable DIN (mg N m ⁻² ; top 10 cm)	44.2 $\pm$ 4.4	58.2 $\pm$ 8.4
Net ammonification (μg m ⁻² d ⁻¹ ; top 10 cm)	11 $\pm$ 221	145 $\pm$ 57
Net nitrification (μg m ⁻² d ⁻¹ ; top 10 cm)	-5 $\pm$ 82	3 $\pm$ 4
Net DIN production (μg m ⁻² d ⁻¹ ; top 10 cm)	6 $\pm$ 291	148 $\pm$ 55
Porewater NH ₄ ⁺ -N, top of water table (μg L ⁻¹)	527 $\pm$ 69	565 $\pm$ 72
Porewater NO ₃ ⁻ -N, top of water table (μg L ⁻¹)	90 $\pm$ 15	68 $\pm$ 15
Porewater DIN, top of water table (μg L ⁻¹)	616 $\pm$ 72	620 $\pm$ 83
Porewater DON, top of water table (μg L ⁻¹)	920 $\pm$ 37	1222 $\pm$ 69

Table 2

Comparisons between ecosystem structural or functional parameter responses to N addition between Mariana Lake Poor Fen and Mariana Lake Bog.

Ecosystem structural or functional parameter	Response to N addition	
	Mariana Lake Poor Fen	Mariana Lake Bog
N ₂ -fixation	Inhibition; no threshold	Inhibition at N dep > 3.1 kg N ha ⁻¹ yr ⁻¹
<i>Sphagnum</i> linear growth	<i>S. fuscum</i> : no response <i>S. angustifolium</i> : no response <i>S. magellanicum</i> : progressive stimulation in 3 of 5 yrs	<i>S. fuscum</i> : no response
<i>Sphagnum</i> NPP	<i>S. fuscum</i> : no response <i>S. angustifolium</i> : no response <i>S. magellanicum</i> : stimulation in 3 of 5 yrs	<i>S. fuscum</i> : progressive inhibition in 2 of 5 yrs
<i>Sphagnum</i> stem N	<i>S. fuscum</i> : no response <i>S. angustifolium</i> : progressive increase in 2 of 5 yrs <i>S. magellanicum</i> : progressive increase in all 5 yrs	<i>S. fuscum</i> : progressive increase in all 5 yrs
<i>Sphagnum</i> N assimilation	<i>S. fuscum</i> : no response <i>S. angustifolium</i> : no response <i>S. magellanicum</i> : progressive increase in all 5 yrs	<i>S. fuscum</i> : increase in 2011, decrease in 2013, 2015; no response in 2012, 2014
Midsummer <i>Sphagnum</i> capitulum N	<i>S. fuscum</i> : no response <i>S. angustifolium</i> : no response <i>S. magellanicum</i> : no response	<i>S. fuscum</i> : no response
<i>Sphagnum</i> plant density in monospecific stands	<i>S. fuscum</i> : no response <i>S. angustifolium</i> : no response <i>S. magellanicum</i> : progressive increase in all 5 yrs	<i>S. fuscum</i> : progressive decrease in all 5 yrs
<i>Sphagnum</i> frequency	<i>S. fuscum</i> : progressive decrease in all 5 yrs <i>S. angustifolium</i> : progressive increase in all 5 yrs <i>S. magellanicum</i> : progressive increase in all 5 yrs	<i>S. fuscum</i> : progressive decrease in 2 of 5 yrs <i>S. angustifolium</i> : no response <i>S. magellanicum</i> : progressive increase in 2 of 5 yrs
Dominant shrub frequency	No response in yrs 1 and 2 Progressive increase in yrs 3–5	No response in yrs 1 and 2 Progressive increase in yrs 3–5
All vascular plant frequency	No response in yrs 1 and 2 Progressive increase in yrs 3–5	No response in yrs 1 and 2 Progressive increase in yrs 3–5
Root biomass – top 30 cm of peat	Progressive increase	Progressive increase
Root production	Progressive increase over one or two growing seasons	Progressive increase over one or two growing seasons
Cellulose decomposition <i>k</i> values	Progressive increase in <i>k</i> in all yrs at average rate of 0.0073 $\pm$ 0.0015 yr ⁻¹ per k N ha ⁻¹ yr ⁻¹ addition	Progressive increase in <i>k</i> in all yrs at average rate of 0.0226 $\pm$ 0.0020 yr ⁻¹ per k N ha ⁻¹ yr ⁻¹ addition
<i>Sphagnum</i> decomposition, mass loss over 5 months	Progressive decrease both of the 2 yrs of measurement	No response in either of the 2 yrs of measurement
Vascular plant decomposition, mass loss over 5 months	Progressive decrease both of the 2 yrs of measurement	No response in either of the 2 yrs of measurement
Peat extractable NH ₄ ⁺ -N (mg N m ⁻² ; top 10 cm)	No response	Progressive, but slight, increase in all yrs
Peat extractable NO ₃ ⁻ -N (mg N m ⁻² ; top 10 cm)	No response	No response
Peat extractable DIN (mg N m ⁻² ; top 10 cm)	No response	No response
Net ammonification (μg m ⁻² d ⁻¹ ; top 10 cm)	Increased in one of the four yrs	No response
Net nitrification (μg m ⁻² d ⁻¹ ; top 10 cm)	No response	Variable between yrs

