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Epiphytic macrolichen communities correspond to patterns of sulfur and nitrogen deposition in the northeastern United States

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Abstract. Atmospheric deposition of sulfur (S) and nitrogen (N) has decreased steadily in the northeastern U.S. since the federal 1970 Clean Air Act was passed, yet deposition remains elevated above natural background levels throughout the region. Epiphytic macrolichens are highly sensitive to air pollution and their status is a good indicator of ecological health. We used deposition modeling for 2000–2013 and multiple metrics of lichen status (i.e., species composition, species richness, thallus condition, lichen sensitivity indices, lichen elemental analysis) to assess air pollution effects at 24 plots in four federally-mandated Class I areas. The areas (Lye Brook Wilderness, VT; Great Gulf and the Presidential Range-Dry River Wildernesses, NH; and Acadia National Park, ME) encompass a range of high to low deposition sites. We developed thallus condition scores and sensitivity groups and indices for S and N based on species patterns using deposition estimates gleaned from a larger, independent data base. Non-metric multidimensional scaling ordinations differentiated forest structure effects on lichen community composition from more complex deposition and elevation effects. Annual mean and cumulative deposition of N correlated strongly with decreases in lichen species richness and N-sensitive species, and poorer thallus condition. Cumulative dry deposition of S yielded the best fit to decreases in thallus condition, poorer community-based S Index values, and absence of many S-sensitive species. Multiple metrics provided consistent evidence that higher depositional loading was associated with greater adverse effects. In general, stronger correlations between present day lichen metrics and cumulative deposition (post-2000), compared to current deposition, emphasize the long-term nature of emissions impacts and continued need to control S and N emissions to restore the ecological health of lichen communities and linked biota.

Keywords. Thallus condition, cumulative deposition, air pollution, epiphyte, cyanolichen, sensitivity rating.

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Deposition of most sulfur (S)- and nitrogen (N)-containing air pollutants has decreased in the northeastern U.S. over the past several decades in response to reductions in emissions of S and N oxides (SO_x and NO_x) achieved through implementation of the U.S. Clean Air Act and similar Canadian legislation. However, reduced nitrogen (NH_x) was not included in that legislation and deposition is locally variable (Vet et al. 2014). Total annual N deposition is often used in calculations of critical loads (Pardo et al. 2011) and correlates better with epiphytic lichen responses than wet deposition alone or any single N-containing depositional compound (Johansson et al. 2010; Jovan et al. 2012). However, the relative harm of different N

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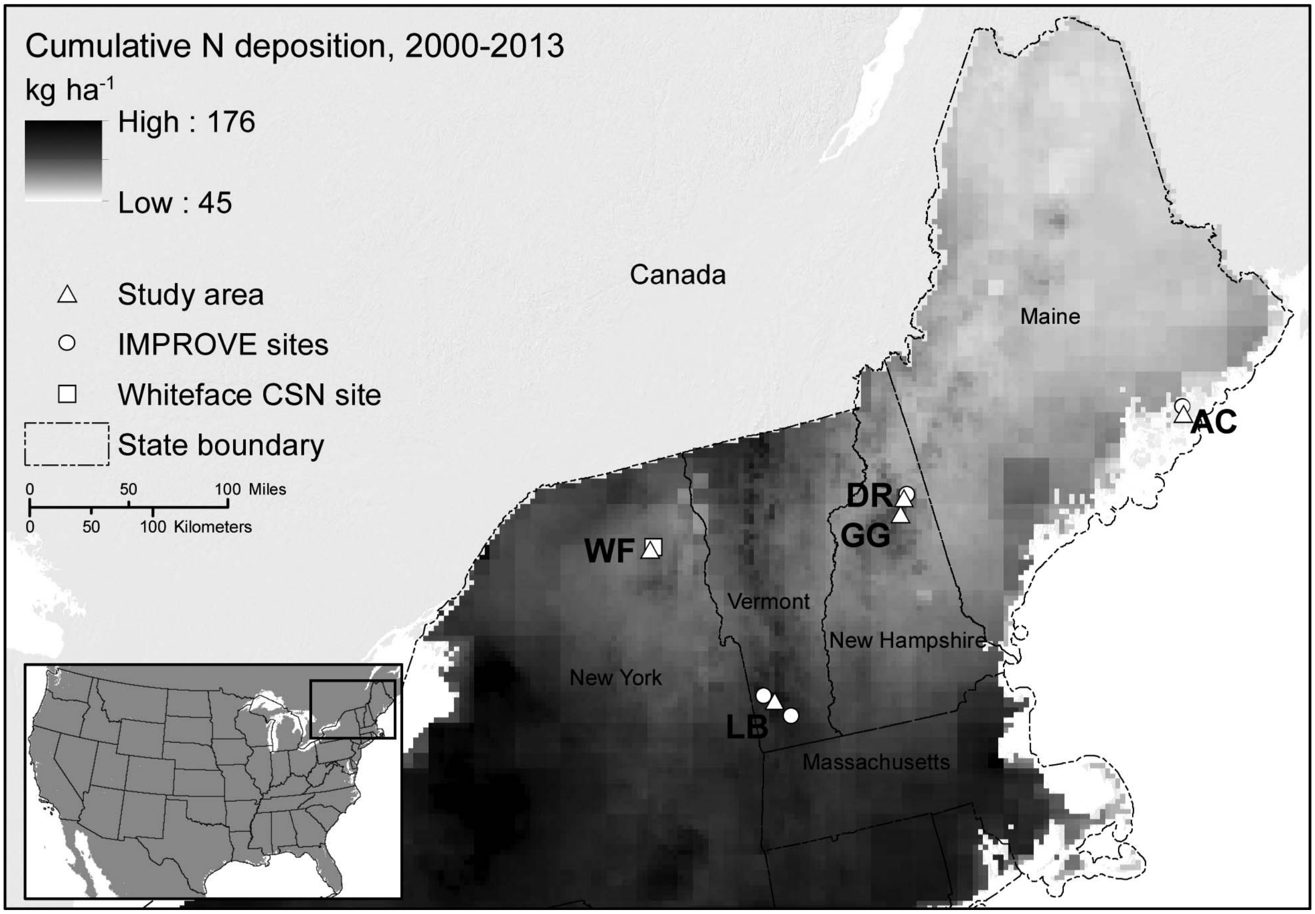

