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Experimental nitrogen addition alters structure and function of a boreal bog: critical load and thresholds revealed

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Abstract. Bogs and fens cover 6% and 21%, respectively, of the 140,329 km^2 Oil Sands Administrative Area in northern Alberta. Development of the oil sands has led to increasing atmospheric N deposition, with values as high as 17 kg $N \cdot ha^{-1} \cdot yr^{-1}$; regional background deposition is <2 kg $N \cdot ha^{-1} \cdot yr^{-1}$. Bogs, being ombrotrophic, may be especially susceptible to increasing N deposition. To examine responses to N deposition, over five years, we experimentally applied N (as NH_4NO_3) to a bog near Mariana Lake, Alberta, unaffected by oil sands activities, at rates of 0, 5, 10, 15, 20, and 25 kg $N \cdot ha^{-1} \cdot yr^{-1}$, plus controls (no water or N addition). Increasing N addition: (1) stimulated N_2 fixation at deposition <3.1 kg $N \cdot ha^{-1} \cdot yr^{-1}$, and progressively inhibited N_2 fixation as N deposition increased above this level; (2) had no effect on *Sphagnum fuscum* net primary production (NPP) in years 1, 2, and 4, but inhibited *S. fuscum* NPP in years 3 and 5; (3) stimulated dominant shrub and *Picea mariana* NPP; (4) led to increased root biomass and production; (5) changed *Sphagnum* species relative abundance (decrease in *S. fuscum*, increase in *S. magellanicum*, no effect on *S. angustifolium*); (6) led to increasing abundance of *Rhododendron groenlandicum* and *Andromeda polifolia*, and to vascular plants in general; (7) led to increasing shrub leaf N concentrations in *Andromeda polifolia*, *Chamaedaphne calyculata*, *Vaccinium oxycoccos*, *V. vitis-idaea*, and *Picea mariana*; (8) stimulated cellulose decomposition, with no effect on *S. fuscum* peat or mixed vascular plant litter decomposition; (9) had no effect on net N mineralization rates or on porewater NH_4^+-N, NO_3^--N, or DON concentrations; and (10) had minimal effects on peat microbial community composition. Increasing experimental N addition led to a switch from new N being taken up primarily by *Sphagnum* to being taken up primarily by shrubs. As shrub growth and cover increase, *Sphagnum* abundance and NPP decrease. Because inhibition of N_2 fixation by increasing N deposition plays a key role in bog structural and functional responses, we recommend a N deposition critical load of 3 kg $N \cdot ha^{-1} \cdot yr^{-1}$ for northern Alberta bogs.

Key words: bog; boreal; critical load; N_2 fixation; nitrogen; NPP; peat; peatland; Sphagnum.

Introduction

The biogeochemical cycle of nitrogen (N) in northern temperate, boreal, and subarctic peatland ecosystems has been altered substantially over large regions of North America and Europe due to anthropogenic activities. As a result, much current understanding of peatland N cycling has been derived from studies that have taken place in biogeochemically altered regions of the globe

(Heijmans et al. 2001, Hoosbeek et al. 2002). Reactive N compounds have increased over historical levels globally and annual rates of atmospheric N deposition are projected to increase into the mid-21st century (Galloway et al. 2008). Some remote regions of the globe have had historically low atmospheric N deposition (<2 kg $N \cdot ha^{-1} \cdot yr^{-1}$) and may remain relatively pristine into the future (Galloway et al. 2008). Northern Alberta, Canada may have been such a region, if not for development of the Alberta oil sands beginning in the 1970s.

Peatlands are a major feature of the northern Alberta landscape (Raine et al. 2002, Lee and Cheng 2009) with bogs covering 6% and fens covering 21% of the

140,329 km^2 Oil Sands Administrative Area (Wieder et al. 2016a). Development of the oil sands resource has led to increasing rates of atmospheric N deposition, with values as high as 17 kg N·ha^{-1}·yr^{-1} in the heart of the oil sands mining region (Wieder et al. 2016a). Given the expanding influences of elevated N on the global environment, it is important to understand how N deposition may affect peatlands that historically have not been impacted by local, regional, and/or long-range atmospheric N deposition. Pristine or nearly pristine systems provide valuable background information about ecosystem function (cf. Hedin et al. 1995, Sheppard et al. 2004) and are of fundamental importance in providing thresholds or critical loads for N, i.e., levels of atmospheric N deposition above which the effects on receiving ecosystems are deemed unacceptable (Bobbink et al. 2010, Bobbink and Hettelingh 2011).

In northern Alberta, given the expansion of oil sands development in recent years, we sought to understand the effects of low to moderate N additions (0–25 N·ha^{-1}·yr^{-1}) on bog ecosystem structure and function with the goal of providing a critical load recommendation for N-sensitive peatlands. Bogs, being ombrotrophic, may be especially susceptible to increasing N deposition. In Europe, transboundary air pollution conventions designed to decrease or reverse unwanted effects of N, notably the Gothenburg Protocol, make use of critical loads (Tipping et al. 2013). Critical loads of N for European raised and blanket bogs have been set at 5–10 kg N·ha^{-1}·yr^{-1} (Bobbink and Hettelingh 2011).

Critical loads for bogs in Europe have been set based on greenhouse studies, field studies of bogs along ambient N deposition gradients, and field experimental studies in which known additions of N have been related to observable short-term changes in plant species composition, net primary production (NPP), and other biogeochemical effects (Bobbink and Hettelingh 2011, Tipping et al. 2013). Field N fertilization studies of bogs in Europe have ranged geographically from the subarctic to boreal to temperate regions, with study site differences in air temperature (mean annual temperature from −8° to 9°C) and precipitation (total annual precipitation from 304 to 1,680 mm/yr) regimes, present and historical atmospheric N deposition (2–52 kg N·ha^{-1}·yr^{-1}), the quantity of N added (as high as 100 kg N·ha^{-1}·yr^{-1}), and the form of N experimentally applied (NH$_4$$^+$-N vs. NH$_4NO_3$; cf. Tamm 1954, Aerts et al. 1991, 1992, Williams et al. 1999, Gunnarsson and Rydin 2000, Berendse et al. 2001, Heijmans et al. 2001, van Heerwaarden et al. 2003, Limpens et al. 2003a, 2004, Nordbakken et al. 2003, Tomassen et al. 2004, Gerdol et al. 2007, Wiedermann et al. 2007, Lund et al. 2009, Sheppard et al. 2011, Bragazza et al. 2012). Far fewer field experimental N addition studies have occurred in North American bogs, all in temperate or subboreal areas, and these too have had study site differences in air temperature (mean annual temperature from 3° to 8°C) and precipitation (total annual precipitation

from 700 to 1,030 mm/yr) regimes, present and historical atmospheric N deposition (4–8 kg N·ha^{-1}·yr^{-1}), the quantity of N added (16–100 kg N·ha^{-1}·yr^{-1}), and the form of N experimentally applied (NH$_4$NO$_3$ vs. NH$_4$Cl vs. urea; Bartsch and Schwintzer 1994, Chapin et al. 2004, Iversen et al. 2010, Bubier et al. 2011). Across all of these studies, there is considerable variation in the quantity of water used in experimental N applications, the concentrations of N in experimental applications, the number of levels of N addition, the degree of replication of treatments, the number of years over which experimental N application occurred, and species, community, and ecosystem-scale response variables. Thus, in assessing bog responses to increasing atmospheric N deposition, there are numerous potentially confounding factors that complicate the ability to draw generalizations (cf. Limpens et al. 2011). Further, plant, microbial and peat responses to N loading are likely to vary temporally, spatially, and by ecosystem type. Most ecological processes operate and interact on different temporal and spatial scales; therefore, responses to increasing N loading are likely to be complex.

Nonetheless, intersite variability in experimental approach and ecosystem responses were somewhat reconciled through the proposed triphasic response of *Sphagnum*-dominated peatlands to increasing atmospheric N deposition (Lamers et al. 2000, Berendse et al. 2001, Heijmans et al. 2002): (1) at low rates, N deposition stimulates *Sphagnum* production, (2) as N deposition increases, N no longer limits *Sphagnum* production, but is taken up by growing mosses, resulting in increases in the organic N concentration in living *Sphagnum* tissues, (3) at high N deposition, the living *Sphagnum* layer becomes N-saturated such that some of the atmospherically deposited inorganic N bypasses the living *Sphagnum* and leaches downward into the peat, where it may fuel either vascular plant production or heterotrophic microbial activity. *Sphagnum*-dominated peatlands differ from other terrestrial ecosystems in the critical role of *Sphagnum* mosses that form a nearly uniform cover at the peat/atmosphere boundary and act as scavengers of atmospheric N. *Sphagnum* mosses can be viewed as gatekeepers of new N additions in ombrotrophic bogs. The triphasic response conceptual framework has not been validated in boreal bogs that historically have received extremely low rates of atmospheric N deposition (<2 kg N·ha^{-1}·yr^{-1}), such as those occurring in northern Alberta, Canada.

Here we report on patterns of N additions, concentrations, and pools in various ecosystem components, and on plant-peat-microbial responses to a five-year field experiment, with seven levels of experimental N addition (up to 25 kg N·ha^{-1}·yr^{-1}), at a boreal bog in northern Alberta, Canada. We chose a typical and remotely located boreal site, Mariana Lake Bog, which has not been subjected to increasing atmospheric N deposition from oil sands development, or from other major anthropogenic N additions since the initiation of peat formation 5,000–7,500 yr ago (cal yr BP; Nicholson and

Vitt 1990, Yu et al. 2014). Our overarching objectives were two-fold: (1) to evaluate whether the triphasic response developed from European research applies to a boreal bog in northern Alberta, Canada with historically low N deposition, and (2) to develop science-driven recommendations for setting a critical load for N for bogs in Alberta with broader relevance to currently pristine boreal bogs. Following from and building on the first objective, we developed a series of specific hypotheses:

1) Atmospheric N deposition represents the major pathway by which new N is added to Mariana Lake Bog.

2) Biological N_2 fixation is relatively unimportant as a source of new N additions to Mariana Lake Bog.

3) *Sphagnum fuscum*, the dominant peat-forming moss, is N limited and hence its growth will be stimulated by increasing N deposition above background levels, up to a N deposition level above which *S. fuscum* growth is no longer N limited.

4) Beyond the N deposition level where *S. fuscum* growth is no longer N limited, increasing N deposition will have no effect on *S. fuscum* plant density (plants/m^2) or capitulum (top 1 cm of individual *Sphagnum* plants) mass density (g/m^2), but will result in increasing N concentrations in *S. fuscum* capitula and stems (2 cm below capitula) up to a N deposition level beyond which neither capitulum nor stem N concentrations will continue to increase. At this point, the *Sphagnum* moss layer will be N saturated.

5) As N deposition levels increase above those that cause the *Sphagnum* moss layer to be N saturated, the aboveground and belowground production of N-limited dominant ericaceous shrubs and of *Picea mariana* will be stimulated. As N deposition increases to the point where the growth of ericaceous shrubs, perennial herbs, or *P. mariana* is no longer N limited, plant leaf/needle N concentrations may increase. Increased shrub/*P. mariana* growth, with or without increasing leaf/needle N concentrations, will lead to an increasingly greater sequestration of atmospherically deposited N by vascular plants.

6) With increasing N deposition, the plant community composition will change toward a lower abundance of *Sphagnum* mosses and a higher abundance of vascular plants.

7) As N deposition increases, decomposition rates of vascular plant litter, *Sphagnum*, and a uniform substrate (cellulose filter paper) will increase.

8) As N deposition increases to a level that exceeds the capacity of the bog vegetation to take up N, net N mineralization in surface peat will be inhibited by higher NH_4^+-N availability, while net nitrification will be stimulated by higher NH_4^+-N availability.

9) As N deposition increases, peat microbial community structure will change. In particular, the fungal : bacterial ratio will increase.

10) As N deposition increases to a level that exceeds the capacity of the bog vegetation to take up N,

atmospherically deposited N will move downward through the peat, resulting in increasing concentrations of porewater dissolved N at the top of the bog water table. At this point, the bog will be considered to be N saturated.

METHODS

Study area

The Mariana Lake peatland complex (55°53′43″ N, 112°05′41″ W; elevation 695 m above sea level) is located 100 km south-southwest of Fort McMurray, Alberta, Canada, about 1 km east of Highway 63. The complex includes an ombrotrophic bog and a large acidic (poor) fen, bordered by upland forest to the northeast and southeast, continuous poor fen to the west, and a gravel road with culverts along the northeast that separates the study area from a larger peatland complex (detailed site description and map provided in Graham et al. 2015). Vegetation at Mariana Lake Bog is representative of Alberta bogs generally (cf. Benscoter and Vitt 2008, Wieder et al. 2009, Graham et al. 2015). The peat surface is covered by *Sphagnum* species, predominantly *S. fuscum*, *S. angustifolium*, and *S. magellanicum*. Vascular plant species include the tree, *Picea mariana*, ericaceous shrubs *Andromeda polifolia*, *Chamaedaphne calyculata*, *Rhododendron groenlandicum*, *Vaccinium vitis-idaea*, *Vaccinium oxycoccos*, and *Kalmia polifolia*, perennial herbs *Rubus chamaemorus* and *Maianthemum trifolium*, and the sedge *Eriophorum vaginatum*.

At Wandering River, Alberta (55°30′ N, 112°30′ W; 80 km south of the Mariana Lake peatland complex along Highway 63) mean annual temperature averages 0.2°C, with monthly mean temperatures ranging from −24.5°C (January) to 16.0°C (July); total annual precipitation averages 490 mm, 365 mm as rainfall and 123 mm as snowfall (30 yr means; data *available online*).[8] Meteorological data were collected at Mariana Lake Bog using a WatchDog weather station (Spectrum Technologies, Aurora, Illinois, USA). Missing meteorological data were in-filled using the interpolated weather data since 1961 for Alberta Townships resource (ACIS, undated). For June through September of each year, we calculated potential evapotranspiration (PET) from hourly temperature data using the Hargreaves approach (Hargreaves and Allen 2003), and an aridity index as PET divided by precipitation (cf. Winter and Woo 1990).

Experimental design

In the bog, we established 21 7.2 m^2 experimental plots along three 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 equivalent to 0, 5, 10, 15, 20, and 25 kg N·ha^{-1}·yr^{-1}. For

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[8] http://theweathernetwork.com

each application, about 13,000 L of water were trucked to the site, pumped into a collapsible, portable water tank, and pumped to a platform in the bog to fill six 200-L tanks, with NH_4NO_3 added to these tanks to achieve the appropriate N dose. For each application, we sprayed each plot with 200 L of appropriately dosed N. The total amount of water added to the N treatment plots was 231 mm/yr, roughly equivalent to one-half of mean annual precipitation of the area. Nitrogen in the water delivered to the site had a mean N concentration of 0.5 mg/L (NH_4^+-N, 0.44 mg/L; NO_3^--N, 0.06 mg/L), adding an additional 1.1 kg $N \cdot ha^{-1} \cdot yr^{-1}$ to each plot that received water. Atmospheric deposition at the site added 0.6, 0.9, and 1.5 kg ha^{-1} yr^{-1} of NH_4^+-N, NO_3^--N, and DIN (dissolved inorganic N; see *Atmospheric N deposition*). Thus, the annual total N doses for the C, 0, 5, 10, 15, 20, and 25 kg $N \cdot ha^{-1} \cdot yr^{-1}$ treatments were 1.5, 2.6, 7.6, 12.6, 17.6, 22.6, and 27.6 kg $N \cdot ha^{-1} \cdot yr^{-1}$.

Atmospheric N deposition

We quantified atmospheric N deposition using ion exchange resin tube collectors (Fenn et al. 2003, Wieder et al. 2016b), filled with mixed bed cation/anion exchange resin (Amberlite IRN 150; DuPont, Wilmington, Delaware, USA) that had been rinsed with deionized water prior to deployment in the field. We placed five collectors in the open and five among clumps of *Picea mariana* trees (throughfall). In addition, two resin tubes, sealed at the top, and placed in the field within PVC sleeves, served as blank controls. A plastic funnel with a collection area of 400 cm^2 channeled precipitation into the resin tubes. We collected and replaced tubes in mid-May and mid-October, to coordinate with initial and final cranked wire measurements for calculating *Sphagnum fuscum* net primary production (NPP; Sphagnum fuscum *growth, N assimilation and N storage*). We refer to mid-May through mid-October as the "growing season" and mid-October through mid-May as the "non-growing season." For the October–May periods, we affixed a 1-m extension tube to each collection funnel to capture snowfall, which upon melt would drain into 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). We subtracted volume-weighted concentrations in blank resin tube extracts from concentrations in extracts from exposed resin tubes and corrected deposition values for laboratory-determined extraction efficiencies (96.9% and 98.2% for NH_4^+-N and NO_3^--N, respectively; Wieder et al. 2016b).

To assess deposition differences between open and throughfall collectors, we used a repeated measures ANOVA, with each sampling period as a blocked effect. To assess deposition differences between seasons (growing season vs. non-growing season), we used a repeated-measures ANOVA, with position (open vs. throughfall) and year as blocked effects.

Nitrogen fixation

We measured N_2 fixation using the acetylene reduction technique (Hardy et al. 1968) with field modifications. At the beginning of each field season, we collected one core of *Sphagnum fuscum*, ~8 cm deep and 7 cm in diameter in each plot, wrapped each core in mesh screening, and placed cores into the peat, allowing for repeated measurements over a growing season. We measured N_2 fixation in these cores five times in 2012, five times in 2013, twice in 2014, and twice in 2015, with all measurements between June and August. For incubations, we weighed the moss cores, placed them upside down in gas-tight 500-mL glass screw-top jars with attached stopcocks, injected 20 mL of acetylene, and returned the jars to the surface peat for 24 h with the bottom of the jar facing upward to expose the moss capitula to sunlight. Using 10-mL syringes with stopcocks, we collected headspace samples at the beginning and end of each 24-h incubation period; linearity of ethylene production over time was established previously. 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 using a Shimadzu 2014 gas chromatograph (Shimadzu Scientific US, Columbia, Maryland, USA) with a flame ionization detector and HayeSep T column (Shimadzu Scientific US) with 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) and 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 0.29 moles of ethylene produced per mole of N_2 fixed, based on a robust set of previous $^{15}N_2$ fixation measurements in *S. fuscum* moss cores in Alberta bogs (Vile et al. 2014).

Sphagnum fuscum *growth, N assimilation and N storage*

Net primary production (NPP) of *Sphagnum fuscum* is the product of linear growth of stems (cm/yr) and stem mass density (SMD), with SMD defined as the mass of 1-cm lengths of *Sphagnum* stems beneath the capitula per square meter. We measured linear growth using the cranked wire method (Clymo 1970). We set 30 cranked wires in each of the 21 study plots, with all wires placed in hummocks dominated by *S. fuscum*. Each year, from 2011 through 2015, we set cranked wires in May after the surface peat had thawed, and remeasured them in late September or early October.

To measure SMD, we collected surface cores (6.5 cm diam.) from *S. fuscum* hummocks in each plot between 7 and 10 July of each year. From each core, we removed and counted all capitula to determine *S. fuscum* plant density (plants/m^2). We separated 70 stems of *S. fuscum*,

cut them into 2-cm lengths, weighed them after drying at 55°C for 5 d, and calculated SMD as the average mass of a 1-cm stem of an individual *Sphagnum* plant multiplied by plant density.