Table 2 (continued)

Ecosystem structural or functional parameter	Response to N addition	
	Mariana Lake Poor Fen	Mariana Lake Bog
top 10 cm)		
Net DIN production ($\mu\text{g m}^{-2} \text{d}^{-1}$; top 10 cm)	No response	No response
Porewater NH_4^+-N , top of water table ($\mu\text{g L}^{-1}$)	No response	No response
Porewater NO_3^--N , top of water table ($\mu\text{g L}^{-1}$)	No response	No response
Porewater DIN, top of water table ($\mu\text{g L}^{-1}$)	No response	No response
Porewater DON, top of water table ($\mu\text{g L}^{-1}$)	No response	No response

4.8. Microbial community responses

We hypothesized that increasing N addition to the poor fen would result in increased ericaceous shrub growth and cover, which in turn would result in an increase in ericoid mycorrhizal fungal abundance and an increase in the fungal:bacterial ratio in peat. Although fungal abundance increased at N addition levels above $16.6 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, total bacterial abundance also increased at N addition levels above $17.1 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, such that the fungal:bacterial ratio was not significantly affected by N addition (Fig. S2). Total microbial, gram-negative bacterial, and actinomycete abundance also showed an apparent threshold responses to N addition at $16\text{--}17 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ addition levels. We cannot offer a mechanistic explanation as to why these apparent threshold responses occurred or to their potential functional significance.

At the adjacent Mariana Lake Bog, the only significant microbial response to N addition was a decrease in actinomycete abundance (Wieder et al., 2019). Similarly, N addition had minimal effects on peat microbial composition at 5 Alberta bogs representing a post-fire chronosequence (Stuart et al., 2018). Although several studies have

examined peat microbial composition using PLFA analysis, revealing differences between peatland types (bog, poor fen, mesotrophic fen), depth, peat moisture, water table drawdown, temperature, and botanical composition of the peat (Borgä et al., 1994; Sundh et al., 1997; Jaatinen et al., 2007, 2008; Bragazza et al., 2015; Peltoniemi et al., 2015; Mpamah et al., 2017), we know of no other studies that have examined potential effects of N deposition in peatlands.

5. Ecosystem scale synthesis - fate of new N additions and relevance to critical loads

Ecosystem responses to increasing atmospheric N deposition have been examined more extensively for bogs than for poor fens. Nonetheless, a critical load of $10\text{--}15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ was recommended for European poor fens (Bobbink and Hettelingh, 2011). This recommendation was based on findings that increasing N deposition results in reduced NPP and/or cover of some *Sphagnum* species, change in *Sphagnum* species composition, changes in P, K, or Ca concentrations in *Sphagnum*, and increased vascular plant growth and/or cover (Hogg et al., 1995; Jauhiainen et al., 1998; Francez and Loiseau, 1999; Granberg et al., 2001; Malmer et al., 2003; Gunnarsson et al., 2004; Wiedermann et al., 2007). *Sphagnum* mosses were thought to rely on atmospheric deposition for N, while vascular plants obtain N through mineralization in peat (Malmer et al., 2003). Further, poor fens were thought to function as a filter for atmospherically deposited N, following the triphasic response paradigm (Lamers et al., 2000; Malmer and Wallén, 2005).