Figure 1. Map of northern New England and New York (NY) showing cumulative deposition (2000–2013) from TDEP for nitrogen at our study areas (four Class I areas): Lye Brook Wilderness (LB), Presidential Range-Dry River Wilderness (DR), Great Gulf Wilderness (GG) and Acadia National Park (AC). Locations of aerosol measuring stations including the station at Whiteface Mountain (WF) in the Adirondack Mountains of NY west of our main study area are also shown. Data sources: NADP (2014), US DOC (2014).

compounds, e.g., reduced N versus oxidized N, to epiphytic lichens remains unclear (Bobbink et al. 2010; Hauck 2010).

For New England states, Hinds & Hinds (2007) demonstrated differences in state-level macro-lichen flora that corresponded with known deposition patterns. Recently, Will-Wolf et al. (2015) used 2002 Community Multi-Scale Air Quality model (CMAQ) total deposition estimates to examine relationships between deposition and Forest Inventory and Analysis (FIA) Lichen Indicator data across New York (NY) and New England. In their data set, lichen composition responded to acidifying S and N inputs, but it proved difficult to separate effects of S from N (Will-Wolf et al. 2015). In our study, we used FIA lichen data and 2002 CMAQ deposition associated with lichen survey sites to develop response curves at the species level and air quality scores for the survey sites. However, our primary focus was evaluating the status of epiphytic macrolichens in four federally mandated Class I areas (areas where air quality will be monitored long-term): Lye Brook Wilderness (LB) in the southern Green Mountains of VT; Great Gulf Wilderness (GG) and the Presidential Range-Dry River Wilderness (DR) in the White Mountains of NH and Acadia National Park (AC) in coastal ME. Deposition in these areas is representative of the regional range for non-urban forests (**Fig. 1**).

A number of studies from the U.S. have recommended specific values that relate N deposition to effects on epiphytic lichen communities. For northern forests, Pardo et al. (2011) recommended a lichen critical load for N of 4–6 kg ha^{-1} yr^{-1}. Concentrations of ammonium nitrates and sulfates in ambient air fine-particulates have correlated strongly with lichen N content and species composition shifts in the western U.S. (Geiser et al. 2010; Root et al.

2015). These researchers also recommended use of IMPROVE aerosol data as a screening tool for exceedance of N critical levels in Class I areas with an annual critical level from 0.37 to 0.51 μg N m^{-3} (Geiser et al. 2010; Root et al. 2015). Healthy lichen communities from "clean" sites in the northwest U.S. corresponded with N concentrations in the lichen *Platismatia glauca* in the range of 0.55 to 0.80% N by dry weight and this range was suggested as a background reference for N effects (Dillman et al. 2007; Glavich & Geiser 2008). However, none of these values have been empirically evaluated for the northeastern U.S., a region lacking in 'background' sites (sites not been impacted by air pollution).

Unlike the situation in the western U.S., in the Northeast, nutrient N deposition effects have been compounded and perhaps overshadowed by a legacy of acidic deposition, primarily from sulfuric and nitric acids. In the Northeast, deposition decreases northeasterly from Vermont (VT) through New Hampshire (NH) to Maine (ME) (NADP 2014a,b). Recent improvements to the CMAQ total deposition estimates employ a "hybrid" approach that combines CMAQ output with measured wet and dry deposition, and accounts for topography (Schwede & Lear 2014). Difficult to model variables, such as cloud water deposition at coastal and high elevation plots and forest structure interactions, can significantly influence local deposition to forest environments (Cleavitt et al. 2011; Templer et al. 2015; Weathers et al. 2006), and make accurate modeling of deposition less certain. Epiphytic macrolichens obtain S and N, important and internally mobile macronutrients, from the air and precipitation in dynamic equilibrium with deposition (Boonpragob et al. 1989; Boonpragob & Nash 1990). Nitrogen concentrations in lichens can be used to estimate current levels of total N deposition (Root et al. 2013), making lichen elemental analysis a valuable ground check for modeling data. Lichen response to pollution exposure is a multi-stage process with cumulative effects (Johansson et al. 2010). As pollution increases in toxicity, lichens undergo physiological stress that subsequently affects growth and can cause necrosis of the thallus (Arhoun et al. 2000). Therefore, detailed scoring of thallus condition for all species may be a valuable indicator of pollution stress and help to identify sensitive species.

Species differ in their sensitivity to pollution and therefore pollution tends to homogenize and depauperate the lichen flora characterized by the progressive loss of species that are sensitive and intermediate in tolerance (Cleavitt et al. 2011; Liška & Herben 2008). Another way of categorizing the differential sensitivity of lichen species is through functional groups. Functional groups have proved useful in predicting pollution effects on lichens (e.g., Hawksworth & Rose 1970; Nash & Gries 2002). In particular, cyanobacteria-containing species have been found to be a very sensitive group of lichens in other ecological studies (Hawksworth & Rose 1970; Hinds & Hinds 2007; Kobylinski & Fredeen 2014; Richardson & Cameron 2004).