From cores collected in early July and early October from 2013 through 2015, we calculated capitulum mass density (CMD, g/m^2) as the dry mass of all capitula from a core, scaled up to a per square meter basis. Capitula and stems were dried, homogenized in a Thomas Wiley Mini-Mill (Thomas Scientific, Swedesboro, New Jersey, USA), and analyzed for N concentration on a Flash EA 1112 Series CN Soil Analyzer (Thermo Fisher Scientific).

We calculated the amount of N annually utilized by *S. fuscum* to satisfy NPP requirements (g N·m^{-2}·yr^{-1}) as the product of *S. fuscum* NPP and the N concentration in the 0–2 cm stem sections. For both summer and fall collections, we calculated the quantity of N stored in *S. fuscum* capitula (g N/m^2) as the product of CMD and N concentration in the capitula.

Aboveground shrub NPP

We developed allometric equations for non-destructive measurement of aboveground NPP for the three dominant shrub species, *Andromeda polifolia*, *Chamaedaphne calyculata*, and *Rhododendron groenlandicum*. We destructively sampled 50 new growth segments (shoots with leaves), thereby collecting all mass from the most recent bud scar collected in late July 2014, for each of the three species. For each growth segment, we measured total length (mm), counted the number of leaves, measured the length and width of each leaf (mm), and dried each of the growth segments at 55°C for 5 d before weighing. From these measurements, for each species, we developed an allometric equation that gave the best linear fit to the measured mass per growth segment (MPS; mg/segment). These allometric equations (all significant, $P < 0.01$) are for *A. polifolia*:

$$MPS = 0.646$$
$$\times \sqrt{(0.5 \times ALL \times 0.5 \times ALW \times \pi \times SL \times LPS)}$$
$$- 17.2; R^2 = 0.86$$

for *C. calyculata*:

$$MPS = 0.523$$
$$\times \sqrt{(0.5 \times ALL \times 0.5 \times ALW \times \pi \times SL \times LPS)}$$
$$- 20.0; R^2 = 0.92$$

and *for R. groenlandicum*:

$$MPS = 0.712$$
$$\times \sqrt{(0.5 \times ALL \times 0.5 \times ALW \times \pi \times SL \times LPS)}$$
$$- 10.0; R^2 = 0.85$$

where ALL is the average length of leaves per segment (mm), ALW is the average width of leaves per segment

(mm), SL is segment length (cm), and LPS is the number of leaves per segment.

In the second week of June 2014, we determined the density of the new growth segments (segments/m^2) by counting all the new growth segments for each of the selected species in each 7.2 m^2 plot. In the last week of July 2014, we measured new growth segment lengths, number of leaves, and leaf dimensions for five individual segments of each of the species per plot, and calculated MPS. We calculated plot-level aboveground NPP (g·m^{-2}·yr^{-1}) for each species as the product of the mean MPS (g·segment^{-1}·yr^{-1}) and the density of new growth segments (segments/m^2).

In 2015, we revised the allometric growth metrics to allow for more shoots per plot to be measured by overcoming the time constraints imposed by measuring the dimensions of each leaf per shoot. Using the average leaf area (calculated as the area of an ellipse using the leaf dimensions) of all measured leaves for each species per plot, the revised allometric equations (all significant, $P < 0.01$) for mass per growth segment (MPS$_b$, mg/segment) are for *A. polifolia*:

$$MPS_b = 0.3 \times \sqrt{(ALA + LLA)^2 \times SL \times LPS}$$
$$+ 0.2; R^2 = 0.85$$

for *C. calyculata*:

$$MPS_b = 0.01 \times \sqrt{(ALA + LLA)^2 \times SL \times LPS}$$
$$+ 8.3; R^2 = 0.91$$

and for *R. groenlandicum*:

$$MPS_b = 0.02 \times \sqrt{(ALA + LLA)^2 \times SL \times LPS}$$
$$+ 4.3; R^2 = 0.85$$

where ALA is the average leaf area for a new growth segment (mm^2), LLA is the area of the largest leaf on a segment (mm^2), SL is segment length (cm), and LPS is the number of leaves per segment.

In late July 2015, we measured five plants of each species per plot as in 2014, in addition to 15 new growth segments per species measuring length of the new shoots, number of leaves, and dimensions of the largest leaf. In the second week of June 2015, we counted shoot densities in the same manner as in 2014. We found no significant differences in predicted new growth segment masses between the original and revised models. In 2015, we calculated aboveground NPP for each species as the product of shoot density and the mean of the 20 derived new shoot masses.

Plant tissue nitrogen concentrations

We analyzed *Sphagnum fuscum* samples taken from cores used to calculate SMD, and additional samples collected in the fall before mean daily temperatures fell below 0°C (typically between September 25 and October 6), for N concentrations in capitula and in the 0–2 cm stem segment just below the capitula. Between July 10 and July 17 of each year, we collected samples of new vascular plant growth (current year growth leaves or needles of *Andromeda polifolia*, *Chamaedaphne calyculata*, *Rhododendron groenlandicum*, *Picea mariana*, *Vaccinium oxycoccos*, *Rubus chamaemorus*, and *Maianthemum trifolium*). We collected current year leaves of *Eriophorum vaginatum* and *Vaccinium vitis-idaea* in 2013–2015, and current year leaves of *Kalmia polifolia* in 2015. We marked individuals from which leaves/needles were collected to avoid resampling in subsequent years. All samples for C and N analysis were sorted for removal of debris, dried for a minimum of 6 d at 55 °C, homogenized in a Thomas Wiley Mini-Mill, and analyzed on a Flash EA 1112 NC Analyzer.

Picea mariana *growth*

In September 2013–2015, we measured annual leader extension of *Picea mariana* on four trees (0.5–2.5 m tall) per plot. To convert leader extension to tree production, we used the allometric equation for *Picea mariana* growth in an Alberta bog developed by Munir et al. (2015): growth (kg·tree^{-1}·yr^{-1}) = 0.0085 × (leader extension, cm/yr)$^{2.2088}$. Across a post-fire bog chronosequence in Alberta, on average, 22% of bog *P. mariana* aboveground biomass was contributed by needles (Wieder et al. 2009). Density of *P. mariana* trees is variable both within and between Alberta bogs, ranging from <1 to over 37 trees/m^2 (>0.4 cm diam.; Wieder et al. 2009). Using a visually estimated *P. mariana* density at Mariana Lake Bog of 1 individual (>0.5 m tall)/m^2, and N concentrations in *P. mariana* needles, we calculated annual N assimilation attributable to new needle production.

Root biomass and production

We measured root biomass in the top 30 cm of peat in 2014. On 31 May 2014, we extracted two cores (7.6 cm diameter, 30 cm deep) from each plot using a PVC pipe with a sharpened metal cylindrical tip. We manually extracted roots from cores divided into two 15-cm sections using tweezers by submerging each section in a shallow dish filled with water to help tease the roots apart.

To quantify root production, we used the ingrowth bag approach (Finér and Laine 2000). On 1 June 2014, we inserted two 1-m mesh tulle bags (7.6 cm diameter; 30 cm tall) filled with Premier organic peatmoss (Premier Tech, Riviere-du-Loup, Quebec, Canada) vertically into the peat in each plot. We removed one bag on 5

October 2014 and the other on 23 September 2015, from which we calculated 1-yr and 2-yr root production. Upon retrieval, we cut each of these bags into 15-cm sections and froze them. Due to the extreme number of fine roots throughout the 15 cm core sections, we cut the midsection of each core into a 2-cm disc using a bandsaw. We gently boiled the discs for 30 min, allowing roots to become loosened, manually separated roots from the peat, dried them for 4 d at 55°C, and weighed them. Root biomass in the top 30 cm of peat was reported for 10 Alberta bogs across a 102-yr time since fire chronosequence (Xu 2004, Wieder et al. 2009). Using these data, across all 10 bog sites, root biomass in the 6–9 cm depth interval was not significantly different from root biomass in the 0–3, 3–6, 9–12, or 12–15 cm depth intervals and root biomass in the 21–24 cm depth interval was not significantly different from root biomass in the 15–18, 18–21, 24–27, or 27–30 cm depth intervals.

Plant community composition

Plant cover was determined by the point-intercept method (Levy and Madden 1933, Jonasson 1988). Using a 1.2-m long point frame anchored permanently in each plot with PVC tubes, we characterized bog plant community composition between 17 and 19 July of each year. We recorded all species touched by a vertical 1-mm rod from the top of the canopy to the peat surface from 30 points placed 3.5 cm apart on the frame. For a given species, frequency was calculated as the proportion of the 30 pins that intercepted that species one or more times.

Decomposition

We measured decomposition of filter papers (2012–2014) and later native plant material taken from each plot (2014–2015), using litter bags placed 10 cm below the peat surface. Five filter papers (Whatman 41; 70 mm diameter) were placed inside of nylon jacket liner fabric bags and sealed. 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; in each October we removed 10 bags, five from the previous year and five placed at the beginning 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 fuscum*-derived peat, from roughly 5–15 cm below the capitula. We placed nylon mesh litter bags with mixed vascular plant litter (~1.7 g dry mass; roughly equal masses of *Rhododendron groenlandicum*, *Andromeda polifolia*, *Chamaedaphne calyculata*, *Maianthemum trifolium*, and *Rubus chamaemorus*) or peat ~10 cm below the peat surface in early June (in 2014, three bags per plot of vascular litter and

three bags of *Sphagnum* peat; in 2015, four bags of each material). Upon retrieval in October of each year, we manually removed debris from each bag before drying at 55°C for a minimum of 5 d and weighing.

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 (Robertson et al. 1999, Bayley et al. 2005, Stuart et al. 2018). In the last week of June of each year, we collected peat cores (6.5 cm diameter, 10 cm deep), halved them longitudinally with a serrated bread knife, and placed them into separate polyethylene WhirlPak (Nasco, Fort Atkinson, Wisconsin, USA) bags (initial N core and final N core), removing any live aboveground vascular vegetation from each peat core prior to placement into WhirlPak bags. Within 24 h after collection from the field, we extracted initial N cores (60 min per extraction) with 0.2 mol/L KCl to displace NH_4^+-N and NO_3^--N (modified from Verhoeven et al. 1990). We buried final N cores in the peat profile from the same location from which they were removed and incubated them in the peat matrix for approximately 30 d. Following the field incubation period, we extracted final N cores with 0.2 mol/L KCl.

For 2011–2012 samples, we measured extractable NH_4^+-N and NO_3^--N concentrations on a Seal AA3 Autoanalyzer using phenate and hydrazine reduction methods, respectively; for 2013–2014 samples, we measured extractable NH_4^+-N and NO_3^--N concentrations on an OI Analytical Flow Solution IV instrument (OI Analytical, College Station, Texas, USA) using the alkaline phenol and cadmium reduction methods, respectively. 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 N mineralization from the difference between final and initial extractable NH_4^+-N plus NO_3^--N contents (Robertson et al. 1999).

Bog porewater chemistry

To collect peatland porewater as a function of depth, we used 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 surface. On each sampling date, we used a 60-mL syringe to evacuate the full volume of water from the topmost segment from which water could be collected prior to removing 60-mL of porewater and field filtering through Whatman 41 filter paper 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.

Microbial biomass and community composition

In July 2015, we collected a surface peat core (10 cm diameter) 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 (*i*14:0, *i*15:0, *a*15:0, 15:0, *i*16:0, 16:1ω7c, 16:1ω9c, *i*17:0, *a*17:0, 17:0, 17:0cy, 18:1ω7c, 19:0 cy) followed Frostegård and Bååth (1996), Högberg 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 (*i*14:0, *i*15:0, *a*15:0, *i*16:0, *i*17:0, *a*17:0) 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 Högberg et al. (2007) and Cardinali et al. (2015).

Statistical analyses

We characterized the responses to N addition using linear regression and two-segment piecewise regression, the latter providing an objective assessment of whether a particular parameter exhibited a threshold response to N addition. Segmented regressions were carried out in SAS (v. 9.4; SAS Institute, Cary, North Carolina, USA), providing three initial values spanning an order of magnitude for the *y*-intercept, the slopes at N addition values less than and greater than a N deposition value where the slopes might change, and the N addition value at which slopes might change. For most dependent variables, segmented regression either failed to converge or provided no improvement over linear regression in characterizing the response to N addition, so we report only linear regression results. For biological N_2 fixation and some of the shrub responses to N addition, however, segmented regression described the response to N addition better then linear regression, as assessed by comparing R^2 values.

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 indicating whether an N response, if significant, was consistent across all years. A posteriori comparison 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 1.5, 2.6, 7.6, 12.6, 17.6, 22.6, and

27.6 kg N·ha^{-1}·yr^{-1}, including N in source water used in the fertilizations, N in background atmospheric deposition, and experimentally applied N.

We assessed the effect of water addition to the experimental plots using analyses of variance to compare responses 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. All analyses were conducted using SAS, version 9.3.

To analyze changes in the plant community over the course of the study, we used nonmetric multidimensional scaling (NMDS) with Primer 6 (Clarke and Gorley 2006) using Bray-Curtis similarity, Kruskal fit 1 scheme, and no pretreatment of the data. We correlated environmental vectors (shrub frequency, change in shrub frequency between 2011 and 2015, year, 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 treatment, year (fixed effects), and an interaction between treatment and year, 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 include *Andromeda polifolia*, *Chamaedaphne calyculata*, *Rhododendron groenlandicum*, and *Kalmia polifolia* combined. For all statistical analyses, we determined significance using $\alpha = 0.05$.

RESULTS

Climate

Temperature and rainfall differed between each growing season of the five-year study (Fig. 1). Mean growing season air temperatures and degree-days were lower in
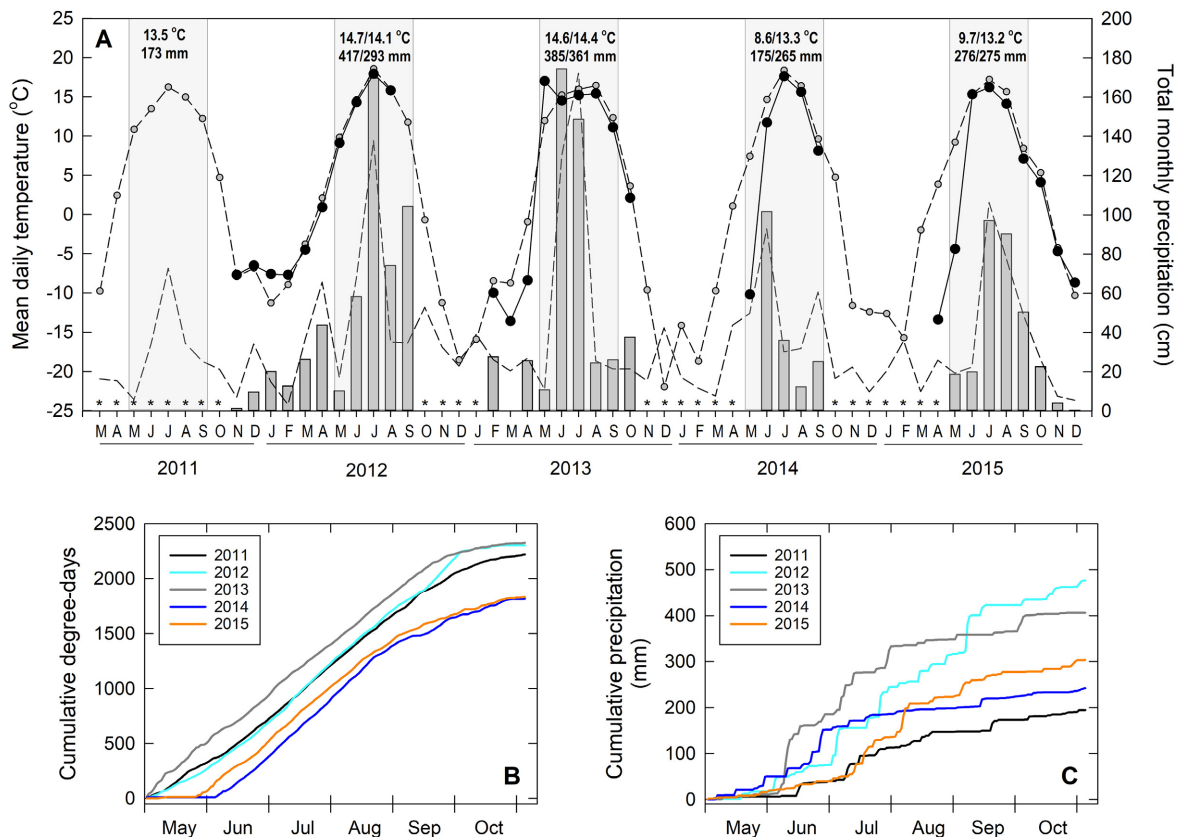

FIG. 1. (A) Mean monthly temperature (black circles) and total monthly precipitation (gray bars) from the weather station at Mariana Lake Bog, along with interpolated temperature (small gray circles, dashed line) and precipitation (no symbols, dashed line) for Alberta Township T080R14W4 (https://agriculture.alberta.ca/acis/township-data-viewer.jsp). Stars indicate months where data were not obtainable from the Mariana Lake Bog weather station. Gray rectangles indicate the growing season (May–September). Mean growing season air temperature and total growing season precipitation are shown at the top of the gray rectangles from the interpolated weather data and from the on-site weather station, separated by a solidus. Also shown are growing season (May–October) cumulative degree-days (B) and cumulative precipitation (C) from the weather station at Mariana Lake Bog.

2014 and 2015 than in the preceding three years due to unusually cool May air temperatures. With respect to rainfall, 2011 was an exceptionally dry year, not only at Mariana Lake Bog, but across the boreal region of northern Alberta (Brimelow et al. 2014). The two wettest years were 2012 and 2013, with 2013 experiencing especially high June precipitation (Fig. 1).

Atmospheric N deposition

Deposition of NH_4^+-N, NO_3^--N, and DIN averaged 187 ± 11, 237 ± 13, and 426 ± 19 $\mu g \cdot m^{-2} \cdot d^{-1}$ (Fig. 2), corresponding to 0.68 ± 0.04, 0.87 ± 0.05, and 1.55 ± 0.07 kg $N \cdot ha^{-1} \cdot yr^{-1}$, with no significant differences between open and throughfall collectors for any of the three measured parameters ($P \geq 0.64$). Deposition of NH_4^+-N was significantly higher during the growing season (averaging 239 $\mu g \cdot m^{-2} \cdot d^{-1}$) than during the non-growing season months (averaging 121 $\mu g \cdot m^{-2} \cdot d^{-1}$; $P = 0.0004$), NO_3^--N deposition was higher in non-growing season (averaging 265 $\mu g \cdot m^{-2} \cdot d^{-1}$) than during the growing season (averaging 209 $\mu g \cdot m^{-2} \cdot d^{-1}$; $P = 0.049$), and DIN deposition did not differ significantly between the growing season and the non-growing season ($P = 0.15$).

Acetylene reduction and N_2 fixation

Mean acetylene reduction rates ranged from 2.2 $mg \cdot m^{-2} \cdot d^{-1}$ (late June 2012) to 12.3 $mg \cdot m^{-2} \cdot d^{-1}$ (late July 2014; Fig. 3A). Averaged across all measurement dates, rates were highest in the 5 kg $N \cdot ha^{-1} \cdot yr^{-1}$ treatment and decreased with increasing N loading (Fig. 3B). Assuming that biological N_2 fixation occurs only during the 140 d growing season, annual N_2 fixation in the C, 0, 5, 10, 15, 20, and 25 kg $N \cdot ha^{-1} \cdot yr^{-1}$ treatments was equivalent to 36 ± 5, 41 ± 7, 45 ± 7, 27 ± 5, 19 ± 4, 24 ± 4, and 17 ± 2 kg $N \cdot ha^{-1} \cdot yr^{-1}$, respectively. The acetylene reduction/N_2 fixation response to N addition was described by a segmented linear regression, with rates increasing as N addition increased to 3.1 ± 1.5 kg $N \cdot ha^{-1} \cdot yr^{-1}$ and decreasing with further increases in N addition (Fig. 3B). Water addition alone had no significant effect on N_2 fixation rates on any of the measurement dates ($P \geq 0.79$).