At Mariana Lake Poor Fen, as N addition increased, we did observe changes in the relative abundances of the three dominant *Sphagnum* species, increases in the frequencies of *Andromeda polifolia*, *Vaccinium oxycoccos*, dominant shrubs and vascular plants (Fig. 6), and an overall change in plant community composition (Fig. 7). However, the ways in which new N inputs were processed were not consistent with what would be predicted under the triphasic response paradigm, the most important difference being the role of biological N_2 -fixation. At Mariana Lake Poor Fen, at N addition up to $7.6 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, N_2 -fixation is greater than atmospheric N deposition as a source of new N (Fig. 13). Because increasing N addition inhibits N_2 -fixation (Fig. 2B), as N addition increases above $7.6 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, atmospheric deposition becomes increasingly

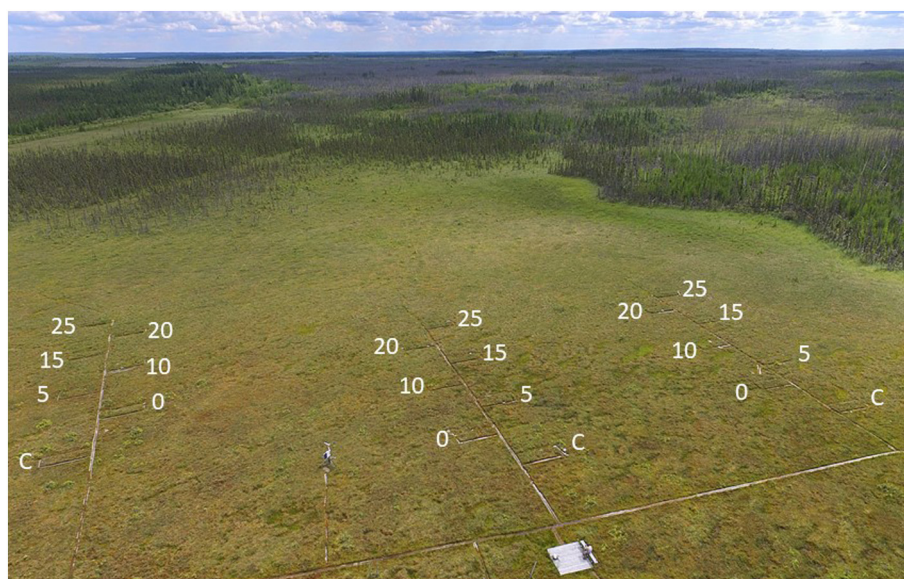


Fig. 12. Drone photograph of the Mariana Lake Poor Fen study site after five years of N addition, showing the visibly evident increase in shrub cover as N addition increases up to $25 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. White numbers adjacent to each plot indicate N addition in $\text{kg N ha}^{-1} \text{ yr}^{-1}$; C indicates control plots.

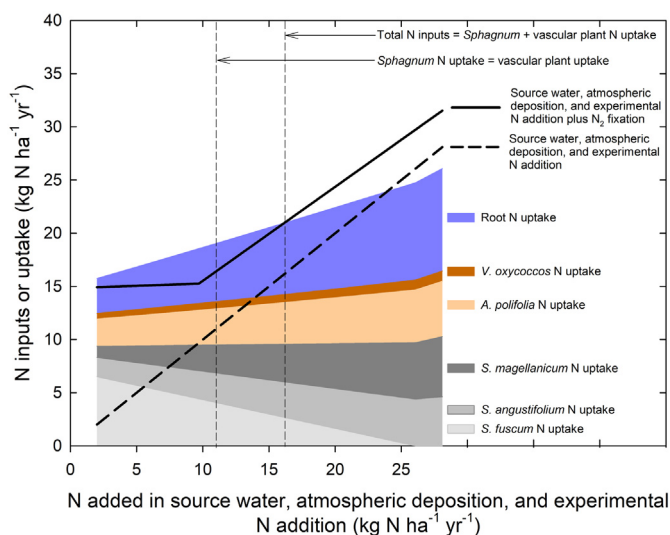


Fig. 13. Schematic representation of N additions and sequestration by *Sphagnum* and vascular plant annual growth based on results at Mariana Lake Poor Fen. To develop this framework, we used N uptake relationships for *S. fuscum*, *S. angustifolium*, and *S. magellanicum* (Fig. 4D, E, F) adjusted for changes in frequencies of these three species (Fig. 6A, D, G). We did not quantify vascular plant NPP, but used NPP values for *Andromeda polifolia* and *Vaccinium oxycoccus* at Bleak Lake Bog, Alberta (18.4 and 4.5 g m⁻² yr⁻¹, respectively; Szumigalski, 1995), along with leaf N concentration (Fig. 5) and frequency (Fig. 6B, E) responses to N addition at Mariana Lake Poor Fen. We calculated root N uptake using the response of root production to N addition at Mariana Lake Poor Fen (Fig. 8D; average of root production over one and two growing seasons), assuming a root N concentration of 7 mg/g (Xu, 2011).