To assess lichen community status and health in mature forest stands of four Class I areas of the northeastern U.S., we related a combination of lichen metrics (i.e., species composition, species richness including functional groups richness, thallus condition, lichen sensitivity indices, lichen elemental analysis) to estimates of S and N deposition. We aimed to: 1) describe the deposition environment of our study plots using hybrid modeled deposition data and concentrations of N and S in lichen thalli; 2) explore relationships between lichen metrics and aspects of the deposition environment, particularly a) the strength and pattern of relationships to current versus cumulative deposition and b) relative fit of lichen metrics to different forms of N deposition; 3) empirically evaluate published critical values for lichen responses to N deposition in the U.S. (note that critical values are not published for S in this region); and 4) enhance protocols for relating lichen metrics to air quality with two novel metrics, thallus condition scores and deposition sensitivity indices.

We anticipated that lichen metrics would show better health of lichen communities from west to east following the regional deposition gradient. However, if lichen communities are recovering in response to the recent declines in deposition, then the lichen communities should correspond best to present day deposition and also be more similar to one another. In contrast, if lichen communities remain primarily affected by the historic deposition environment, then we anticipate a stronger relationship to cumulative deposition and larger differences in community composition between areas indicative of a lag in recovery.

Materials and Methods

Study areas and sampling plots. Approximate locations, study area boundaries, and deposition are

Table 1. Four Class I Areas with plot variables included in the second matrix for species composition ordination including annual precipitation (ppt) derived from PRISM-enhanced NADP data, elevation and forest structure. The broadleaf percent was calculated as: (number of trees that were broadleaf/total number of trees assessed) *100. Broadleaf tree species are abbreviated by the first two letters of the genus and species[1].

Plot label	Year of visit	Annual ppt (cm)	Elevation (m)	Broadleaf (%)	Basal area (m^2 ha)	Visual gap (%)	Main broadleaf species
Maine, Acadia National Park (AC)							
AC11	2005	133.9	287	8.6	32.1	5	BEAL
AC18	2005	133.9	307	0.5	48.2	15	SOAM
AC66	2005	145.9	262	14	38.8	12	ACRU, BEAL
AC67	2005	145.9	211	27	34.0	7	BEAL, ACRU
AC160	2005	140.4	104	31	28.9	11	ACRU
AC162	2005	140.4	148	27	29.8	12	ACRU
AC284	2005	145.8	359	27	23.0	25	ACPE
AC285	2005	145.8	338	21	32.1	10	BEAL, BEPA
ACD1	2006	145.9	198	62	23.8	20	FAGR, ACPE, ACSA
ACD2	2006	145.1	128	87	24.1	6	FAGR, ACPE
ACD3	2006	147.0	135	82	23.3	20	FAGR, ACPE, BEAL
ACD4	2006	145.9	96	51	27.2	15	ACRU, BEAL, ACPE
New Hampshire, WMNF, Presidential Range-Dry River Wilderness (DR)							
DR1	2012	149.2	925	4.0	14.3	5	BECO, ACRU
DR3	2012	186.9	959	39	34.0	5	BECO
DR5	2012	188.9	1135	36	39.6	12	BECO, SOAM
DR6	2012	164.0	1245	5.0	43.0	0	SOAM
New Hampshire, White Mountain National Forest, Great Gulf Wilderness (GG)							
GG1	2011	155.1	857	9.0	24.9	40	BECO
GG2	2013	155.1	785	86	31.1	5	BEAL, ACRU
GG3	2013	227.0	1138	17	45.2	20	BECO
GG4	2013	155.4	594	72	37.4	15	BEAL, ACRU
Vermont, Green Mountain National Forest, Lye Brook Wilderness (LB)							
LB1	2013	152.0	561	34	35.2	5	ACRU, FAGR
LB2	2013	168.2	852	35	21.0	60	ACRU. BEPA
LB3	2013	160.3	809	2.0	34.5	2	BEPA
LB4	2013	169.9	803	85	30.9	5	FAGR. BEAL, ACRU

[1] ACPE, *Acer pennsylvanicum*; ACSA, *A. saccharum*; ACRU, *A. rubrum*, BEAL, *Betula allegheniensis*; BECO, *B. cordifolia*; BEPA, *B. papyrifera*; FAGR, *Fagus grandifolia*; SOAM, *Sorbus americana*.

mapped in **Fig. 1** and descriptive information is provided in **Table 1**. The primary sampling focused on four Class I areas; areas afforded the highest level of air quality protection under the Clean Air Act. Within these four areas we sampled 24 forested plots: seven plots were dominated by broadleaf trees, nine by mixed broadleaf and conifers, and eight by conifers. All three forest types occurred in each area. At Acadia National Park, Maine (AC), 12 plots were sampled in 2005–2006 (Cleavitt et al. 2009) and eight of these were co-located with previous throughfall collectors (Weathers et al. 2006). At White Mountain National Forest, New Hampshire (WMNF; 8 plots) and Green Mountain National Forest, Vermont (GMNF; 4 plots), we revisited locations sampled by Wetmore (1995a,b), detailed in Dibble et al. (2015). The four GMNF plots were sampled in LB in 2013. There were eight WMNF plots with four plots each in two areas sampled from 2011–2013: Presidential Range-Dry River (DR) and Great Gulf Wilderness (GG). All plots were in mature to old-growth forests based on historical documentation.