Sphagnum fuscum growth and N responses to N addition

Across all N treatments and years, *S. fuscum* NPP averaged 224 ± 11 $g \cdot m^{-2} \cdot yr^{-1}$ ($n = 105$). Averaged across the five years, *S. fuscum* NPP was 281 ± 29 $g \cdot m^{-2} \cdot yr^{-1}$ in the control treatment ($n = 15$; Fig. 4A). The slopes of the 2011, 2012, and 2014 regressions of NPP as a function of N addition were nonsignificant; NPP decreased significantly with increasing N addition in 2013 and 2015 (Fig. 4A). Thus, the NPP response to N addition became more pronounced over time. In the 25 kg $N \cdot ha^{-1} \cdot yr^{-1}$ treatment, *S. fuscum* NPP averaged

281 ± 44 $g \cdot m^{-2} \cdot yr^{-1}$ in 2011 and 2012 ($n = 6$) and 163 ± 26 $g \cdot m^{-2} \cdot yr^{-1}$ in 2013–2015 ($n = 9$).

Over all years and N treatments, *S. fuscum* linear growth averaged 2.3 ± 0.1 cm/yr. Linear growth was not affected by N addition in any of the five years (Fig. 4B). *Sphagnum fuscum* stem mass densities (SMD) averaged 97.7 ± 4.2 g/m^2 per 1-cm stem length. While SMD was unaffected by increasing N addition in 2011 and 2012, SMD decreased with increasing N addition in 2013, 2014, and 2015 (Fig. 4C). Over the five years of the study, *S. fuscum* plant density averaged $49,664 \pm 1,492$ plants/m^2, decreasing consistently with increasing N addition across all five years (Fig. 4D). Over all N treatments and years, *S. fuscum* stem N concentrations averaged 9.3 ± 0.1 mg/g. Although stem N concentrations differed between years, consistently across all years stem N concentration increased with increasing N addition, albeit slightly (Fig. 4E). Combining the effects of N additions on *S. fuscum* NPP and stem N concentration, the total quantity of N assimilated by growing moss increased significantly with increasing N addition in 2011, but decreased with increasing N addition in 2013, 2014, and 2015 (Fig. 4F).

Water addition alone (control vs. 0 kg $N \cdot ha^{-1} \cdot yr^{-1}$ treatments) had no effect on *S. fuscum* linear growth, SMD, plant density, or stem N concentrations in any of the five years ($P \geq 0.16$). In 2013, but not in the other four years, *S. fuscum* NPP and N assimilation were significantly higher in the control treatment than in the 0 kg $N \cdot ha^{-1} \cdot yr^{-1}$ treatment ($P = 0.03$ and 0.0006, respectively).

From 2013–2015, we examined the effects of N addition on changes in *S. fuscum* capitulum mass density (CMD), N concentrations, and N contents in plants collected in early July (summer) and early October (fall). In each year, CMD decreased with increasing N addition at equal rates in summer-collected and fall-collected plants, although CMD was consistently higher in fall-collected than in summer-collected plants (Fig. 5A). For both summer- and fall-collected plants, capitulum N concentrations were unaffected by N additions, although N concentrations were consistently higher in summer (14.4 ± 0.3 mg/g) than in fall (10.7 ± 0.1 mg/g; Fig. 5B). Combining CMD and capitulum N concentrations, capitulum N contents overall averaged 1.16 ± 0.04 g/m^2. From 2013–2015, capitulum N contents decreased with increasing N addition, with the response being stronger in 2014 or 2015 than in 2013 (Fig. 5C). Water addition alone had no significant effect on CMD, capitulum N concentrations, or capitulum N contents in summer or fall of any of the three years ($P \geq 0.77$, 0.07, 0.48, respectively).

Vascular plant growth and N responses to N addition

Andromeda polifolia, *Chamaedaphne calyculata*, and *Rhododendron groenlandicum*, and these three dominant shrub species combined, exhibited different responses to
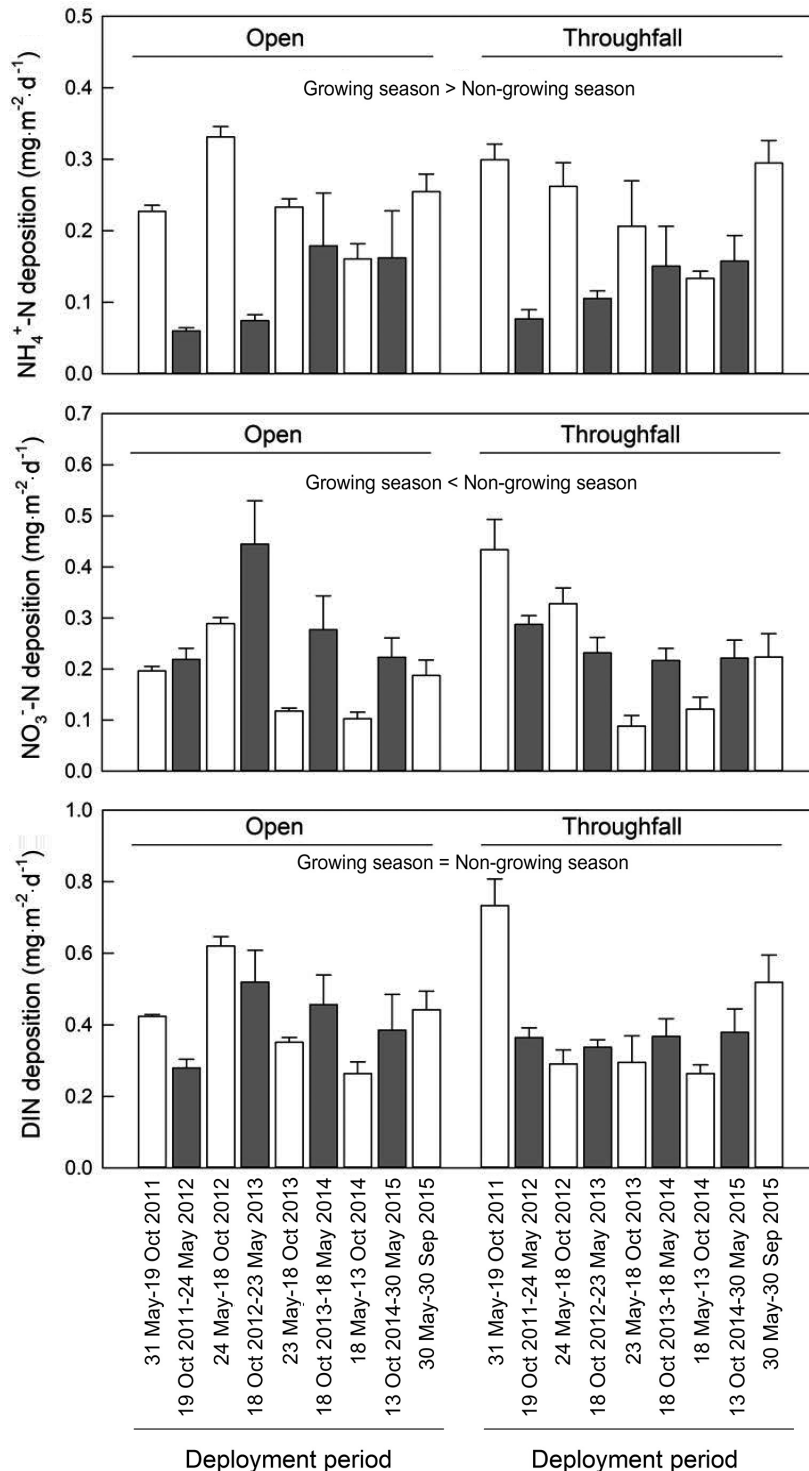
FIG. 2. Atmospheric deposition of NH_4^+-N, NO_3^--N, and DIN (NH_4^+-N plus NO_3^--N) at Mariana Lake Bog, quantified using ion exchange resin collectors placed in the open and among patches of black spruce trees to quantify throughfall. Resins were deployed during the growing season (May–October; white bars) and during non-growing season periods (October–May; dark gray bars). Values are means + SE (n = 5).
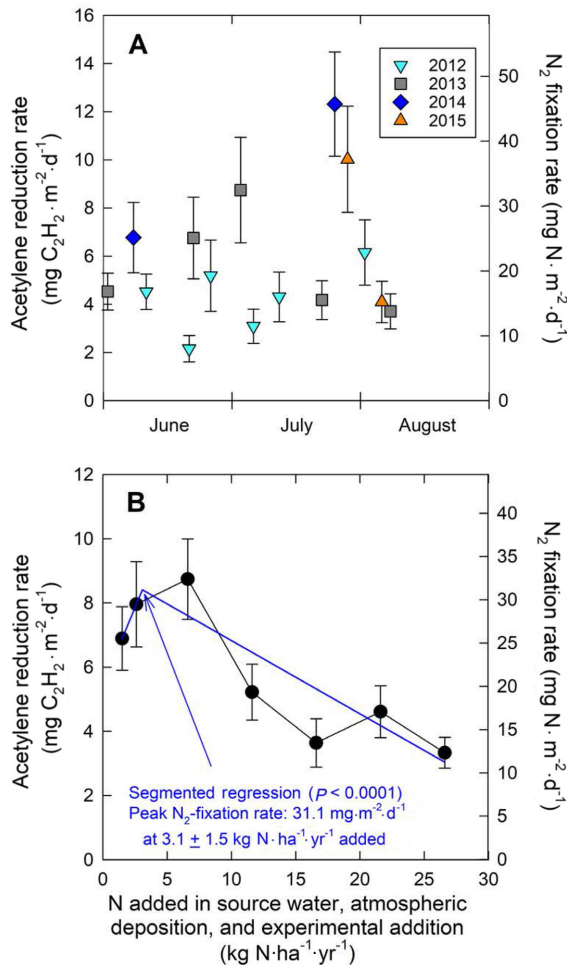
FIG. 3. (A) Rates of acetylene reduction (left axis), converted to N$_2$ fixation (right axis) throughout the growing season (2012–2015) (values are mean ± SE averaged across all N treatments, $n = 21$) and (B) as a function of N addition (values are mean ± SE averaged across all measurement dates, $n = 45$). In panel B, results from a two-segment piecewise linear regression are shown, highlighting the N deposition value at which the slope changes from positive to negative.

increasing N addition (Fig. 6). While NPP of *A. polifolia* and the three shrub species combined increased linearly with N addition (Fig. 6A, D), *C. calyculata* NPP increased as N addition increased up to 17.6 kg N·ha^{-1}·yr^{-1} and subsequently declined (Fig. 6B), and *R. groenlandicum* NPP was unaffected by N addition up to a value of 22.6 kg N·ha^{-1}·yr^{-1} and increased at higher N addition (Fig. 6C). Water addition alone had no significant effect on NPP for any of the species or for the dominant shrubs combined in either 2014 or 2015 ($P \geq 0.47$).

The mass of newly produced shoot segments for *Andromeda polifolia*, *Chamaedaphne calyculata*, *Rhododendron groenlandicum*, and these three dominant shrubs combined all increased with increasing N addition, with no differences in slopes between 2014 and 2015

(Fig. 7A–D). As N addition increased, the number of newly produced shoots (vegetative buds per m^2) increased linearly for *A. polifolia* and the three shrub species combined, with no differences in slopes between 2014 and 2015 (Fig. 7E, H). The number of newly produced shoots increased up to 17.6 kg N·ha^{-1}·yr^{-1} and then decreased for *C. calyculata* and was unaffected for *R. groenlandicum* (Fig. 7F, G). Water addition alone had no significant effect on new segment mass or vegetative bud density for any of the species or for the dominant shrubs combined in either 2014 or 2015 ($P \geq 0.56$).

Leaf N concentration responses to increasing N addition differed between species. Increasing N addition led to increasing leaf N concentrations across all years in *A. polifolia*, *C. calyculata*, *V. oxycoccos*, and *V. vitis-idaea*, with differences in N concentrations between years for *A. polifolia* and *C. calyculata* (Fig. 8). There was no leaf N concentration response to increasing N addition for *E. vaginatum*, *R. chamaemorus*, *M. trifolium*, or *K. polifolia*. Increasing N addition led to increasing leaf N concentration in *R. groenlandicum* in 2012 only.

For the three dominant shrub species, annual NPP was more responsive to increasing N addition than leaf N concentrations, such that the responses of annual assimilation of N by new leaf production to increasing N addition mirrored NPP responses (Fig. 6). Water input alone had no significant effect on leaf N concentration for any of the species ($P \geq 0.18$) or on N assimilation by *A. polifolia*, *C. calyculata*, *R. groenlandicum*, or the three species combined ($P \geq 0.06$) in any of the years of sampling.

For *P. mariana*, annual leader extension and tree growth increased with increasing N addition, with the response becoming more pronounced from 2013 through 2015 (Fig. 9A, B). *Picea mariana* needle N concentrations also increased with increasing N addition, at a modest, but significant rate that was consistent across all years (Fig. 9C). As with the dominant shrubs, *P. mariana* annual growth was more responsive to increasing N addition than leaf N concentrations, such that the response of annual assimilation of N in *P. mariana* through new needle growth mirrored the annual growth response (Fig. 9D). Water addition alone had no significant effect on *P. mariana* leader extension, growth, needle N concentration, or N assimilation in any of the years of measurement ($P \geq 0.54$).

Plant species abundances and community composition

We used absolute frequencies of *Sphagnum* and vascular plants to examine species and community responses to N treatment. Increasing N addition led to decreased frequency of *S. fuscum* in 2013 and 2014 (Fig. 10A) and an increased frequency of *S. magellanicum* in 2014 and 2015 (Fig. 10C), with no significant effect on *S. angustifolium* frequency in any of the five years (Fig. 10B). Combined frequency of *Sphagnum fuscum*, *S. angustifolium*, and *S. magellanicum* was higher in 2011 and 2012 (average across all N treatments for the two years, 0.99 ± 0.005; $n = 42$)
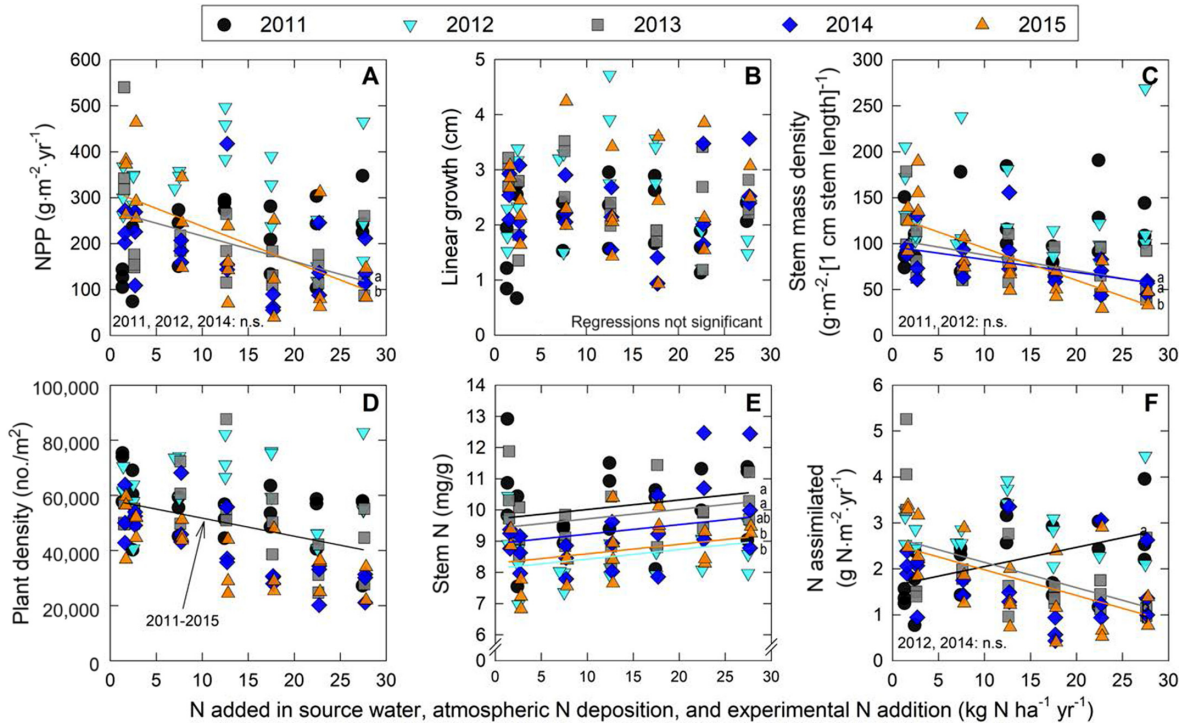
FIG. 4. *Sphagnum fuscum* (A) NPP, (B) linear growth, (C) stem mass density, (D) plant density, (E) stem N concentrations (2-cm stem increment below capitulum), and (F) N assimilated in annual growth as a function of N addition. Significant regressions are shown. Regression slopes with the same lowercase letter do not differ significantly.
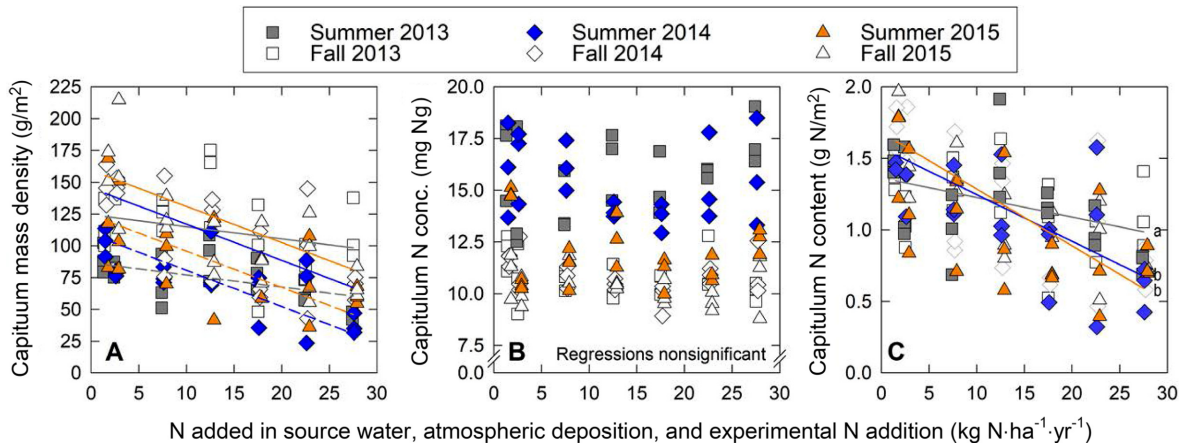

FIG. 5. *Sphagnum fuscum* capitulum (A) mass density, (B) N concentration, and (C) N content measured in the summer and fall of 2013–2015 as a function N addition. Regressions in panel A are all significant, shown as solid lines for fall sampling and dashed lines for summer sampling. Capitulum N content (C) decreased with increasing N addition less strongly in 2013 than in 2014 and 2015 (slopes of the regressions with the same lower case do not differ significantly), with no differences between summer- and fall-collected plants in any of the three years. Regression slopes with the same lowercase letter do not differ significantly.

than in 2013–2015 (average across all N treatments for the three years 0.86 ± 0.02; $n = 63$). Water addition alone had no significant effect on the frequency of any of the *Sphagnum* species in any of the five years ($P \geq 0.87$).