dominant as a source of new N. A similar pattern was obtained at Mariana Lake Bog where background N₂-fixation was much higher (Table 1) and also was inhibited by N addition, so that N₂-fixation was greater than atmospheric deposition at N addition levels up to 21 kg N ha⁻¹ yr⁻¹ (Wieder et al., 2019). At Mariana Lake Poor Fen, the demand for N to support moss and vascular plant growth was slightly higher than N inputs from N₂-fixation and deposition at combined N inputs up to 16 kg N ha⁻¹ yr⁻¹ (Fig. 13), suggesting that at combined new N inputs up to this level, plant growth may require N inputs from dry N deposition, N mineralization in the peat, and/or groundwater or surface water. At combined N inputs above 16 kg N ha⁻¹ yr⁻¹, new N inputs appear to exceed plant growth demands (Fig. 13). Since we did not observe increasing concentrations of DIN and/or DON in Mariana Lake Poor Fen porewaters at N additions >16 kg N ha⁻¹ yr⁻¹, it is likely that some N is taken up by the growth of vascular plant species whose production we did not quantify. At Mariana Lake Bog, N assimilation by *Sphagnum fuscum* and vascular plants, ranging from 35 to 45 kg N ha⁻¹ yr⁻¹ over the entire N addition gradient, was much higher than at the poor fen (Wieder et al., 2019). Further, at Mariana Lake Bog, N₂-fixation was much higher (14–40 kg N ha⁻¹ yr⁻¹) than at the poor fen; at the bog, combined N input from N₂-fixation and deposition exceeded the demand for moss and vascular plant growth at N addition levels up to 21 kg N ha⁻¹ yr⁻¹ (Wieder et al., 2019). At both sites, under ambient N deposition, it is evident that *Sphagnum* NPP relies more on N₂-fixation than on atmospheric deposition. Also at both sites, a consequence of increasing N addition is a shift from new N inputs being sequestered primarily by growing *Sphagnum* to being sequestered primarily by growing vascular plants, with this shift occurring at 10.9 and 13.4 kg N ha⁻¹ yr⁻¹ at the poor fen and bog, respectively (Fig. 13, Wieder et al., 2019).

We note that the relatively low N₂-fixation and hence input of new N in the poor fen, and effective uptake of new N by *Sphagnum* and shrub growth, may preclude the development of a substantial *Picea mariana* and tall ericaceous shrub cover.

As with our work at Mariana Lake Bog, the synthesis for Mariana Lake Poor Fen, summarized in Fig. 13, is not intended to represent a complete N budget. Components that are not included are dry N deposition, potential gaseous losses of N₂/N₂O, N uptake by vascular plant species that represented a relatively minor component of the vegetation, dissolved N inputs from groundwater and/or surface water, gross N mineralization and immobilization in peat, and dissolved N losses in streamflow that are likely to be more important in poor fens than in bogs. Nonetheless, Fig. 13 hopefully represents a reasonable quantitative assessment of the sources and major short-term fates of new N inputs to the poor fen.