We collected additional samples for elemental analysis of lichen thalli in 2009 at Acadia National Park, ME, Whiteface Mountain in the Adirondack Mountains of New York, and WMNF in NH (**Fig. 1**; see ***Lichen elemental analysis*** below). These additional lichen elemental data extend the gradient of deposition we could analyze for the relationship between lichen N content and aerosol N values, which is important to evaluating published critical loads and levels outlined in the Introduction.

Deposition data—plot level deposition of S and N. Sulfur and N deposition for the conterminous U.S. were estimated by combining Community Multi-Scale Air Quality (CMAQ) modeled estimates with ambient wet and dry deposition monitoring data and topography (Schwede & Lear 2014). Annual hybrid total deposition data, referred to as "TDEP", are available at 4 km^2 grid resolution for the years 2000 through 2013, with updates presumably continuing in the future. TDEP total, wet and dry S and N deposition, oxidized and reduced N, and precipitation (Parameter-elevation Relationships on Independent Slopes Model (PRISM), Daly et al. 1994, 2008) data were downloaded from the National Atmospheric Deposition website (NADP 2014a,b) as ESRI ArcGrid™ files. Data were associated with each of the 24 lichen plot locations using ESRI ArcMap™ version 10.2.1. Due to a deposition grid misalignment along the complex Maine coast (**Fig. 1**), several AC plots fell outside the grid, and were assigned nearest grid-cell values. Any associated error in deposition estimates was likely small as S and N deposition in this area were relatively low and exhibited minimal spatial variability. We represented the deposition environment using two year annual means (S_{ann}, N_{ann}) for the year prior to and year of the plot visit (following Jovan et al. 2012), and cumulative deposition (S_{cum}, N_{cum}), summed from all available years, 2000–2013.

Area level comparisons to IMPROVE and CSN aerosol data. The Interagency Monitoring of Protected Visual Environments (IMPROVE) network (Eldred et al. 1990; Hand et al. 2011) collects 24-hour aerosol samples of fine particulates (<2.5 μm) every third day to assess visibility at Class I areas. Relevant monitors were Acadia (ACAD1), Great Gulf (GRGU1) and Lye Brook (LYBR1 and LYEB1). The EPA Chemical Speciation Network (CSN) monitors fine particulates at urban and rural sites nationwide, including Whiteface Mountain, NY. IMPROVE and CSN samples are analyzed for sulfate and nitrate by ion chromatography.

Fine particulate sulfate and nitrate data from ACAD1, GRGU1, LYBR1 and LYBE1 and Whiteface Mountain for 2002–2013 (the period of common data availability) were obtained using the FED Data Query Wizard (http://views.cira.colostate.edu/fed/DataWizard/Default.aspx). Although IMPROVE does not measure reduced N (NH_4) directly, total fine particulate N can be estimated by assuming sulfate and nitrate are balanced by ammonium as $(NH_4)_2SO_4$ and NH_4NO_3, respectively. We calculated mean annual fine particulate concentrations of nitrate and total N (μg m^{-3}) for each site and compared them to mean thallus N concentrations (percent dry weight) in *Platismatia glauca* and *Evernia mesomorpha* as an average for each of four areas, detailed below under ***Lichen elemental analysis***.

Timed macrolichen surveys. Epiphytic macrolichens were surveyed in 0.38 ha circular plots following the U.S. Forest Service FIA lichen communities protocol (McCune et al. 1997; USDA 2004). We conducted two-hour timed surveys, searching for macrolichen species on live and dead tree boles >0.5 m from the ground and on recently fallen branches. We collected the main vouchers of each species with substrate notations and assigned abundance ratings from the number of detections (ocular logarithmic scale of 1–4). Lichen nomenclature followed Esslinger (2012) and Hinds & Hinds (2007). All lichen collections were collected in the field by Cleavitt and Dibble and verified in the laboratory by Jim Hinds. Additional plot measurements included UTM location, elevation, slope, aspect, percent gap, and basal area and percent density of broadleaf and conifer trees (**Table 1**; Cleavitt et al. 2009; Dibble et al. 2015). Lichen diversity on the plots was summarized as lichen richness (number of macrolichen species per plot) and as the number of species in each functional group.

Functional groups. For our lichen flora, we differentiated four groups, cyanobacteria containing lichens (including tri-partite lichens), fruticose chlorolichens lichens, foliose chlorolichens and *Cladonia* species (squamulose). These groups follow well-recognized lichen growth forms with additional separation of cyanobacteria containing species from other foliose species, which has been found useful in a range of ecological studies (Hawksworth & Rose 1970; Hinds & Hinds 2007; Kobylinski & Fredeen 2014; Richardson & Cameron 2004). Lichen functional groups used here related in part to growth form, but also ecological differences: fruticose, foliose, cyanolichens (defined broadly as any species containing cyanobacteria in the thalli even secondarily) and "cladonia" (all squamulose *Cladonia* species) (**Supplementary Table S1**).

Sensitivity indices. We prepared S and N deposition response curves for 85 species using lichen data from U.S. Forest Service FIA lichen communities protocol sites (accessed Sept 2013 at http://gis.nacse.org/lichenair) across the eastern U.S.