Of the three dominant shrubs, *C. calyculata* frequency was unaffected by increasing N addition (Fig. 10D),

while frequencies of *R. groenlandicum* increased with increasing N addition in 2014 and 2015 (Fig. 10E) and frequencies of *A. polifolia* with increasing N addition in 2012 through 2015 (Fig. 10F). Dominant shrubs, all shrubs, and all vascular plant species frequencies all increased with increasing N addition, with slopes of
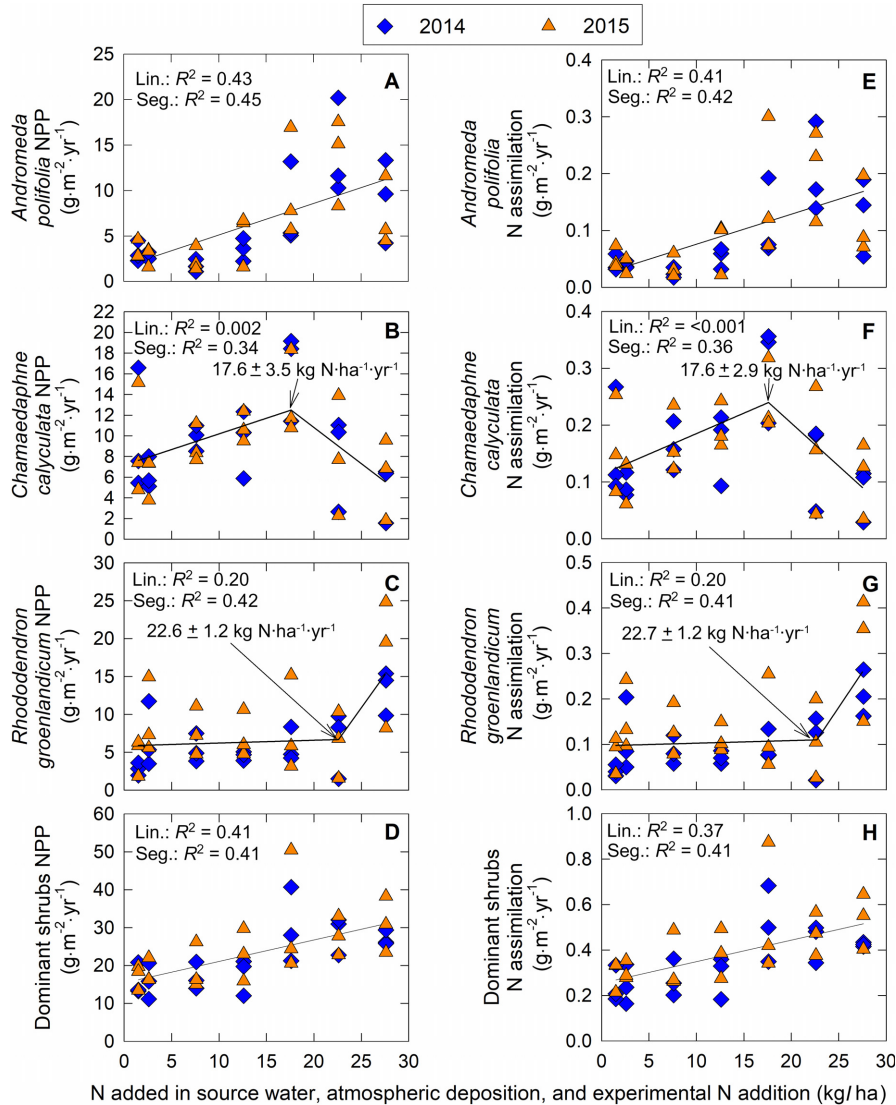
FIG. 6. Individual and combined shrub (A–D) NPP and (E–H) N assimilated through shrub growth as a function of N addition. Segmented (Seg.) regressions were chosen over linear (Lin.) regressions only if there was a substantial improvement in goodness of fit, as indicated by R^2 values. In all panels, regressions did not differ significantly between 2014 and 2015.

those relationships becoming steeper from 2013 through 2015 (Fig. 10G–I). Water addition alone had no significant effect on the frequency of vascular plant species or groups in any of the five years ($P \geq 0.12$).

We examined plant community structure through NMDS ordination and PERMANOVA, which revealed significant N treatment ($P = 0.001$) and year ($P = 0.002$) effects, with no interaction ($P = 1.00$). Nitrogen treatments were generally significantly different from one another (Table 1). Years also were mostly different except that 2015 was not different from 2014 ($P = 0.213$), but was different from all other years ($P \leq 0.004$); 2014 and 2011 also differed ($P = 0.018$). Graphically, the N treatments over the five years form a central cluster on the ordination (Fig. 11); however, the higher N treatments, especially those at 20–25 kg N·ha^{-1}·yr^{-1} are positioned

to the upper right on the ordination while control, 5, and 10 kg N·ha^{-1}·yr^{-1} treatments generally are to the lower left. A similar positioning is evident between years, with 2011 and 2012 located to the lower left, 2013 variable, and 2014–2015 to the upper right. A vector for N treatment is oriented toward the upper right and is associated with vectors for increasing shrub frequency, increasing shrub frequency change, and decreasing *Sphagnum* frequency.

Root responses to N addition

Root biomass in the 0–15 cm and 15–30 cm depth increments in peat increased with increasing N addition; the response was similar in the two depth increments (Fig. 12A). Hence, root biomass integrated over the top 30 cm of peat increased with increasing N addition
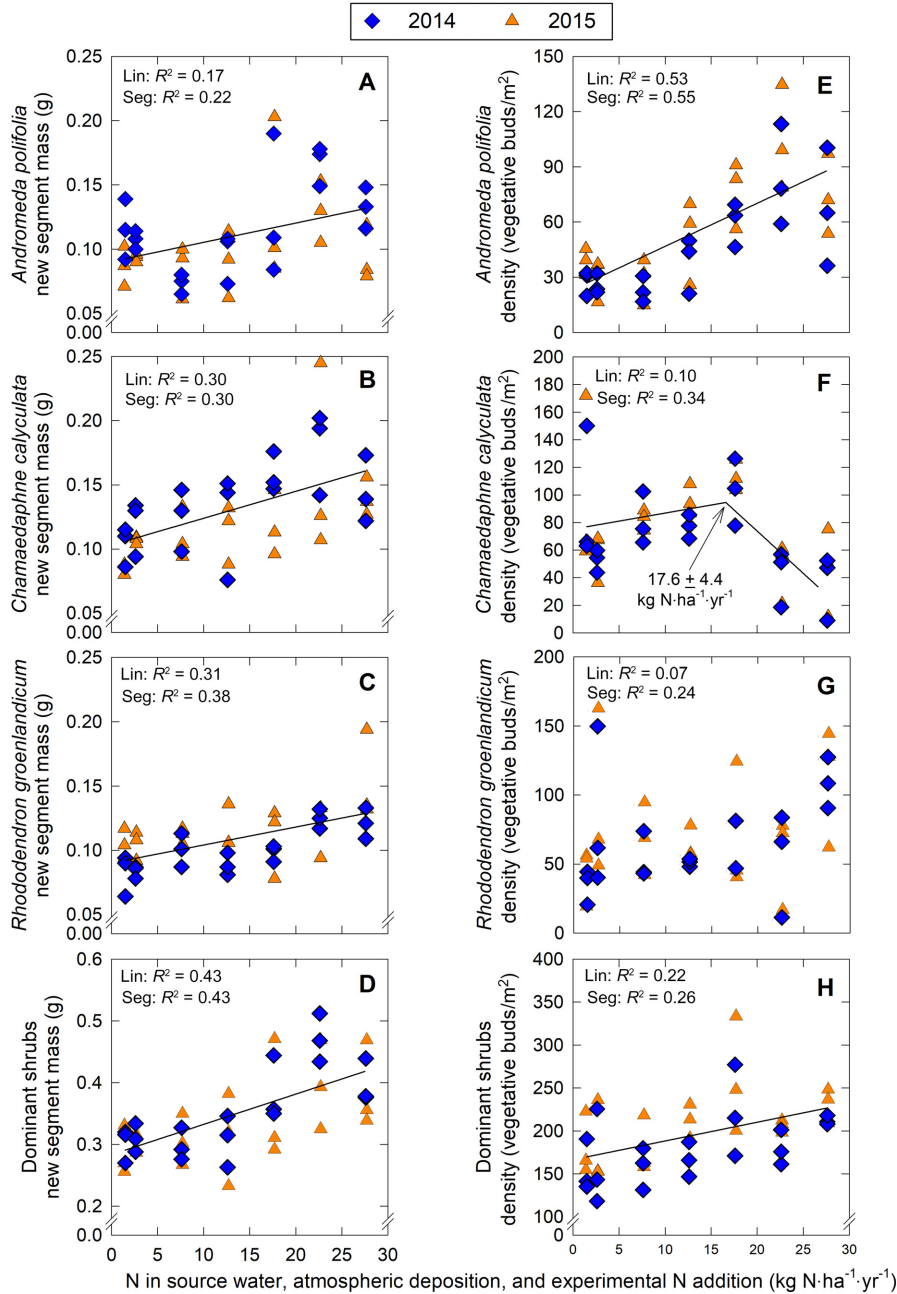
Fig. 7. Aspects of dominant shrub growth (2014–2015) as a function of N addition. Only significant regressions are shown. Segmented (Seg.) regressions were chosen over linear (Lin.) regressions only if there was a substantial improvement in goodness of fit, as indicated by R^2 values. In all panels, regressions did not differ significantly between 2014 and 2015.

(Fig. 12B). Root production, measured using ingrowth bags, increased with increasing N addition only for the 0–15 cm depth increment when ingrowth bags remained in the peat for two growing seasons (Fig. 12C). However, root production integrated over the top 30 cm increased with increasing N addition, with the response being similar whether ingrowth bags were left in the peat for one or two growing seasons (Fig. 12D). Water addition

alone had no significant effect on root biomass ($P > 0.72$) or root production ($P > 0.70$).

Decomposition

Decomposition of cellulose filter paper in surface peat over 17-month periods increased with N addition (Fig. 13A). In control and 0 kg N·ha^{-1}·yr^{-1} treatments,
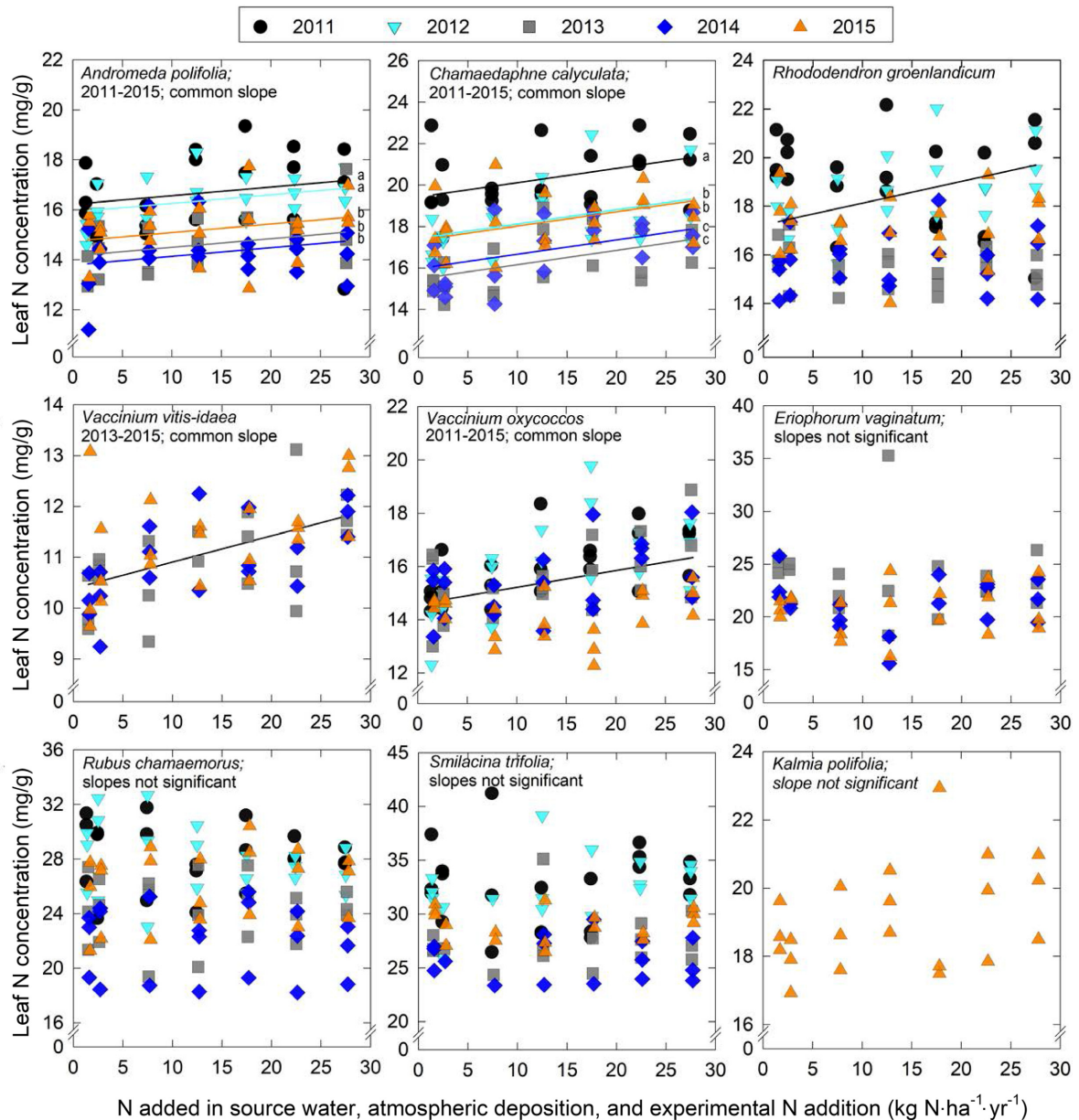
FIG. 8. Leaf/needle N concentrations for nine vascular plant species as a function of N addition. For *A. polifolia* and *C. calycu-lata*, slopes of the regressions were consistent across the five years, but elevations of the regressions differed; elevations of the regressions with the same lowercase letter do not differ significantly.

k values averaged 0.58 yr^{-1}, corresponding to 42% of initial mass lost in the first year, while in the 25 kg N·ha^{-1}·yr^{-1} treatment, k values averaged 1.27 yr^{-1}, corresponding to 72% of initial mass lost in the first year. In contrast, decomposition of *Sphagnum* moss or mixed vascular plant litter was unaffected by N additions (Fig. 13B, C), on average losing 8% and 38% of initial mass, respectively, over 5 months of decomposition. Water addition alone had no significant effect on decomposition of cellulose, *Sphagnum*, or vascular plant litter ($P > 0.15$).

Net N mineralization

Concentrations of initial KCl-extractable NH_4^+-N, NO_3^--N, and DIN in the top 10 cm of peat were unaffected by N additions (Table 2). Initial KCl-extractable NH_4^+-N, but not NO_3^--N, in peat increased from 2011 through 2014; although mean initial DIN concentrations increased from 2011 through 2014, differences between years were not significant (Table 2). Except in 2014 when net ammonification increased significantly with increasing N addition, net ammonification, net nitrification, and net
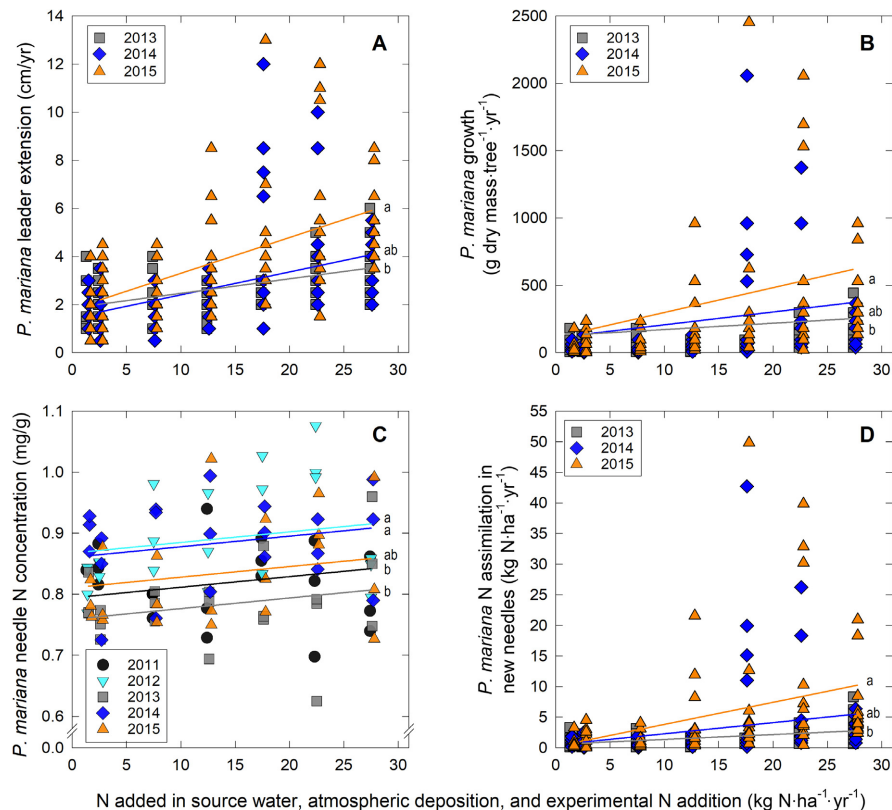
FIG. 9.   *Picea mariana* (A) leader extension, (B) growth, (C) N concentration in needles, and (D) N assimilation in new needles as a function of N addition. In panels A, B, and D, regression slopes with the same lower case letter do not differ significantly; in panel C, slopes of the regressions were consistent across the five years, but elevations of the regressions differed; elevations of the regressions with the same lowercase letter do not differ significantly.

DIN production were unaffected by increasing N addition (Fig. 14). Water addition alone had no significant effect on initial KCl-extractable NH_4^+-N, NO_3^--N, and DIN concentrations ($P \geq 0.79$) or on net ammonification, net nitrification, or net DIN production rates ($P \geq 0.96$).

Porewater N concentrations

Across all N treatments, porewater NH_4^+-N, NO_3^--N, and DON concentrations at the top of the bog water table were substantially higher in 2011 (averaging 1.37 ± 0.03 mg/L, 202 ± 13 µg/L, 1.53 ± 0.05 mg/L, respectively) than in 2012–2015 (averaging 0.13 ± 0.005 mg/L, 9.3 ± 1.1 µg/L, 1.01 ± 0.01 mg/L, respectively, Fig. 15). In 2011, DON constituted $48.9\% \pm 0.7\%$ of total dissolved N; in 2012–2015, DON constituted $87.3 \pm 0.5\%$ of total dissolved N. Porewater NH_4^+-N, NO_3^--N, and DON concentrations were unaffected by N addition in any of the five years (rmANOVA; $P = 0.44$, 0.37, and 0.82, respectively).

Microbial community composition

For the most part, microbial group abundances were not affected by increasing N addition (Fig. 16).