Based on our work at Mariana Lake Bog, we recommended a critical load of 3 kg N ha⁻¹ yr⁻¹; at higher N deposition rates, N₂-fixation is progressively inhibited (Wieder et al., 2019). This was one of the few threshold responses that we observed, and signaled the onset of major changes in N cycling (decreasing NPP, N uptake, and cover of *Sphagnum*; increasing NPP, N uptake, and cover of shrubs; shift from *Sphagnum* dominance of N assimilation to shrub dominance of N assimilation). Although Bobbink and Hettelingh (2011) argued that poor fens should be less sensitive than bogs to increasing N deposition, this appears not to be the case when comparing the Mariana Lake Poor Fen and bog sites. Inputs of new N from N₂-fixation and atmospheric deposition to Mariana Lake Poor Fen are considerably lower than inputs to the bog and correspondingly, both NPP and the capacity for N sequestration by moss and vascular plant growth are also much lower at the poor fen than at the bog. At the bog, N sequestration by annual plant growth exceeded combined N inputs from N₂-fixation and deposition when these inputs reached 18 kg N ha⁻¹ yr⁻¹ (Wieder et al., 2019). In contrast, at the poor fen, combined N inputs from N₂-fixation and deposition exceeded N sequestration by annual plant growth when combined N inputs reached 16 kg N ha⁻¹ yr⁻¹ (Fig. 13), suggesting saturation of the N sequestration capacity of the poor fen. In general, poor fen structural and functional responses to increasing N addition did not exhibit threshold triggers, but rather began as soon as N additions began to increase. All of these factors together lead us to recommend a critical load for poor fens in Alberta of 3 kg N ha⁻¹ yr⁻¹. At N deposition levels above this critical load, poor fens should exhibit increasing shrub cover and decreasing *Sphagnum* cover, which we, like Bobbink and Hettelingh (2011), regard as a harmful consequence given the foundational role of *Sphagnum* mosses in poor fen ecosystems.

Previously, we produced a map of inorganic N deposition in a 3255 km² sampling area centered at the midpoint between the Syncrude and Suncor upgrader stacks (Wieder et al., 2016a). Within this area, 1506 km² currently receives N deposition in excess of 3 kg N ha⁻¹ yr⁻¹, of which 104 km² and 355 km² are bogs and fens, respectively, based on the Alberta Wetland Inventory (Halsey et al., 2003) (fens are not differentiated between poor, rich, and shrubby fens). Thus, it appears that bogs and poor fens are being negatively impacted by N deposition related to oil sands development in northern Alberta.

CRedit authorship contribution statement

R. Kelman Wieder: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing - original draft, Visualization, Supervision, Project administration, Funding acquisition. **Dale H. Vitt:** Conceptualization, Methodology, Investigation, Formal analysis, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Melanie A. Vile:** Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Jeremy A. Graham:** Methodology, Investigation, Resources, Formal analysis, Data curation, Writing - review & editing, Supervision, Project administration. **Jeremy A. Hartsock:** Methodology, Investigation, Data curation, Writing - review & editing. **Jacqueline M.A. Popma:** Methodology, Investigation, Formal analysis, Data curation, Writing - review & editing. **Hope Fillingim:** Methodology, Investigation, Formal analysis,

Data curation, Supervision. **Melissa House:** Methodology, Investigation, Formal analysis, Data curation, Writing - review & editing. **James C. Quinn:** Methodology, Investigation, Resources, Data curation, Supervision. **Kimberli D. Scott:** Methodology, Investigation, Resources, Data curation, Writing - review & editing, Supervision, Project administration. **Meaghan Petix:** Methodology, Investigation, Formal analysis, Data curation. **Kelly J. McMillen:** Methodology, Investigation, Data curation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data statement

Data are archived at the Environmental Data Initiative (nitrogen fixation data: <https://doi.org/10.6073/pasta/304e862ca19052360c84739d0dc78ec5>; cellulose, peat, and mixed vegetation decomposition data: <https://doi.org/10.6073/pasta/c7866f5b50a61227a11bd351f4e5250d>; net N mineralization data: <https://doi.org/10.6073/pasta/fc4f17cb2e51bf1f2ff41b289cbf071d>; phospholipid fatty acid data: <https://doi.org/10.6073/pasta/910d7e43736bffd4e12e00ec2eb56bee>; point frame moss and vascular frequency data: <https://doi.org/10.6073/pasta/1cc585e08d3a8efc80e53cfc2b033612>; nitrogen deposition data: <https://doi.org/10.6073/pasta/60367870c88e92b503c72af7d95cca14>; root biomass and production data: <https://doi.org/10.6073/pasta/a775ba71095ae946acf3157fc4afe438>; *Sphagnum fuscum* growth and N concentration data: <https://doi.org/10.6073/pasta/96e5013c4ff9db6f381324e521270209>; vascular plant leaf N concentration data: <https://doi.org/10.6073/pasta/3cdb645440e83b8fe7a5d40c74fe5b4b>; porewater N concentration data: <https://doi.org/10.6073/pasta/cc53039eee0dd878fad382669c0e123>).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2020.138619>.

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