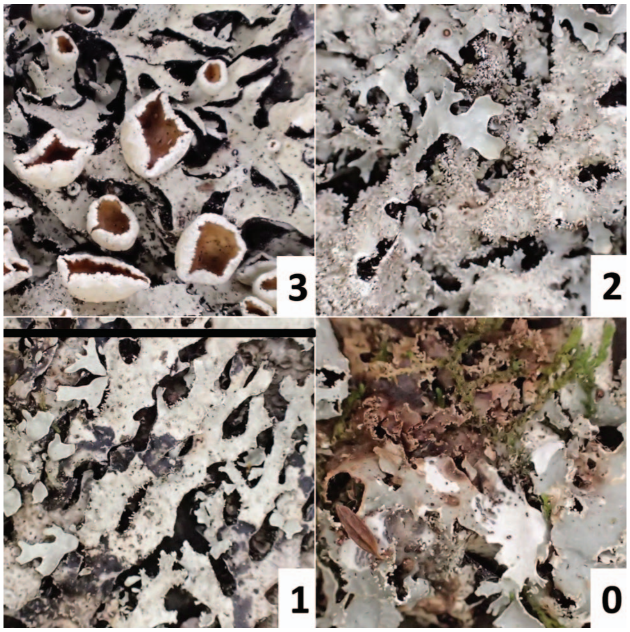

Figure 2. Differences in thallus condition of the foliose lichen species, *Parmelia squarrosa*. The specimens are labeled **0–3** corresponding to our thallus scoring system. Thallus condition scores: **0**) poor, **1**) fair, **2**) good and **3**) robust. See Methods for more details of scoring. Scale bar in **1** is 15 mm and is the same for all images. Photographs were taken by Patricia Hinds on specimens scored by J. W. Hinds.

(Eastern Temperate Forest and Northern Forest Level 1 EPA Ecoregions), which includes 1,956 plot surveys independent of the work done here. For each species present in our study, we extracted CMAQ modeled S and N deposition estimates from 2002 (CMAQv5.0.1; the most recent coverage available to us for these sites) for every detection site in a 1992–2012 collection window and calculated the median deposition. We rated the lichen species by the deposition of S and of N at the median quantile for the distribution of detections along each pollution gradient: 'sensitive' (<5 kg ha^{-1} yr^{-1}), 'intermediate' (5–10 kg ha^{-1} yr^{-1}), or 'tolerant' (>10 kg ha^{-1} yr^{-1}) (**Supplementary Table S1**). For each of our 24 plots, N and S community index scores were calculated as: $100^{*}\ (n_{sensitive} - n_{tolerant})/n_{total}$, where n = the number of species for the plot.

Lichen thallus condition scores. We scored the condition of 510 specimens vouchered during the macrolichen surveys on a four point scale: 0 = very poor (thallus surface with extensive damage: convoluted lobes, bleaching, black speckles, pink blotchy areas, or other discoloration and/or damage); 1 = poor (damage less extensive); 2 = good (within the normal range for the species); and 3 = robust (larger thallus often having reproductive structures; **Fig. 2**). One thallus per voucher was scored and only the main collection for each species on the plot was scored. All scoring was done by J. W. Hinds to minimize observer error. Thallus condition was most variable within DR, GG and LB plots. In these areas, we compared thallus condition of common (defined as present in all three areas in at least two plots per area) and uncommon species (less frequent than as defined for common) to determine whether uncommon species were in worse condition.

Lichen elemental analysis. Samples (20 g) of common species *Hypogymnia physodes*, *Evernia mesomorpha* and *Platismatia glauca* were collected for elemental analysis in August of all years (2009, 2011–2013) following Geiser (2004). In 2009, samples were collected at six locations in AC (two overlapping with lichen survey sites: AC11 and AC18). All collections sites were co-located with previous deposition measurements (McNulty et al. 1990). Samples from GG, DR and LB were collected in 2011–2013 at lichen survey sites (**Table 1**). Nitrile gloves were worn during collection to reduce contamination, and air-dried specimens were cleaned of debris and non-target lichens with a time-limit of two hours per sample, and shipped to the University of Minnesota Research Analytical Laboratory, St. Paul, MN for total N (LECO FP-528 total N analyzer, LECO Corp., St. Joseph, MI) and total S (LECO SC-132 S analyzer) analysis.

Between means comparisons. We made several comparisons of deposition across areas using standard ANOVA. For both lichen element concentrations and lichen thallus condition, Welch's test allowing unequal variances insured that differences in sample size between groups did not affect the ANOVA outcome. Although lichen element profiles can differ by species (Bennett & Wetmore 1997), because not all species were collected at all plots, we grouped species by plot after assessing that there were no species by plot interactions. Significant ANOVAs were followed by Tukey's Honest Significant Difference (HSD) between-pair comparison to differentiate significant subsets ($\alpha = 0.05$).

Correlation and regression analyses. Correlations between variables were explored for relationships of interest and to determine co-linearity of independent variables. To detect changes in deposition over time (2000–2013), we compared linear regression slopes for the four areas using ANOVA and Tukey's HSD post hoc test. Differences in reduction of S versus

N deposition were quantified by regression slopes and percent reduction values, which were examined by paired t-tests. All regressions and ANOVAs were run using JMP Pro 10 (SAS Institute, Cary, NC).

In order to compare the pattern of lichen metric responses to deposition values, linear and nonlinear regression models were compared using three metrics of fit: Akaike Information Criterion (AIC), root mean square error (RMSE), and the R^2. First, curve fits for linear, logistic and exponential curves were compared by AIC. The model with the lowest AIC was regarded as the best model describing the data for that independent-dependent variable pair (Motulsky & Christopoulos 2003). For deposition types with the same pattern of fit for the same dependent metric, a difference in RMSE of greater than 10% was regarded as significantly different.