However, actinomycete abundance decreased with increasing N addition at rates that were similar in 0–5 and 5–10 cm peat. Total microbial abundance, total bacterial abundance, and Gram-negative bacterial abundance were significantly higher in 0–5 cm peat than in 5–10 cm peat, while the Gram-positive to Gram-negative bacterial ratio was higher in 5–10 cm peat than in 0–5 cm peat.

DISCUSSION

The literature regarding the effects of changing N deposition regimes on peatland (bog and fen) structure and function is vast. For the purposes of our work at Mariana Lake Bog, we interpret findings mostly within the framework of previous research conducted in boreal, and to a lesser extent temperate or subarctic, ombrotrophic bogs. With respect to *Sphagnum* growth responses to N addition, we focus on previous research that specifically addresses *S. fuscum*. For plant abundance and plant community composition responses to N addition, we focus on *S. fuscum*, *S. magellanicum*, and *S. angustifolium*, the three dominant moss species, and the three dominant short-statured, ericaceous shrub species at Mariana Lake Bog: *Andromeda*
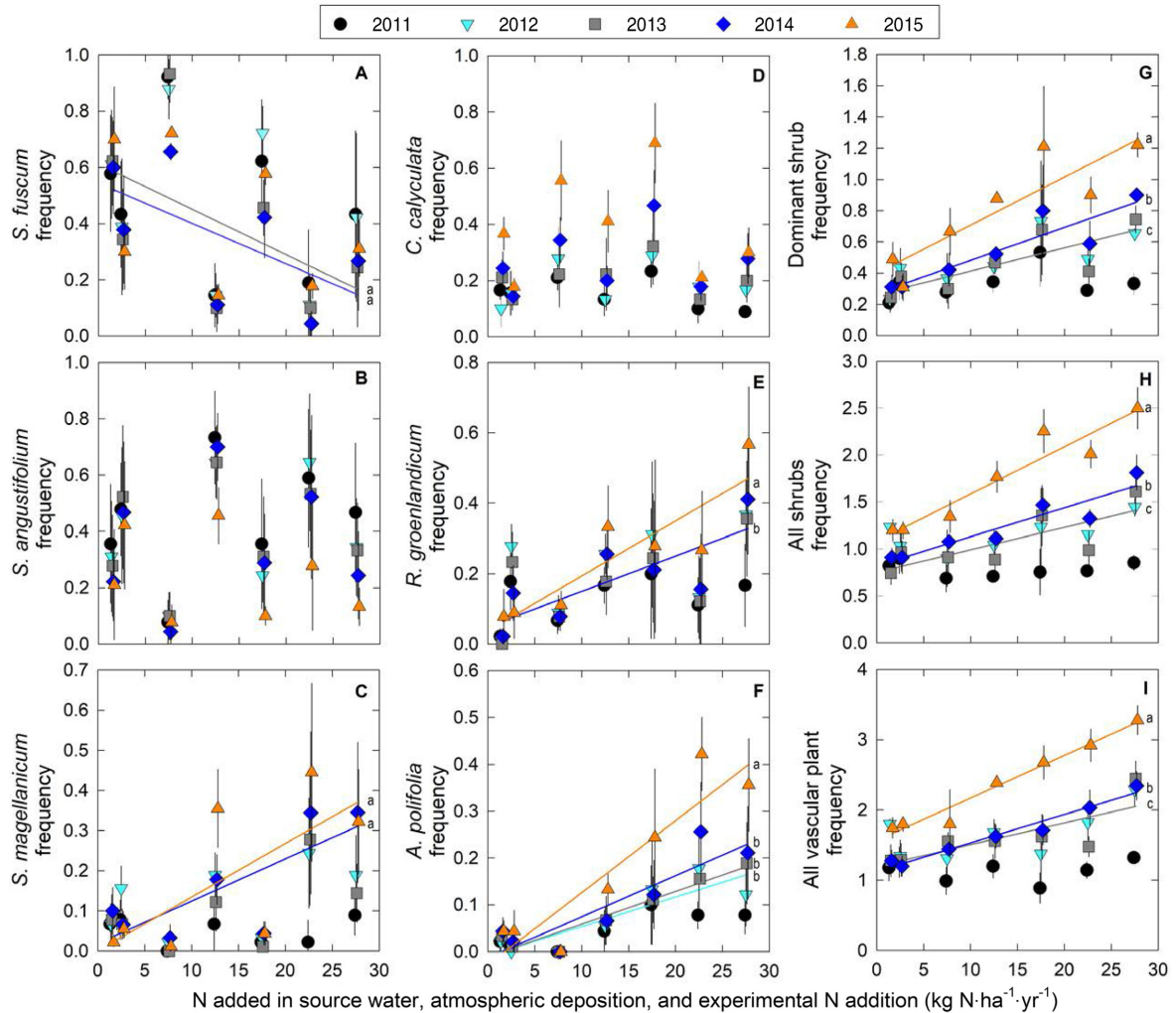
FIG. 10. Frequency of occurrence from point frame measurements for the three most frequently occurring *Sphagnum* species, the three most frequently occurring shrub species, dominant shrubs combined (*C. calyculata*, *A. polifolia*, *R. groenlandicum*), all shrubs (dominant shrubs plus *Vaccinium oxycoccos*, *Kalmia polifolia*, *Vaccinium vitis-idaea*), and all vascular plant species combined (all shrubs plus *Rubus chamaemorus*, *Maianthemum trifolium*, *Eriophorum vaginatum*, *Drosera rotundifolia*, *Picea mariana* <1 m tall) as a function of N addition. Values are mean ± SE; $n = 3$. Regression slopes with the same lowercase letter do not differ significantly.

polifolia, *Chamaedaphne calyculata*, and *Rhododendron groenlandicum*.

Inputs of new nitrogen at Mariana Lake Bog

Our initial hypotheses that atmospheric N deposition represents the major source of new N to bogs and that biological N_2 fixation is a minor source of new N were not supported. Atmospheric N deposition at Mariana Lake Bog (averaging 1.5 kg·ha^{-1}·yr^{-1}; Fig. 2) is typical for northern Alberta sites unaffected by N emissions from oil sands development (Vitt et al. 2003, Wieder et al. 2010, 2016a, b, Fenn et al. 2015).

The major input of new N to the Mariana Lake Bog, however, is not atmospheric deposition, but biological N_2 fixation (Fig. 3). At Mariana Lake Bog, N_2 fixation

was quite variable, both within and between measurement dates (Fig. 3), but the overall mean rate of 21.4 ± 1.4 kg N·ha^{-1}·yr^{-1}, is similar to the mean rate of 25.8 ± 2.4 kg N·ha^{-1}·yr^{-1} for four bogs in the Fort McMurray area of northern Alberta (Vile et al. 2014). Rates of N_2 fixation exhibited a pattern of increasing (compared to the control treatment) at low N addition and subsequently decreasing with increasing N addition (Fig. 3). Control treatments received no added N or water, so the increase in N_2 fixation rates at low N loading (0 kg N·ha^{-1}·yr^{-1}) may be in part a result of water addition. However, in environments where the DIN availability is very low, as in Alberta bogs (cf. Fig. 15), increasing atmospheric N deposition could stimulate N_2 fixation (cf. Reed et al. 2011). Nonetheless, decreases in N_2 fixation rates at N loadings above

TABLE 1. Results of pair-wise PERMANOVA *t* tests for plant community composition differences between each nominal N treatment.

Nominal N treatment (kg·ha⁻¹·yr⁻¹)	Nominal N treatment (kg·ha⁻¹·yr⁻¹)					
	0	5	10	15	20	25
C	1.615	*2.199*	*2.987*	1.447	*2.685*	*2.276*
0		*3.200*	1.541	*1.703*	*1.888*	*1.624*
5			*4.746*	2.223	*4.324*	*3.938*
10				2.764	1.461	*1.739*
15					*2.693*	*1.782*
20						*1.716*

Notes: *t* values are shown; statistically significant ($P \leq 0.05$) pairwise differences are indicated in boldface, italic font.

3.1 kg N·ha⁻¹·yr⁻¹ are indicative of a suppression of biological N_2 fixation with increasing N addition.

Inhibition of N_2 fixation by high N availability and/or deposition has been demonstrated in boreal feather-mosses (*Pleurozium schreberi* and *Hylocomium splendens*) where the N_2 fixers are predominantly cyanobacteria (Gundale et al. 2011, Ackermann et al. 2012, Rousk et al. 2014) and in *Sphagnum* mosses, where the N_2 fixers may be predominantly methanotrophs (Vile et al. 2014, Kox et al. 2016). The inhibition of N_2 fixation that we observed at Mariana Lake Bog is nearly equivalent to the increase in N loading through

experimental N addition. As N addition increased from 3.1 to 27.6 kg N·ha⁻¹·yr⁻¹, N_2 fixation decreased from 31.1 to 11.3 kg N·ha⁻¹·yr⁻¹. While increasing N deposition may not substantively change total inputs of new N to bogs, the form of new N additions shifts to inorganic N in deposition, to organic N produced by the microorganisms that are fixing N_2 and incorporating the fixed N into organic N compounds.

Sphagnum fuscum responses

Our initial hypothesis that *S. fuscum* growth is N-limited and therefore should be stimulated by experimental N addition was not supported. Rather we found an inhibition of *S. fuscum* growth with increasing N addition in the third and fifth year of N addition (Fig. 4A). The NPP response was related to decreasing *S. fuscum* stem mass density (Fig. 4C) along with plant density (Fig. 4D), with no effects on linear growth (Fig. 4B).

Our *S. fuscum* NPP (averaging 224 ± 11 g·m⁻²·yr⁻¹) and linear growth (averaging 2.3 ± 0.1 cm/yr) rates are comparable to published rates for bogs, including our previous work that showed no *S. fuscum* growth responses to atmospheric N deposition, although site differences in N deposition were small with a maximum value of 1.2 kg N·ha⁻¹·yr⁻¹ (Wieder et al. 2010, 2016*b*). Vitt et al. (2003) reported *S. fuscum* NPP to be 600 g·m⁻²·yr⁻¹ at an Alberta bog where modeled N
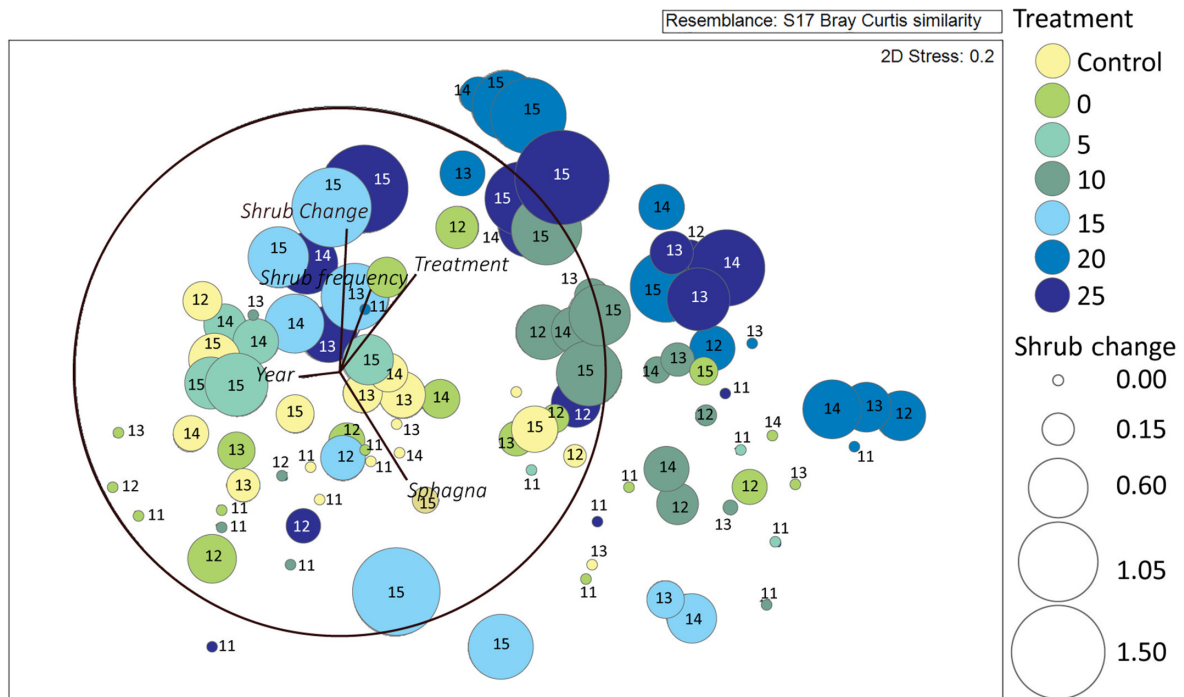

FIG. 11. Results of nonmetric multidimensional scaling, differentiating the N treatments and years, with vectors for increasing shrub frequency, increasing change in shrub frequency between 2011 and 2015, and decreasing *Sphagnum* frequency. Each circle represents a replicate plot, color coded by N treatment and sized according to change in shrub frequency between 2011 and 2015. Year of sampling for each plot is indicated adjacent to each circle.
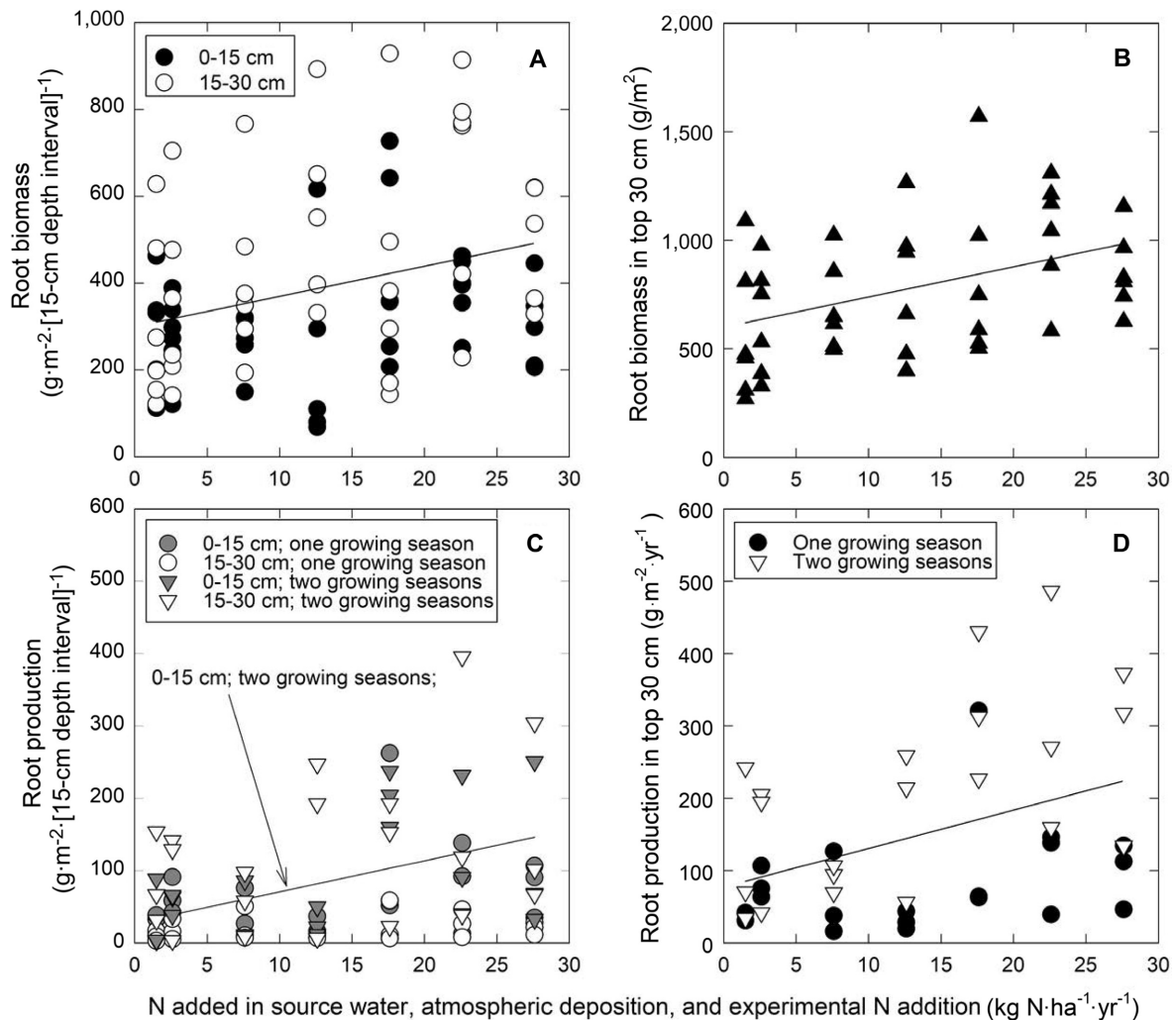
Fig. 12. (A) Root biomass in the 0–15 and 15–30 cm depth increments, (B) root biomass in the top 30 cm of peat, (C) root production in the 0–15 and 15–30 cm depth increments over one and two growing seasons, and (D) root production in the top 30 cm of peat over one and two growing seasons. Significant regressions are shown as solid lines. In panel D, regressions were not significantly different between one and two growing seasons.

deposition was 4.04 kg N·ha^{-1}·yr^{-1}, in contrast to 183 g·m^{-2}·yr^{-1} at 4 other bogs where N modeled deposition was <0.5 kg N·ha^{-1}·yr^{-1}, concluding that *S. fuscum* NPP is stimulated at low N deposition, and then is inhibited as N deposition increases above a critical N deposition value of 14.8 to 15.7 kg N·ha^{-1}·yr^{-1}. In a global synthesis of *Sphagnum* NPP, Gunnarsson (2005) reported average NPP of *S. fuscum* to be low (~190 g·m^{-2}·yr^{-1}), with little variability.

Two field N fertilization studies in Alberta bogs have produced contrasting results, one indicating a stimulation of *S. fuscum* NPP (Li and Vitt 1997) and another an inhibition of *S. fuscum* NPP with experimental N addition (Thormann and Bayley 1997). In a greenhouse study, with *S. fuscum* from the Ahvensalo mire, Finland, optimal deposition for growth in length was 10 kg·ha^{-1}·yr^{-1} and for

production was 30 kg·ha^{-1}·yr^{-1}, while deposition of 100 N kg·ha^{-1}·yr^{-1} almost completely inhibited both elongation and production (Jauhiainen et al. 1994).