Because the relationships for S_{ann} and lichen metrics were reversed from all other deposition and lichen metric comparisons, we realized: 1) the need to correct for sea sulfate contribution to wet sulfate inputs at coastal AC plots compared with all other inland plots, and 2) the much more biologically meaningful and statistically better fit relationship between dry S deposition rather than total S or wet S. To address the first point, we corrected for wet sea salt sulfate deposition (estimated as Na^+ divided by 4) from the Acadia NADP site (ME98) for the 2004–2006 years. By this calculation, 15% of AC wet S_{ann} was from sea salt; therefore, S_{ann} values at AC were adjusted to remove this sea salt influence. Similarly for S_{cum}, AC wet S was corrected for 18.12% sea sulfate contribution to total wet sulfate. For the second point, we analyzed relationships for both total and dry S throughout, but show only those data for variables that yield reasonable fits to the data.

Ordination. Species and plot data were analyzed using Non-metric Multidimensional Scaling (NMS) in PC-ORD ver. 6.0 (MJM Software, Gleneden Beach, OR). NMS is a non-parametric ordination method that uses an iterative search for rankings and placement of the analyzed variables to find a minimal stress solution (McCune & Mefford 1999). NMS is particularly suitable for ecological data where many species do not occur at most survey sites (McCune & Mefford 1999). All NMS analyses were run using Sorensen's distance measure with 100 runs using real data and 50 runs of randomized data. The instability criterion was 0.00001 (the probability that a similar final stress could have been found by chance) with 500 as the maximum number of iterations. The final solution was chosen based on the dimensionality with the lowest mean stress from a run comparing randomized to real data (McCune & Grace 2002; McCune & Mefford 1999).

The data set included 24 plots and abundance scores of the 76 macrolichens occurring in 2 or more plots. This deletion of only the rarest species is quite conservative yet helped to minimize 'noise' that can weaken real ecological gradients present in the data (McCune & Grace 2002). Variables in the secondary matrix were elevation, relative abundance of broadleaf and conifer trees, annual precipitation, tree basal area, visual percent gap, total species richness, fruticose and cyanolichen species richness, S and N sensitivity index scores, cumulative and annual deposition values for total S and N. Secondary matrix variables displayed in the joint bi-plot had correlation coefficients of $r^2 \geq 0.405$ ($p = 0.05$ for $N = 24$) to at least one ordination axis.

Supplemental analysis of ordination scores for the individual species was examined by several species groupings of interest: indicator classes for S and N deposition and functional group (**Supplementary Table S1**). This approach is similar to flipping the ordination to be in species rather than plot space, but further allowed the differences in ordination scores between species groupings to be tested and displayed using one-way ANOVA followed by Tukey HSD for post-hoc subsets.

Results

Plot level deposition of S and N. Cumulative S and N deposition increased from AC<GG=DR<LB, with N_{cum} deposition ratios of 1:2:3 between areas, respectively (**Fig. 3A**). Annual total S did not exhibit large variability across the areas, due in part to the fact that the relatively cleaner AC plots were sampled in earlier, higher deposition years (2005–06) than plots in the other areas (2011–13). Most of this S was in the form of wet deposition and AC remained the lowest site for dry deposition of S_{ann} (**Fig. 3B**), despite the earlier sampling period. Annual total, oxidized, and reduced N followed a similar pattern across sites; oxidized N was always greater than reduced N (**Fig. 3C**). The only area where N_{cum} deposition was lower than S_{cum} deposition was AC (**Fig. 3A**). N_{cum} and N_{ann} were tightly correlated ($r^2 = 0.90$; $F_{1,\,22} = 199.27$; $p < 0.0001$); whereas S_{cum} and S_{ann} were uncorrelated. Wet deposition dominated all areas; S and N wet:dry ratios ranged from 1.25:1 at LB to 3:1 at AC because of larger contribution of dry deposition at LB. AC was the only area below the recommended lichen critical load of 4–6 kg N ha^{-1} yr^{-1} (Pardo et al. 2011; **Fig. 3C**). Reduced N deposition was three times lower at AC than the other three areas at the time of plot visits (**Fig. 3C**).

Total annual deposition of S and N decreased from 2000-2013, but S decreases were greatest at LB (**Fig. 4A & B**; comparison of slopes: $F_{3,12} = 97.83$ (S); 116.7 (N); $p<0.0001$; post-hoc LB significantly greater). LB annual deposition of S and N was at least twice as high as other areas in 2000 (**Fig. 4A & B**). Sulfur deposition decreased more than N deposition at all areas (**Fig. 4A & B**; paired t-test of slopes: df = 16, t = 13.91, $p < 0.0001$). However, the amount of relative decrease in S and N differed between areas. At AC, S decreased 2.5–2.75 times as much as N, while decreases were closer to equivalent at the other areas (**Fig. 4A & B**). There was a strong correlation between S_{cum} and N_{cum} ($r^2 = 0.91$; $F_{1,22} = 218.52$; $p < 0.0001$), although this relationship weakens with annual means ($r^2 = 0.32$; $F_{1,22} = 10.17$; $p = 0.0042$). The ratio of oxidized to reduced N forms also decreased significantly over the 14 years at all plots, exceeding 2:1 in the early 2000's (2.83:1 in 2001), but tending toward 1:1 relationship (1.34:1 in both 2012 and 2013) in more recent years, consistent with better controls on NO_x emissions sources.

Aerosol concentration and deposition. At our northeastern areas, the upper end of the range of annual critical levels identified at western sites (0.51 µg N m^{-3}; Geiser et al. 2010) was exceeded (**Fig. 4C**). Recent aerosol N concentrations have fallen below 0.51 µg N m^{-3} at all sites. The most recent 2013 data have fine particle N concentrations of 0.41, 0.47, 0.29 and 0.31 µg N m^{-3} at the Whiteface, LB, GG and AC areas, respectively (**Fig. 4C**).

Species richness and functional groups. We documented 94 species of macrolichens across the 24 plots (**Supplementary Table S1**); species richness was significantly higher at AC (35.1 ± 0.96 species/plot) than other areas (**Fig. 5**). Because AC plots are much closer to the ocean than plots in the other areas, the question of oceanic species contributing to higher lichen diversity at AC plots must be addressed. We regarded the following eight species as having oceanic distributions in our study area: *Everniastrum catawbiense*, *Hypotrachyna afrorevo-*

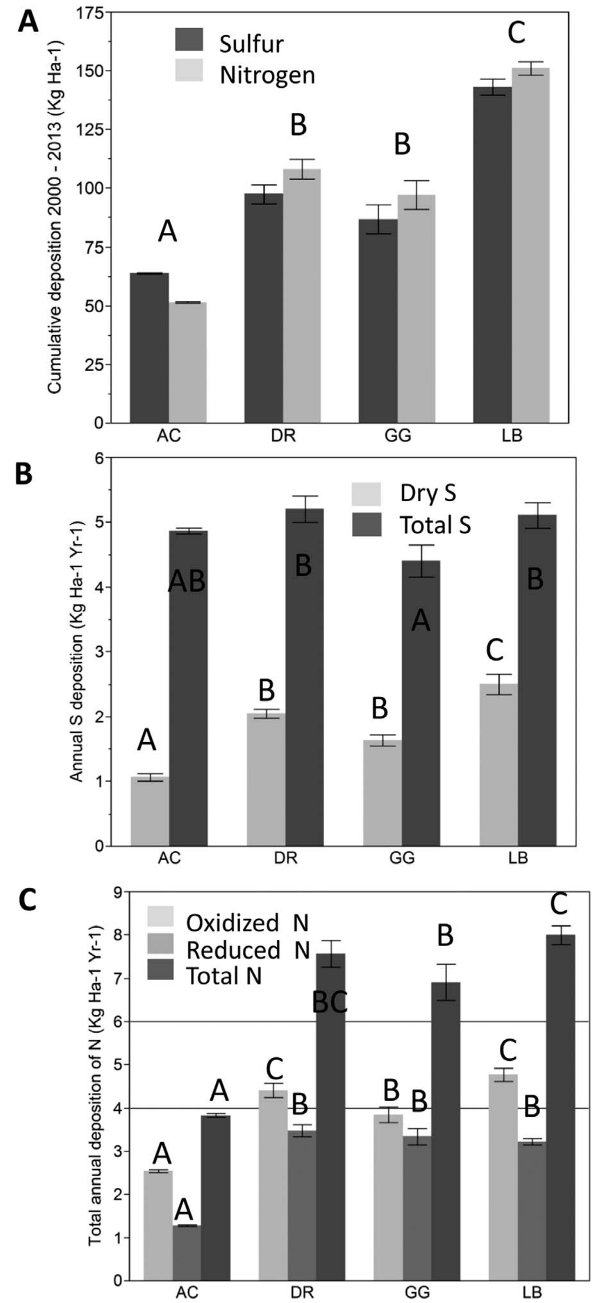

Figure 3. Modeled TDEP values for 24 plots averaged by Area: Acadia National Park (AC), Presidential Range-Dry River Wilderness (DR), Great Gulf Wilderness (GG) and Lye Brook Wilderness (LB). Bars represent mean values and the error bars represent ± 1SE. Bars with different letters are significantly different at $\alpha = 0.05$ by Tukey's HSD. **A.** Cumulative deposition for 2000–2013 for S and N deposition. **B.** Annual total deposition of S as a bi-annual mean including the year prior to and year of plot visits. Wet and total S deposition are compared across the four main study areas. **C.** Annual total deposition of N as a bi-annual mean including the year prior to and year of plot visits. Total N deposition is partitioned into oxidized and reduced forms of N. The reference lines at 4 and 6 kg ha^{-1} yr^{-1} correspond to recommended critical limits for total N (Pardo et al. 2011). For all graphs, bars represent the mean (1SE) and bars of the same color with different letters denote significantly different means by Tukey HSD (α – 0.05).