In Alberta bogs, climate variability may have a strong influence on *S. fuscum* growth. At Mariana Lake Bog, both NPP and linear growth of *S. fuscum* were positively correlated with September and growing season precipitation, and negatively correlated with the growing season aridity index (PET/rainfall); NPP also was positively correlated with precipitation in other months, air temperature in some months, and growing season degree-days (Table 3). Previously, we reported that *S. fuscum* NPP was positively correlated with August, September, and total growing season rainfall and negatively correlated with a growing season aridity index (Wieder et al. 2016b). More generally, air temperature and
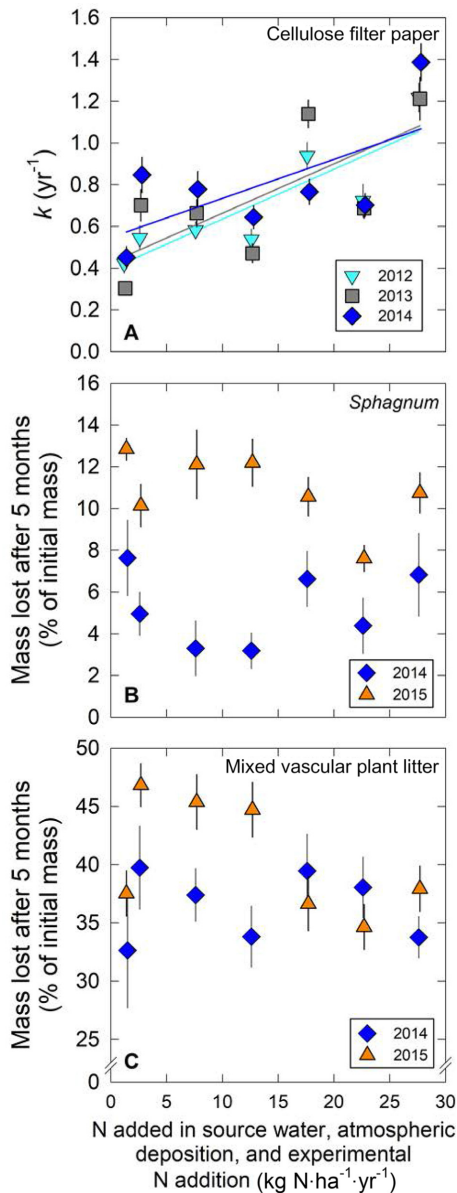
TABLE 2. Initial KCl-extractable NH_4^+-N, NO_3^--N, and dissolved inorganic nitrogen (DIN) concentrations in the top 10 cm of peat (mg N/m^2) prior to field incubation for quantification of net N mineralization rates.

Year	Initial concentrations (mg N/m^2)		
	NH_4^+-N	NO_3^--N	DIN
2011	23.8 ± 4.3[c]	7.3 ± 1.0[c]	31.2 ± 5.3[a]
2012	22.3 ± 1.6[c]	24.0 ± 0.9[a]	46.3 ± 2.3[a]
2013	40.6 ± 1.5[b]	12.5 ± 0.6[b]	53.0 ± 1.8[a]
2014	54.0 ± 3.7[a]	9.3 ± 1.0[bc]	63.2 ± 4.6[a]

Notes: For the three N forms, analysis of covariance to assess homogeneity of slopes indicated a nonsignificant N addition effect ($P \geq 0.81$), and a nonsignificant N addition by year interaction ($P \geq 0.34$). Significant differences in intercepts (differences between years) were obtained for NH_4^+-N and NO_3^--N ($P \leq 0.0005$), but not for DIN ($P = 26$). Values are means averaged across all nominal N treatments ± standard errors ($n = 21$); for each N form, values with the same letter superscript do not differ significantly (a posteriori comparisons with Tukey's adjustments).

FIG. 13. (A) Decomposition of cellulose filter paper (exponential decay k values, mean ± SE) 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 additions. Decomposition of (B) *Sphagnum* and of (C) mixed vascular plant litter placed in the peat in May of 2014 and 2015 and retrieved in October of the same year; values are means ± SE ($n = 3$ in 2014; $n = 4$ in 2015). Significant regressions are shown.

precipitation variables appear to have a strong effect on *Sphagnum* growth (Gunnarsson 2005).

The ground layer of *Sphagnum* is a fundamental component of bog ecosystems (Vitt et al. 1995, Vitt 2006). In western Canadian bogs, *S. fuscum* is dominant, typically covering 100% of the peat surface in hummocks (Belland and Vitt 1995). Peat accumulation in bogs over the long term is the result of NPP exceeding decomposition

throughout the peat profile. Further, *Sphagnum* mosses decompose more slowly than vascular plant tissues, such that peat is composed primarily of moss remains (Kuhry and Vitt 1996). Therefore, the decrease in *S. fuscum* NPP with increasing N addition that we report for Mariana Lake Bog has implications for peat accumulation in bogs. What remains uncertain, however, is whether decreased *S. fuscum* growth is a direct effect of increasing N loading and/or an indirect effect mediated through decreased N_2 fixation and/or the increased shrub growth responses.

We initially hypothesized that increasing N loading would have no effect on *S. fuscum* plant density or capitulum mass density, but should lead to increasing N concentrations in both *S. fuscum* capitula and stems. Responses were not as expected. Nitrogen addition resulted in lower *S. fuscum* plant densities (Fig. 4D) and lower capitulum mass (Fig. 5A), such that capitulum N storage (g N/m^2) decreased with increasing N addition (Fig. 5C). While N addition had no effect on capitulum N concentrations, N concentrations were higher in summer than in fall (Fig. 5B).

At 15 bogs along a European N deposition gradient, increasing N deposition did not affect *S. fuscum* capitulum biomass (g/m^2), but was related to a decrease in *S. fuscum* stem volumetric density (g/dm^3; Bragazza et al. 2004). At two Swedish mires, experimental N application did not affect *S. fuscum* capitulum densities (Gunnarsson and Rydin 2000). Experimental application of N directly to *S. fuscum* capitula in a greenhouse study resulted in increasing capitulum N concentrations, but had no significant effects on either capitulum mass, moss production, or linear growth (Granath et al. 2012). Thus, capitulum responses to increasing N loading have not been consistent.

Traditionally, the calculation of NPP of *Sphagnum* mosses has assumed that capitula masses remain
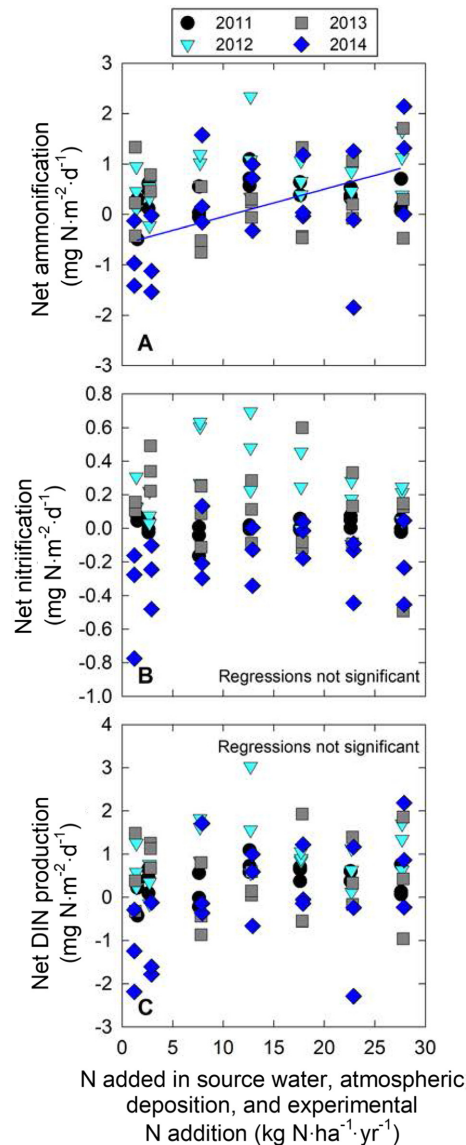
FIG. 14. Net (A) ammonification, (B) nitrification, and (C) DIN production in the top 10 cm of peat, as a function of N addition, measured in 2011–2014. The only significant regression was for net ammonification in 2014.

constant over the growing season (cf. Clymo 1970, Pakarinen 1978). Previously, we reported that for *S. fuscum* in Alberta, stem mass (mg/1 cm length of stem) decreases exponentially as stem density (stems/cm^2; quantified as number of capitula per area) increases (Wieder et al. 2016b), suggesting that densely packed *S. fuscum* plants produce less bulky stems than less densely packed plants. These results, combined with the *S. fuscum* plant density, stem, and capitulum responses at Mariana Lake Bog (Figs. 4, 5), suggest that increasing N loading affects not only *S. fuscum* NPP, but also the way in which *S. fuscum* grows.

At the outset of the Mariana Lake Bog study, we anticipated that N concentrations in *S. fuscum* capitula and stems would follow the triphasic response, namely that capitulum N concentrations should be 7–8 mg/g when N deposition is <15 kg N·ha^{-1}·yr^{-1}; as N deposition increases to over 18 kg N·ha^{-1}·yr^{-1}, capitulum N concentrations should increase to 12 mg/g (Lamers et al. 2000). At Mariana Lake Bog, *S. fuscum* stem N concentrations increased only slightly with increasing atmospheric N addition (Fig. 4E), while capitula showed no response (Fig. 5B); across all years and N treatments, stem and capitulum N concentrations averaged 9.3 ± 0.1 and 13.6 ± 0.2 mg N/g, respectively (n = 105). Previously, we reported that across northern Alberta, *S. fuscum* N concentrations (apical 2 cm sections) were 13–18 mg/g, and unaffected by ambient N deposition levels (Wieder et al. 2016b). Our observed lack of or minimal response of *S. fuscum* N concentrations to experimental N addition at Mariana Lake Bog runs counter to several European studies that have reported increases in *Sphagnum* N concentrations with increasing atmospheric N deposition along spatial geographic gradients (Lamers et al. 2000, Bragazza and Limpens 2004, Jiroušek et al. 2011). However, none of these gradient studies specifically focused on a single *Sphagnum* species. The triphasic *Sphagnum* N concentration response (Lamers et al. 2000) was based on 14 different *Sphagnum* species, although any potential species effect was not examined. There is ample literature to suggest that the response of *Sphagnum* mosses to increasing N, and/or nutrient, availability differs between taxonomic groups and/or species (Pakarinen 1978, Press et al. 1986, Gunnarsson et al. 2004, Lee et al. 1990, Kooijmann and Kanne 1993, Aerts et al. 1992, Jiroušek et al. 2011, Limpens et al. 2003b, Lutke Twenhöven 1992, Lee and Studholme 1992, Bragazza et al. 2004).

It seems evident that in Alberta bogs, the absence of a growth response or a substantial tissue N response of *S. fuscum* to increasing experimental N addition is related to the dominant source of new N being biological N$_2$ fixation, a process not considered in the triphasic response model or in the setting of critical loads for bogs in Europe (Bobbink and Hettelingh 2011). With the high rates of biological N$_2$ fixation at Mariana Lake Bog, *S. fuscum* appears to have a ready supply of N. Experimental N addition may inhibit N$_2$ fixation (Fig. 3B), but without affecting total inputs of new N through N$_2$ fixation plus experimental N addition. We suggest that while the triphasic response to increasing N deposition (Lamers et al. 2000) may apply to regions experiencing long-term, chronic high N deposition, it is likely not an appropriate model for bogs in northern Alberta, Canada.

Aboveground vascular plant responses

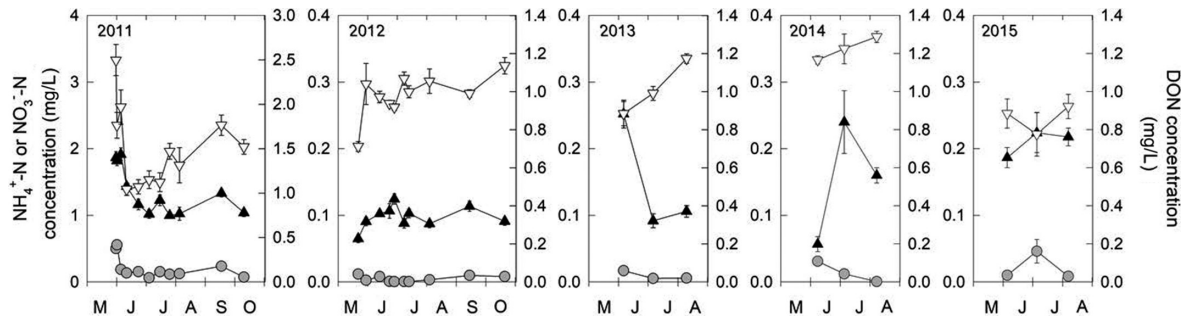We hypothesized that as N deposition levels increase above those that cause the *Sphagnum* moss layer to be N

FIG. 15. Concentrations of NH_4^+-N (black triangles), NO_3^--N (gray circles), and DON (white inverted triangles) at the top of the bog water table. Values are means ± SE ($n = 3$). Arrows along the x-axes indicate N addition dates. Note the different y-axis scales for N concentrations for 2011 than for 2012–2015.

saturated (threshold 1), the NPP of N-limited dominant ericaceous shrubs and of *Picea mariana* will be stimulated. As N deposition further increases to a level exceeding the N uptake capacity of ericaceous shrubs and/or *Picea mariana* (threshold 2), vascular plant leaf/needle N concentrations will increase. Thus, thresholds of N deposition should sequentially trigger responses.

We saw no clear evidence of such thresholds. At Mariana Lake Bog, values for NPP of *A. polifolia*, *C. calyculata*, and *R. groenlandicum* (averaging 6.2 ± 0.8, 9.1 ± 0.7, 7.6 ± 0.8 g·m^{-2}·yr^{-1}) are within the range of reported values for these species growing in bogs (Appendix S1: Table S1), although we note that sparse data on NPP of these species show wide intraspecific variability. Growth of *A. polifolia*, the three ericaceous shrubs combined (Fig. 6), and *P. mariana* (Fig. 9) all increased linearly with increasing N loading. Further N concentrations in *A. polifolia* and *C. calyculata* leaves (as well as in *Vaccinium vitis-idaea* and *Vaccinium oxycoccos*; Fig. 8) and *P. mariana* (Fig. 9) needles also increased linearly with increasing N loading, although differences in leaf N concentrations for *A. polifolia* and *C. calyculata* were greater between years than the increases in N concentrations across the entire N deposition range (Fig. 6). Rather than threshold responses, at Mariana Lake Bog, stimulation of ericaceous shrub and *P. mariana* growth begins as soon as N deposition increases above background levels.

Stimulation of *P. mariana* growth by N (and/or P) fertilization has been previously reported (Alban and Watt 1981, Payandeh 1989, Wells 1993). At Mariana Lake Bog, the effect of N addition on *P. mariana* NPP became more pronounced over time (Fig. 9B). The three years of measurement at Mariana Lake Bog were quite different climatically. The growing season of 2013 was warm and wet in contrast to 2014 and 2015, which were both relatively cool because of unseasonally cold temperatures in May (Fig. 1). While in 2014, considerable rainfall occurred in late June, in 2015 most of the growing season rainfall occurred later in the year. *P. mariana* growth can be affected by soil moisture and aeration in uplands

(Jeglum 1974, Lieffers and Rothwell 1986, Dang and Lieffers 1989, Hillman et al. 1990, MacDonald and Yin 1999, Roy et al. 1999) and by drainage in peatlands (Grigal et al. 1985, Munir et al. 2016). We cannot determine the extent to which the progressively steeper *P. mariana* growth response to N addition (Fig. 9B) represents a cumulative effect of added N or is related to interannual differences in temporal patterns of air temperature and rainfall throughout the three growing seasons.

The ericaceous shrub and *P. mariana* responses to increasing N addition at Mariana Lake Bog are not consistent with the widely held view that growth of *Sphagnum* moss growth relies on atmospherically deposited N, while growth of bog shrubs relies on N obtained through mineralization in peat (cf. Malmer et al. 1994). If this were true, when N deposition increases to levels exceeding the capacity of the bog *Sphagnum* layer to scavenge N, inorganic N would move downward in the peat profile where it would stimulate vascular plant growth (Lamers et al. 2000, Berendse et al. 2001, Tomassen et al. 2003). From the limited published literature on the growth of *A. polifolia*, *C. calyculata*, and *R. groenlandicum* in bogs, however, the NPP response to experimental N addition has been variable and/or inconsistent (Appendix S1: Table S1).

At Mariana Lake Bog, assuming that greater NPP leads to greater biomass of shrubs and *P. mariana*, a consequence of increasing N deposition could be the progressive shading of the *Sphagnum* moss layer, decreasing *Sphagnum* NPP (Clymo 1973, Hayward and Clymo 1983, Heijmans et al. 2001). Indeed, at Mariana Lake Bog, *S. fuscum* NPP declines as a function of both increasing dominant shrub NPP and dominant shrub frequency (Fig. 17). Increasing vascular plant frequency appears to affect *S. fuscum* NPP by decreasing plant density. Regressions of *S. fuscum* plant density as a function of vascular plant frequency were significant ($P < 0.03$) for all five years (Fig. 18), with slopes generally increasing over time as vascular plant abundance increased. Regressions of *S. fuscum* stem mass density or of *S. fuscum* capitulum mass as a function of vascular
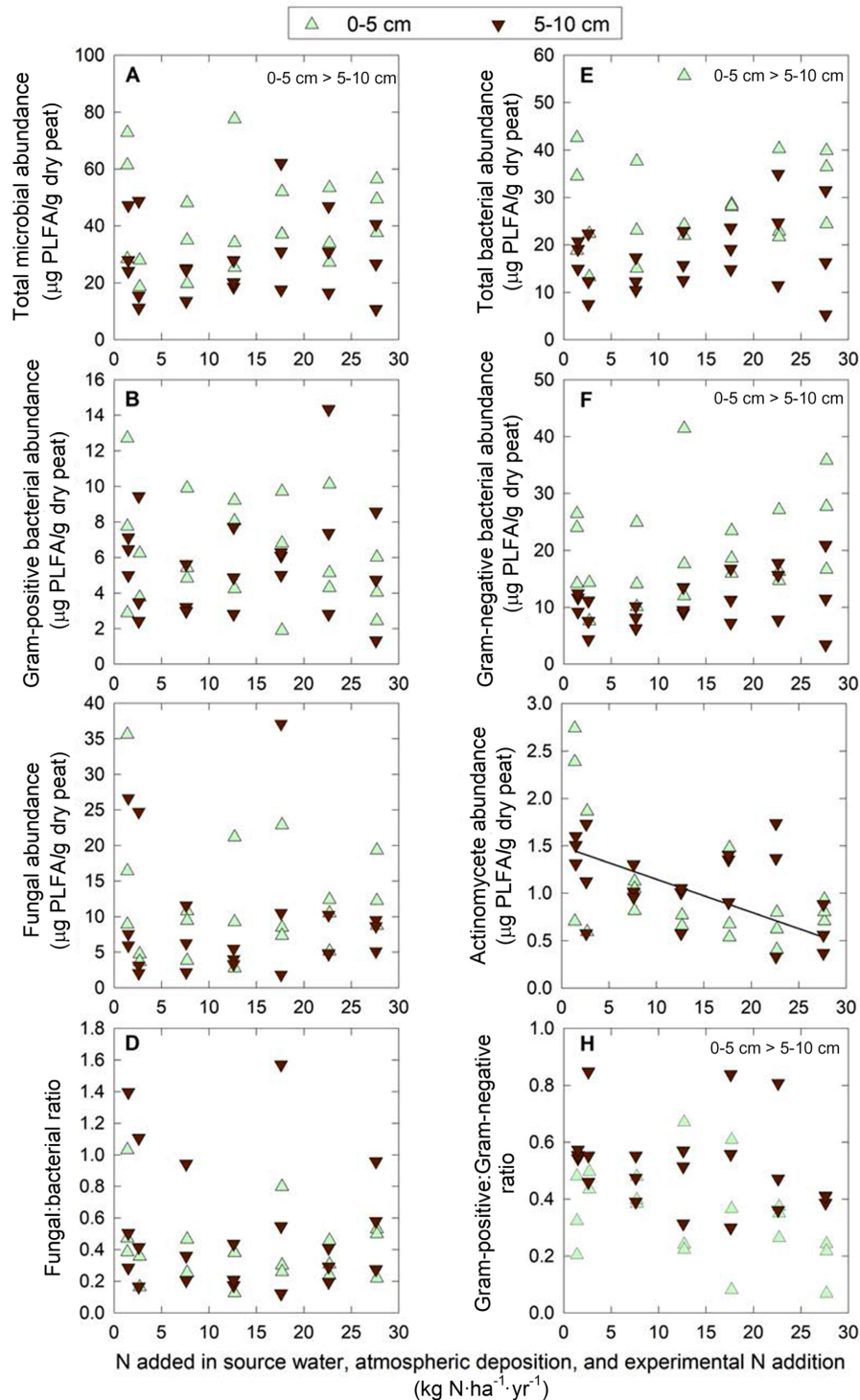
FIG. 16. Microbial abundances based on PLFA analysis. Significant regressions are shown and significant differences between 0–5 cm and 5–10 cm depth increments are indicated.

plant frequency were not significant in any of the five years ($P \geq 0.25$, $P \geq 0.07$, respectively). If increasing N loading to bogs leads to greater shrub abundance, which

in turn leads *S. fuscum* hummocks with less densely packed individuals without affecting the mass of individual plants, it is possible that the water-holding capacity

TABLE 3. Correlations (Pearson's r and associated P values, $n = 105$) between *Sphagnum fuscum* net primary production (NPP) and environmental variables at Mariana Lake Bog; significant correlations are indicated in boldface italic r and P values.

Parameter	Vertical growth (cm/yr)		NPP (g·m^{-2}·yr^{-1})		Stem mass (mg/1-cm stem)		Capitulum density (no./m^2)	
	r	P	r	P	r	P	r	P
Mean monthly temperature (°C)								
May	0.0031	0.9749	0.1595	0.1042	−0.0599	0.5433	*0.4315*	*<0.0001*
June	0.1594	0.1044	0.1731	0.0775	0.0116	0.9069	0.0888	0.3675
July	0.0416	0.6733	*0.2102*	*0.0314*	0.1249	0.2045	0.1163	0.2374
August	0.0134	0.8919	0.1359	0.1668	−0.0508	0.6067	*0.3328*	*0.0005*
September	0.0341	0.4298	*0.2605*	*0.0073*	−0.0232	0.8146	*0.5164*	*<0.0001*
Growing season degree days (°C-d)	0.0444	0.6527	*0.2567*	*0.0082*	−0.0380	0.7003	*0.3697*	*0.0001*
Monthly precipitation (mm)								
May	0.1725	0.0784	0.1248	0.2046	0.0205	0.8357	−0.0457	0.6436
June	0.0133	0.8929	*−0.2257*	*0.0206*	*−0.2162*	*0.0268*	−0.0820	0.4059
July	0.2024	0.0384	*0.3190*	*0.0009*	−0.0297	0.7636	*0.3810*	*<0.0001*
August	0.1670	0.0886	*0.2673*	*0.0059*	0.1279	0.1936	0.0479	0.6274
September	*0.1929*	*0.0487*	*0.4191*	*<0.0001*	0.1124	0.2536	*0.3312*	*0.0006*
Growing season total precipitation (mm)	*0.2403*	*0.0136*	*0.2837*	*0.0034*	−0.0559	0.5709	*0.5179*	*<0.0001*
Growing season aridity index (PET/precip)	*−0.2557*	*0.0085*	*−0.2531*	*0.0092*	0.0634	0.5205	*−.2252*	*0.0209*

Note: PET, potential evapotranspiration.

of the hummocks decreases as well (cf. Wagner and Titus 1984), contributing to decreasing NPP of a species whose growth appears to be tightly linked to climatic variables.

We did not observe a threshold response for leaf/needle N concentrations for any of the species at Mariana Lake Bog. Of the 10 vascular plant species examined, only five showed increasing leaf/needle N concentration with increasing experimental N addition: *A. polifolia*, *C. calyculata*, *Vaccinium vitis-idaea*, *Vaccinium oxycoccos* (Fig. 8), and *P. mariana* (Fig. 9). However, the increases in N concentration were generally small, and often less than inter-annual variation. A review of leaf/needle N concentrations in the 10 vascular plant species (Appendix S1: Table S2) reveals that vascular plant leaf/needle N concentrations at Mariana Lake Bog are comparable to published values, although these values often exhibit wide intraspecific variability; where there have been experimental N addition or ambient N deposition gradient studies, responses have been inconsistent.

The mechanisms by which vascular plant species take up, store, and internally retranslocate N, have long interested peatland scientists (cf. Chapin 1980, Chapin et al. 1986, Aerts and Chapin 2000). In bogs, vascular plant species may rely on different sources of N, depending on factors such as growth form, mycorrhizal associations, vertical root distributions, and position relative to the bog water table (Nordbakken et al. 2003). Of particular relevance here is whether vascular plant species in bogs exhibit increasing N leaf/needle concentrations with increasing experimental N addition. Such a response could indicate luxury consumption of N, viz. N accumulation in leaf/needle tissues, typically after a flush of N availability, above levels that directly promote growth (Chapin 1980, Aerts and Chapin 2000). At Mariana Lake

Bog, there is no strong evidence of luxury consumption. Although a stimulation of aboveground growth of bog vascular plants may be a general response to increasing N deposition, we do not have a species-specific mechanistic understanding of how growth and leaf/needle N concentrations respond to increasing N deposition.

Root responses

We hypothesized that as N deposition levels increase above those that cause the *Sphagnum* moss layer to be N saturated, belowground production of N-limited dominant ericaceous shrubs and of *Picea mariana* would be stimulated. Although, at Mariana Lake Bog, root biomass and production increased with increasing N loading, there was no evidence to support a threshold response (Fig. 12). Further, because both root biomass and root production increased with increasing N deposition, root turnover (production/biomass; per yr) was not affected by N additions ($P = 0.67$ and 0.12, respectively), averaging 0.11 ± 0.01 and 0.07 ± 0.01 yr^{-1} based on 1-and 2-yr production values.

There is a paucity of information regarding root biomass and production in bogs. Root biomass and production at Mariana Lake Bog are within the range of published values for bogs (Appendix S1: Table S3). As with shrub aboveground production, root biomass and production are quite variable across bogs (Appendix S1: Table S3), with sites being climatically different, measurements made in different places within bogs, and with different size classification of roots, making generalization difficult. We are aware of only one effort to quantify bog root responses to experimental N addition. At Mer Bleue Bog, Ontario, under different N, P, and K treatments, fine root (<1 mm) biomass varied from 90 ± 60

FIG. 18. Regressions of *Sphagnum fuscum* plant density as a function of total vascular plant frequency. Regressions were carried out assuming a common intercept using PROC MODEL in SAS (v. 9.3). Regressions for 2011, 2012, 2013, 2014, and 2015 were all significant ($P \leq 0.025$).
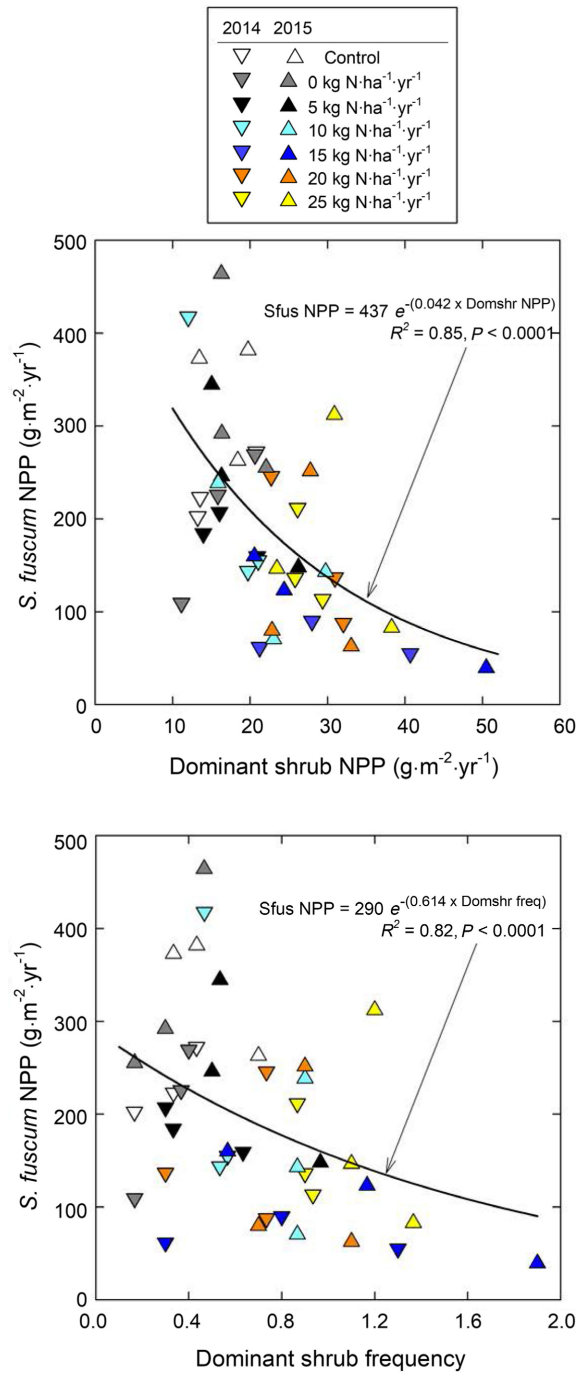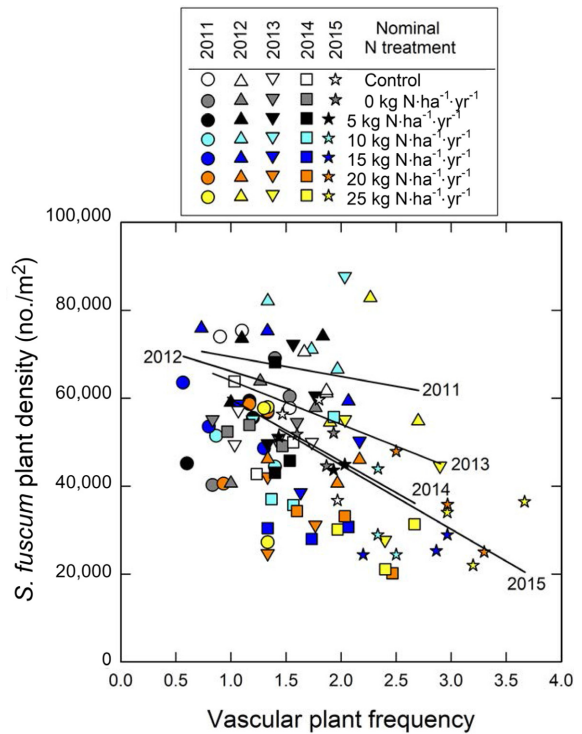

FIG. 17. *Sphagnum fuscum* NPP as a function of dominant shrub NPP and dominant shrub frequency. Dominant shrub frequency is the sum of the absolute frequencies of *A. polifolia*, *C. calyculata*, and *R. groenlandicum*.

to 173 ± 113 g/m^2, while coarse root (>1 mm) biomass varied from 482 ± 417 to 930 ± 706 g/m^2 (Xing et al. 2011). The large within-treatment variability in this study precluded identification of clear fertilization effects, although there was a general pattern of a decreasing large root to fine root ratio with increasing fertilization.

Given the rather consistent finding that increasing N deposition stimulates aboveground vascular plant biomass and production, along with our results that root biomass and production at Mariana Lake Bog are stimulated, further work on belowground responses seems warranted. While the aboveground response to increasing N deposition has likely implications for moss production, belowground responses could have implications for bog carbon cycling (e.g., increased root contribution to total peat C stocks and net C accumulation, increased CO_2 emission from root biomass), along with general rhizosphere effects on bog biogeochemistry (cf. Fritz et al. 2011, Agethen et al. 2018).

Plant community composition responses

Initially, we hypothesized that increasing N deposition would lead to changes in plant community composition, specifically leading to a lower abundance of *Sphagnum* mosses and a higher abundance of vascular plants. Indeed, at Mariana Lake Bog, increasing N loading led to changes in both *Sphagnum* species frequencies (decrease in *S. fuscum*, increase in *S. magellanicum*, no response of *S. angustifolium*) and vascular plant

frequencies (increases in *R. groenlandicum*, *A. polifolia*, the three dominant shrubs combined, all shrubs, and all vascular plants; Fig. 10). Multivariate analysis further illustrated changes in the plant community, especially separating the 20 and 25 kg N·ha^{-1}·yr^{-1} treatments and especially in 2014 and 2015, from lower N loadings and earlier years (Fig. 11).

A prevailing paradigm in bog ecology is that increasing N deposition/availability results in shifts in the relative importance (cover, biomass, net primary production) of plant species. It is widely held that when N deposition exceeds the uptake capacity of *Sphagnum* mosses, downward movement of N into the peat results in increasing shrub growth, biomass, and/or litter production, ultimately resulting in lower *Sphagnum* production and abundance, and potentially compromising the bog net C sink function (Lamers et al. 2000, Berendse et al. 2001, Limpens et al. 2006, 2011, Bubier et al. 2007). Changes in bog plant community composition ascribed to N deposition have been described from comparative studies along N deposition gradients, site-specific historical assessment of vegetation, and experimental N addition in the laboratory or in the field (Berendse et al. 2001, Heijmans et al. 2001, Chapin et al. 2004, Wiedermann et al. 2007, Juutinen et al. 2010). Across these studies, bogs have differed widely in ambient N deposition and in species composition; experimental N additions have ranged from 20 to 64 kg·ha^{-1}·yr^{-1}, with differences in the number of years over which N additions have occurred. Nonetheless, increasing N deposition often results in higher shrub abundance and lower *Sphagnum* abundance, sometimes with the nearly complete elimination of *Sphagnum* (cf. Wiedermann et al. 2007, Juutinen et al. 2010).

At Mariana Lake Bog, we observed changes in vegetation even at low experimental N loadings, with vegetation progressively changing over the 5 yr of the study (Figs. 10, 11). These results suggest that bogs that have persisted under very low ambient N deposition may be especially sensitive to increasing N deposition in terms of plant species relative abundances and plant community composition.

Decomposition responses

We hypothesized that as N deposition increases, decomposition of vascular plant litter, *Sphagnum*, and a uniform substrate (cellulose filter paper) would increase.

This hypothesis was based on the premise that as N deposition increases beyond the capacity of *Sphagnum* and vascular plants to take it up, DIN availability in near-surface peat would increase, thereby stimulating decomposition of vascular plant litter. At Mariana Lake Bog, stimulation of cellulose decomposition by increasing N deposition did not exhibit an N deposition threshold response; vascular plant litter and *Sphagnum* decomposition were unaffected by N loading (Fig. 13).

Pure cellulose, typically filter paper, has been widely used in decomposition studies, with the premise that it acts as a substrate of uniform quality, and hence has the potential to separate the effects of environmental factors from the effects of plant litter quality on decomposition rates (Berg et al. 1975). Published cellulose decomposition rates for bogs are quite variable (Appendix S1: Table S4). Nonetheless, there is some evidence that increasing N deposition/availability stimulates cellulose decomposition in surface bog peat (Brække and Finér 1990, Hiroki and Watanabe 1996, Jiroušek et al. 2015), as we found at Mariana Lake Bog.

Compared to other *Sphagnum* mosses or other peatland mosses, *S. fuscum* decomposition is quite slow (Johnson and Damman 1991, Turetsky et al. 2008, Lang et al. 2009, Bengtsson et al. 2016), a consequence of species-specific differences in organic matter composition (Turetsky et al. 2008, Bengtsson et al. 2018). At Mariana Lake Bog, *S. fuscum* decomposition rates are within the range of published values (Appendix S1: Table S5), and are unaffected by N loading (Fig. 13), a result consistent with other studies (Breeuwer et al. 2008, Bragazza et al. 2012). Similarly, vascular plant litter decomposition was unaffected by N addition at Mariana Lake Bog (Fig. 13). In general, there is little or weak evidence that increasing N deposition stimulates vascular plant litter decomposition in bogs (cf. Breeuwer et al. 2008, Bragazza et al. 2012). It is well established that vascular plant litter decomposes more rapidly than *Sphagnum* (cf. Moore and Basiliko 2006, Breeuwer et al. 2008, Lang et al. 2009), as we observed (Fig. 13).

There appears to be no general consensus on the extent to which increasing atmospheric N deposition may affect decomposition of *Sphagnum* species generally (cf. Jiroušek et al. 2015), of *S. fuscum* in particular, or of vascular plant litter in boreal bogs. At Mariana Lake Bog, the absence of a response of *S. fuscum* or mixed vascular plant litter decomposition to N addition (Fig. 13) suggests that decomposition of these materials in Alberta bogs is not N limited. Although increased shrub growth in response to increasing N additions would produce a greater quantity of vascular plant litter, this may not result in a substantial increase in peat accumulation rates, given that vascular plant litter is not a major contributor to long-term peat accumulation (Kuhry and Vitt 1996). Further, the absence of *Sphagnum* and vascular plant litter decomposition responses to increasing N additions is consistent with the absence of net N mineralization and porewater DIN concentration responses at Mariana Lake Bog.

Net N mineralization and porewater DIN concentration responses

We hypothesized that as N deposition increases to a level that exceeds the capacity of the bog vegetation to take up N, net N mineralization in surface peat would be inhibited by higher NH_4^+-N availability, net nitrification would be stimulated by higher NH_4^+-N availability (cf. McGill and Cole 1981, Robertson and Groffman 2015),

and concentrations of DIN in porewater at the top of the water table would increase, as DIN bypasses interception by the ground layer vegetation (cf. Bragazza and Limpens 2004). None of these hypotheses was supported.

Pools of KCl-extractable NH_4^+-N and NO_3^--N (Table 2), as well as net ammonification, net nitrification rates, and net DIN production rates in Mariana Lake Bog surface peat were low (Fig. 14), comparable to values from other bogs in northern Alberta (Bayley et al. 2005, Wood et al. 2016, Stuart et al. 2018), and generally unaffected by N loading, as was found by Stuart et al. (2018) for Alberta bogs along a post-fire chronosequence with experimental N addition. In Alberta bogs, given the wide C:N ratio of the *Sphagnum* peat, experimentally added NH_4^+-N and NO_3^--N apparently appear to be rapidly immobilized (cf Hartsock et al. 2019). This immobilization prevents experimentally added DIN from moving downward through the peat to the bog water table. As a result, concentrations of NH_4^+-N and NO_3^--N at the top of the bog water table, although variable between and within years (Fig. 15), were unaffected by experimental N addition. It is possible, however, that when bogs are chronically exposed to high N deposition over long periods of time, the N immobilization capacity of the peat may be progressively diminished, which could lead to increasing DIN concentrations in bog porewater (cf. Bragazza and Limpens 2004).

Microbial community responses

We expected that as N deposition increases, peat microbial structure would change, and in particular, that as the abundance of mycorrhizal vascular species increases, the peat fungal:bacterial ratio would increase. At Mariana Lake Bog, we obtained little evidence to support N addition effects on microbial community composition (Fig. 16).

Microbial community composition and structure in bog peat is not well understood. Based on PLFA-FAME analyses, microbial communities have been shown to differ between peatland types (ombrotrophic bogs, oligotrophic fen, mesotrophic fen), with depth within peatlands, with peat moisture, and with the botanical composition (*Carex* vs. moss derived) of peat (Borgå et al. 1994, Sundh et al. 1997, Jaatinen et al. 2007, Bragazza et al. 2015). Along a disturbance-recovery gradient, peat PLFA profiles differed between the beginning and end of the growing season, but did not change between the five stages of peatland restoration (Andersen et al. 2010). Stuart et al. (2018) found few differences in peat microbial communities between five Alberta, Canada bogs along a bog postfire chronosequence, with little effect of experimental N addition (up to 20 kg·ha^{-1}·yr^{-1}) at any of the bog sites. It is possible that the absence of an N addition effect on peat microbial community composition is a reflection of increasing

DIN deposition being offset by decreasing biological N_2 fixation, such that total new N additions to Mariana Lake Bog are not dramatically affected. However, we are not inclined to conclude that N addition has no effects on bog peat microbes. It may be that more sensitive/targeted techniques, such as high-throughput pyrosequencing (Ramirez et al. 2012), 16s RNA clone library analysis and rRNA-targeted fluorescence in situ hybridization (FISH; Dedysh et al. 2006, Bragina et al. 2014), or whole genome shotgun sequencing (Rusin 2016) may be required to reveal bog microbial community responses to N loading.

Ecosystem-scale synthesis

Fate of new N additions and relevance to critical loads

Decades of research conducted across Europe have produced widespread agreement that increasing atmospheric N deposition has negative consequences for bog ecosystem structure and function. This research led to setting a critical load for N deposition for raised and blanket bogs of 5–10 kg·ha^{-1}·yr^{-1} (Bobbink and Hettelingh 2011). Bog responses that were especially important in recommending this critical load range included altered growth and species composition of mosses, increase in vascular plant abundances, increased N concentrations and quantities in peat, and increased N concentrations in bog porewaters, although other responses were considered as well (Bobbink and Hettelingh 2011). In terms of understanding the fate of N added to bog ecosystems through increasing atmospheric DIN deposition, two prevailing principles have dominated. First, *Sphagnum* mosses rely only on atmospherically deposited N, whereas bog vascular plants can also derive N made available through mineralization processes in peat (Malmer et al. 1994). Building on this, the second principle is the "triphasic" response to increasing atmospheric N deposition put forth by Lamers et al. (2000). Under the triphasic response scenario, at N deposition below 10 kg·ha^{-1}·yr^{-1}, atmospherically deposited N is scavenged by the *Sphagnum* moss layer, supporting growth. As N deposition increases to a point exceeding the growth requirements of the *Sphagnum* layer (10–20 kg N·ha^{-1}·yr^{-1}), N concentrations in *Sphagnum* tissues increase. Further increases in N deposition lead to a failure of the moss filter, such that a portion of the deposited N moves downward into the peat profile, with the consequences of increasing DIN, and possibly DON, concentrations in bog porewater and of stimulating vascular plant growth. Stimulation of vascular plant growth may result in the shading of *Sphagnum* mosses, leading to lower moss growth and cover. These threshold responses are graphically depicted in Fig. 19A. Although our results from Mariana Lake Bog are in some ways consistent with the European model, they also provide a quite different mechanistic view of the biogeochemical processing of atmospherically deposited N.
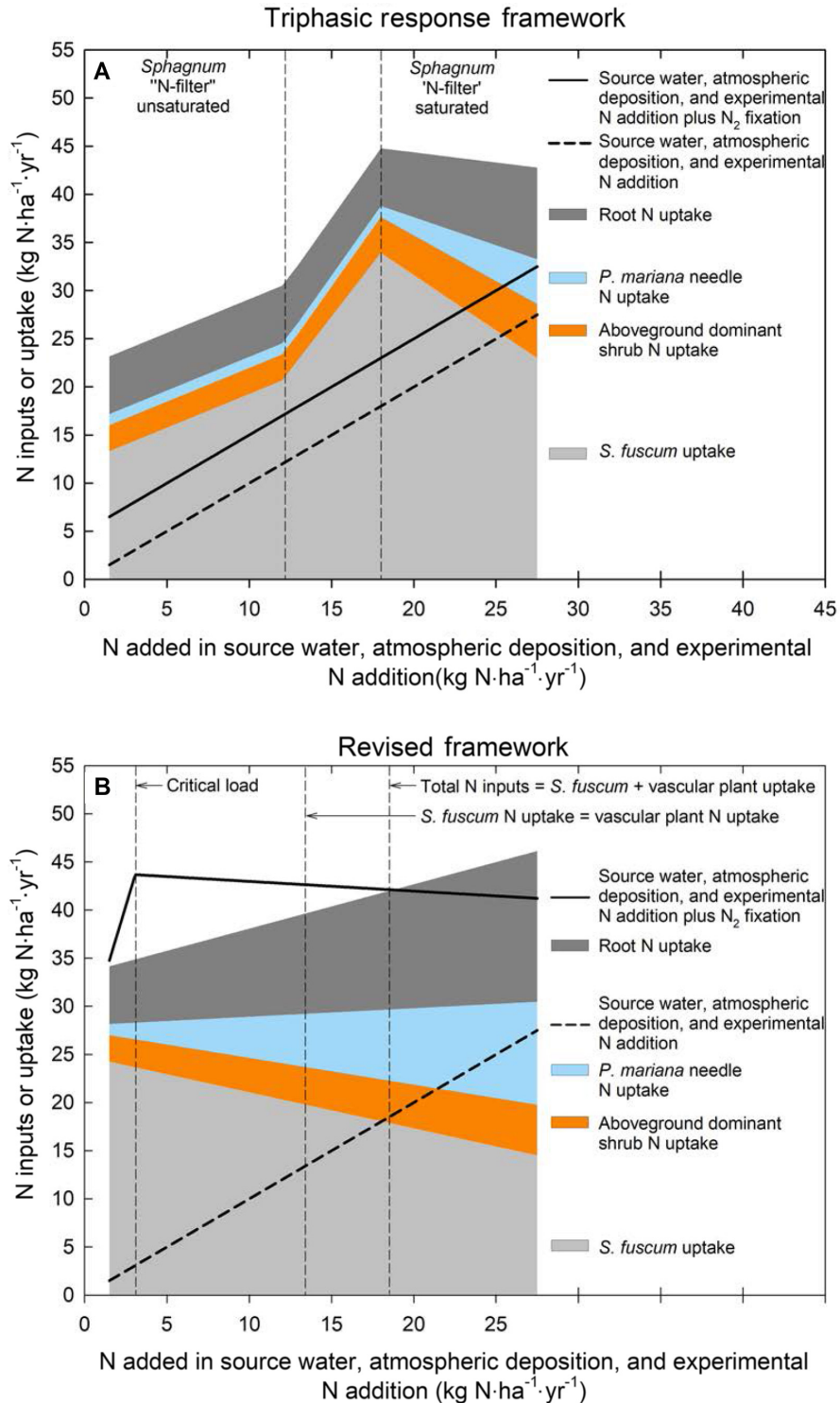
FIG. 19.   Schematic representation of N additions and sequestration by *Sphagnum fuscum* and vascular plant annual growth under (A) the triphasic response framework (Lamers et al. 2000) and (B) our revised framework based on results at Mariana Lake Bog. In panel A, the biological N_2 fixation value of 4 kg N·ha^{-1}·yr^{-1} is from Limpens et al. (2006). To apply the triphasic response model, we used an N concentration for *S. fuscum* at low N addition of 7 mg/g (Lamers et al. 2000) and an NPP of 190 g·m^{-2}·yr^{-1} (Gunnarsson 2005), allowing NPP to increase at a modest 10 g·m^{-2}·yr^{-1} per kg N·ha^{-1}·yr^{-1} increase in N addition with no change in N concentration. At N additions from 12–18 kg N·ha^{-1}·yr^{-1}, *S. fuscum* NPP remains constant, but stem N concentrations increase to 11.5 mg/g (Lamers et al. 2000). At N additions above 18 kg N·ha^{-1}·yr^{-1}, *S. fuscum* NPP decreases at a modest

Fɪɢ.19. (*continued*)

10 g·m^{-2}·yr^{-1} with no change in stem N concentration, such that N uptake decreases. We assumed no stimulation of shrub, *P. mariana*, or root uptake at N additions up to 18 kg N·ha^{-1}·yr^{-1}, using N uptake values from our control treatments from (Figs. 6H, 9D, and 12D, assuming a root N concentration of 7 mg/g; Xu 2011). At N additions above 18 kg N·ha^{-1}·yr^{-1}, we allowed shrub, *P. mariana*, and root uptake to increase using the slopes in Figs. 6H, 9D, and 12D. For the revised framework (B), we used N uptake relationships for *S. fuscum* (Fig. 4F, 2013–2015), dominant vascular plants (Fig. 6H), *P. mariana* needles (Fig. 9D), and roots (Fig. 12D, assuming a root N concentration of 7 mg/g; Xu 2011).

As has been shown in numerous studies of European bogs, at Mariana Lake Bog, we characterized marked changes in moss and vascular plant species composition with increasing N deposition. Increases in *S. magellanicum* frequency and decreases in *S. fuscum* frequency, as well as increases in the frequencies in *R. groenlandicum*, *A. polifolia*, all shrubs, and all vascular plants (Fig. 10), became increasing evident over the five years of experimental N addition. Thus, using plant community composition (Fig. 11) as an indicator of ecosystem structure, increasing N addition causes changes in vegetation both at Mariana Lake Bog and European bogs.

However, our assessment of the mechanisms by which new N is added to and processed within Mariana Lake Bog (Fig. 19B), bears little similarity to the commonly held European views. The European conceptualization has made no mention of the role of biological N$_2$ fixation as a source of new N to bogs. Under pristine conditions (control treatments) it is evident that biological N$_2$ fixation represents the major source of new N (96% of new N) to Mariana Lake Bog, and indeed to other bogs in northern Alberta (Vile et al. 2014). Further, under pristine conditions, the N requirements to support the annual growth of *S. fuscum* at Mariana Lake Bog (control treatments averaged over the 5 yr 27.5 ± 0.3 kg N·ha^{-1}·yr^{-1}, *n* = 15) cannot be supported by atmospheric N deposition alone (1.5 kg N·ha^{-1}·yr^{-1}), contrary to the view that moss growth relies solely or primarily on atmospheric N deposition (Malmer et al. 1994). As experimental addition of DIN increased beyond 3.1 kg·ha^{-1}·yr^{-1}, the decrease in biological N$_2$ fixation (Fig. 3B) occurred along with a decrease in *S. fuscum* NPP (in the third and fifth year of N addition; Fig. 4A), suggesting that *S. fuscum* may be more reliant on biologically fixed N than on DIN additions. Such a strategy could be advantageous especially in regions like northern Alberta where both background N deposition and growing season rainfall are low, with rain events being temporally sporadic. By relying on biological N$_2$ fixation, there could be a temporally consistent source of new N to support *Sphagnum* growth. If this is indeed the case, for biologically fixed N to support *Sphagnum* growth, there would need to be a transfer of biologically fixed N from within the cells of the fixers to *Sphagnum* cells; such a process has been unexplored.

Because N$_2$ fixation appears to occur in the *Sphagnum* moss layer (Vile et al. 2014), it may be reasonable to regard this layer as having the first opportunity to access new N additions to a bog, whether from atmospheric deposition or N$_2$ fixation. At Mariana Lake Bog, across all N treatments, after accounting for N sequestration of new N through *S. fuscum* growth (Fig. 4F), there is a positive balance of unused new N that could then be available for vascular plant growth (Fig. 19B). However, because experimental N addition inhibits N$_2$ fixation (Fig. 3B; Reed et al. 2011), the quantity of unused new N available for vascular plant growth does not clearly increase with increasing N addition at levels above 3.1 kg N·ha^{-1}·yr^{-1} (Fig. 19B). If it is the case that *S. fuscum* preferentially utilizes biologically fixed N, this unused new N may be predominantly in the form of DIN. Under this scenario, with increasing DIN deposition, new N additions would become increasingly available for vascular plant growth not because of a failure of the moss filter *per se*, but through a more complex set of interactions centered on the preferential reliance on biologically fixed N by growing *Sphagnum*, the inhibition of N$_2$ fixation by increasing DIN deposition, and the resulting decrease in *Sphagnum* growth with increasing N deposition (Fig. 19B).

At Mariana Lake Bog, aboveground ericaceous shrub (Figs. 6D), aboveground *P. mariana* (Fig. 9B), and root (Fig. 12D) production all increase with increasing experimental N addition, and as such N assimilation into these vascular plant compartments increases as well (Figs. 6H, 9D, 19B). At N deposition up to 17 kg N·ha^{-1}·yr^{-1}, the unused new N available for vascular plant growth after *Sphagnum* sequestration is more than sufficient to support vascular plant growth, such that N made available through mineralization is not necessarily required (Fig. 19B). Overall, across all N treatments, we did not observe increasing concentrations of DIN or DON in peatland pore water at the top of the water table (Fig. 15), leading us to argue that N added to the Mariana Lake Bog via N$_2$ fixation and by experimental DIN addition are effectively retained within the vegetation and/or peat above the water table (cf. Hartsock et al. 2019). Nonetheless, a major effect of experimentally increasing N deposition at Mariana Lake Bog is a shift from a system in which overall annual N assimilation is dominated by *Sphagnum fuscum* to a system in which most of the annual N assimilation is through the vascular plant component of the vegetation (Fig. 19B).

We offer some caveats about our synthesis and interpretation. First, the synthesis (Fig. 19B) is intended to summarize the potential fate of new N added to the bog annually; it is not intended to be a complete N budget

for the bog. Among the items that would be required to construct a complete budget would be: N released by decomposition/mineralization of annually produced vascular plant litter, gross N mineralization and immobilization/consumption, and potential losses of N_2O, although the latter is expected to be quite small. In terms of the fate of new N added to the bog, we quantified production of only the three dominant ericaceous shrub species. We did not quantify N assimilation through annual growth of other ericaceous shrub (*Vaccinium vitis-idaea*, *Vaccinium oxycoccos*, *Kalmia polifolia*), perennial herb (*Rubus chamaemorus*, *Maianthemum trifolium*), and graminoid (*Eriophorum vaginatum*) species. Further, we have not considered N assimilation by large (>1 m tall) *P. mariana* trees, or N assimilation into the new growth of woody portions of shrubs. We acknowledge that by not including these components, we have underestimated, we assume slightly, annual N assimilation by vascular plants at Mariana Lake Bog.

With respect to a critical load recommendation, we note that when we initiated this study, we anticipated finding responses akin to the European triphasic conceptualization (Lamers et al. 2000). We chose 7 N treatment levels, up to 25 kg·ha^{-1}·yr^{-1}, with the anticipation that responses to N addition would be sufficiently strong to be able to be detected even though our level of replication of the N treatments was small (three plots per treatment). We expected that we would be able to identify a sequence of responses to increasing N deposition, with individual responses being sequentially triggered at increasing N deposition thresholds. These N deposition thresholds would be revealed objectively through the use of two-segment piecewise regressions.

The one response variable for which a clear threshold response to increasing N addition was obtained was biological N_2 fixation, with a threshold of 3.1 kg N·ha^{-1}·yr^{-1} (Fig. 3B). Other responses to increasing N deposition, for the most part, did not indicate clear N deposition thresholds, but rather exhibited linear changes across the entire N deposition range (two-segment piecewise regressions either failed to converge or did not explain the variability substantially better than linear regressions). Responses of this sort include declining *S. fuscum* NPP and *S. fuscum* N assimilation (that became evident in years 3–5 of experimental N addition; Fig. 4F), increasing dominant shrub NPP and dominant shrub N assimilation (Fig. 6D, H), increasing root biomass and root production in the upper 30 cm of peat (Fig. 12B, D), decreasing frequency of *S. fuscum* and increasing frequency of *S. magellanicum*, *Rhododendron groenlandicum*, *Andromeda polifolia*, dominant shrubs, all shrubs, and all vascular plants (that also became more evident in years 3–5 of experimental N addition Fig. 10). Thus, we conclude that for Mariana Lake Bog, and for bogs in general in northern Alberta, even the slightest increase in atmospheric deposition above a background level of 1.5 kg N·ha^{-1}·yr^{-1} is likely to exert consequential changes in ecosystem

structure and function that individually or collectively could be viewed as deleterious. Nonetheless, given the critical role that biological N_2 fixation plays in supplying new N to Alberta bogs, and our suggested changes to how new N is processed as increasing N deposition progressively inhibits N_2 fixation (Fig. 19B), we recommend a critical load for bogs in northern Alberta of 3 kg N·ha^{-1}·yr^{-1}, recognizing that deleterious effects may in fact occur at lower N deposition levels.

We acknowledge that our recommended critical load is lower than that of 5–10 kg·ha^{-1}·yr^{-1} recommended for European bogs (Bobbink and Hettelingh 2011). We have discussed that our findings in general are not consistent with the triphasic response that may indeed characterize European bogs. Peatlands in Alberta have persisted in an environment where N deposition has likely been quite low (<2 kg·ha^{-1}·yr^{-1}) for thousands of years. While some European peatlands also receive low N deposition, many have been receiving exceptionally high N deposition (40 kg N·ha^{-1}·yr^{-1} or greater) for decades. It is difficult to assess the extent to which studies that challenge bogs with historically very low N deposition to experimental N addition, as we did at Mariana Lake Bog, relate to studies that use natural N deposition gradients or to studies that experimentally add N to sites with high chronic ambient N deposition. Further, we cannot with confidence predict how bogs like Mariana Lake Bog would respond to longer term N additions. Nonetheless, our findings suggest that even small increases in atmospheric N deposition are likely to influence the structure and function of bogs in northern Alberta, where historical N deposition has been quite low. If N deposition were to increase above 3 kg·ha^{-1}·yr^{-1}, biological N_2 fixation would decline, initiating a shift in the balance of new N additions toward inorganic rather than organic (biologically fixed) N, changing plant community composition by favoring shrub production, shifting the balance of the assimilation of new N additions from *Sphagnum* to vascular plants, and decreasing ecosystem-wide *Sphagnum* abundance, production, and important role in assimilation of new N additions.

at the Meanook Biological Research Station, until its closing in 2014.

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DATA AVAILABILITY

Data sets have been archived with the Environmental Data Initiative (*Sphagnum fuscum* capitulum data: https://doi.org/10.6073/pasta/87829794cd0fb0d58a850e21895cc866; cellulose decomposition: https://doi.org/10.6073/pasta/0c3d3acd8942dde30138cbc9700e61a2; vascular plant leaf N concentrations: https://doi.org/10.6073/pasta/6c4232a59c7e3c4380052c03a63b48d5; net N mineralization: https://doi.org/10.6073/pasta/8f015cca0ee4c38db2d1b13bda2db96c; N_2 fixation: https://doi.org/10.6073/pasta/ab9b82764876ec8f2a86c57d57290b42; *Picea mariana* growth and nitrogen data: https://doi.org/10.6073/pasta/6f83a1109497c097c8db32cc38e43d76; PLFA data: https://doi.org/10.6073/pasta/2776b5bd019ad3e119ed41b7d66e4746; point-frame plant frequency data: https://doi.org/10.6073/pasta/53d50ac0e39d49bbac053d4839fdebb6; nitrogen deposition from ion exchange resin collectors: https://doi.org/10.6073/pasta/2e674709c5af0c316ce1792b67513dbf; root biomass and nitrogen data: https://doi.org/10.6073/pasta/2a0587cbdcf10b4063d45649aada8e64; shrub growth and nitrogen assimilation: https://doi.org/10.6073/pasta/cfd9088078e596e4db801d8bf19e45d3; Sphagnum and vascular plant litter decomposition: https://doi.org/10.6073/pasta/51acf9e230a25a1cda7f7e2e7eb0bf79; *Sphagnum fuscum* cranked wire data: https://doi.org/10.6073/pasta/ca2279cf25469abffbc43873e907163b; *Sphagnum fuscum* NPP and N assimilation: https://doi.org/10.6073/pasta/4459709a4a43792d88be102034415d80; pore water nitrogen concentrations: https://doi.org/10.6073/pasta/20e551f527bff2ed362c8da95f4050ee)