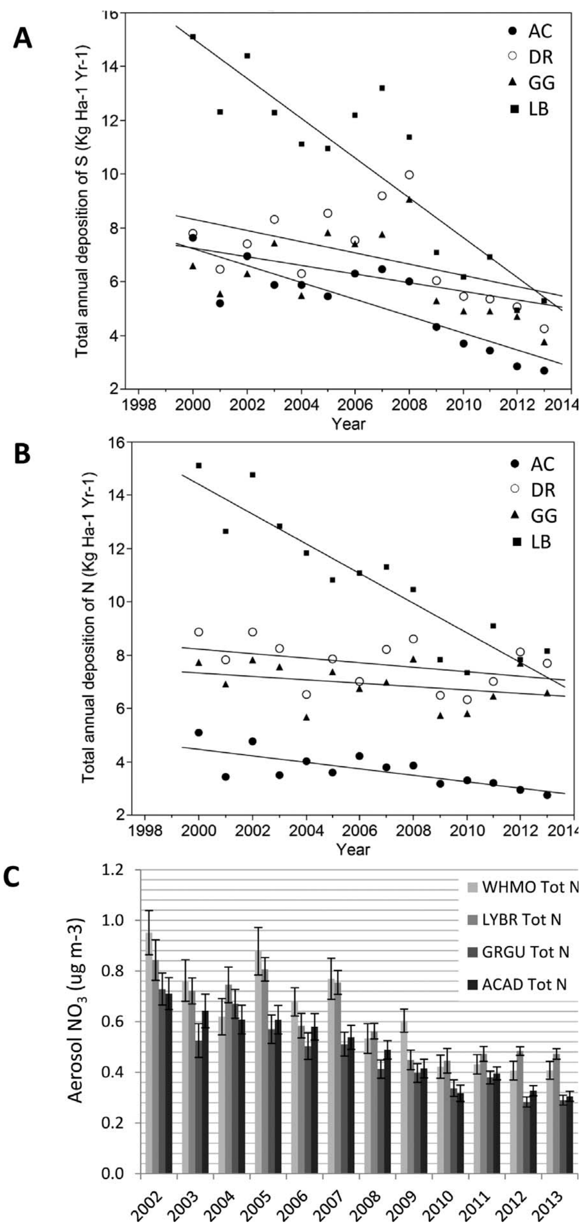

Figure 4. Changes in deposition over time. **A.** The decrease in total annual deposition of S from modeled TDEP data in four Class I areas, Lye Brook Wilderness (LB), Great Gulf Wilderness (GG), Presidential Range-Dry River Wilderness (DR), and Acadia National Park (AC). Points represent the mean values of the study plots within the four areas. **B.** The change in total annual deposition of N between 2000 and 2013 at the four main study areas. Points represent the mean values of the study plots within the four areas. **C.** Decrease in total inferred N inputs for aerosol particulates ($PM_{2.5}$) at four monitoring stations (3 IMPROVE, 1 CSN, detailed in Methods) in proximity to plots where lichen were collected for elemental analysis: Whiteface Mountain, NY (station WHMO), Lye Brook Wilderness, VT (station LYBR), Great Gulf Wilderness, NH (station GRGU) and Acadia National Park, ME (station ACAD). Bars represent mean values and the error bars represent $\pm$ 1SE.

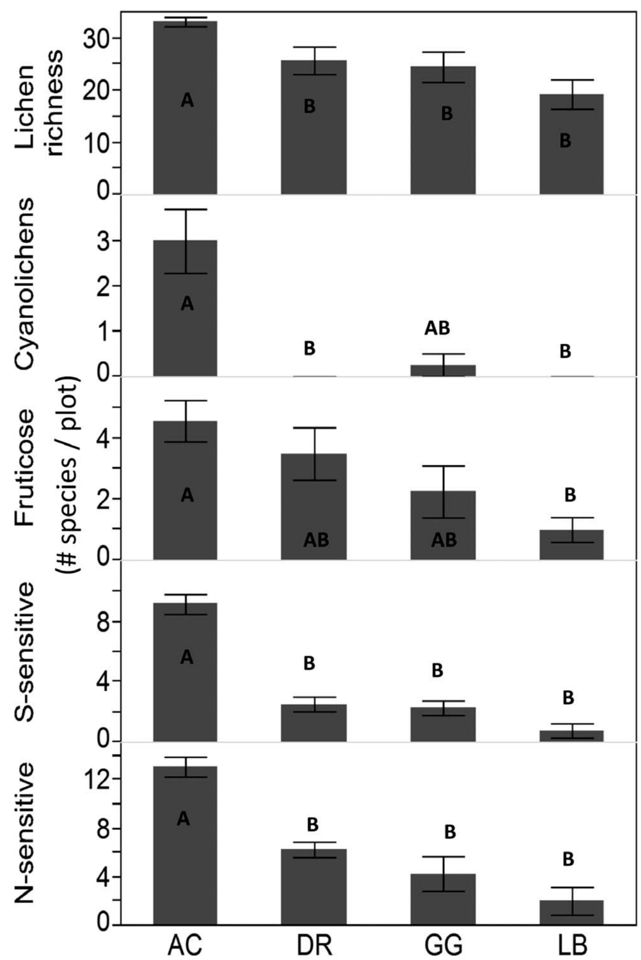

Figure 5. Lichen diversity partitioning for 24 plots in four Class I areas of the northeastern U.S.: Acadia National Park (AC), Presidential Range-Dry River Wilderness (DR), Great Gulf Wilderness (GG) and Lye Brook Wilderness (LB). From top to bottom: number per plot of cyanolichens, fruticose, N-sensitive, S-sensitive and total species per area. For all graphs, bars represent the mean (1SE) for plots within areas and bars with different letters denote significantly different means by Tukey HSD (α – 0.05).

luta, *H. revoluta*, *Nephroma laevigatum*, *Parmotrema perlatum*, *Usnea cornuta*, *U. flavocardia* and *U. subrubicunda*. Therefore, we subtracted the occurrence of these species from plot species richness for AC and reanalyzed area differences, which remained significant with AC plots (33.2 $\pm$ 0.90 species/plot), significantly richer than the other three areas.

Only eight species of cyanobacteria-containing lichens were found and only one of these (*Lobaria pulmonaria* at GG1) occurred outside of AC, resulting in significantly higher diversity of cyanolichens at AC (**Fig. 5**). There were 17 species of fruticose lichens, 16 species in the genus *Cladonia* and 53 foliose lichens. Both cyanolichens ($F_{3,20}$ = 5.11; p = 0.0087) and fruticose lichens ($F_{3,20}$ = 3.73; p = 0.028) were

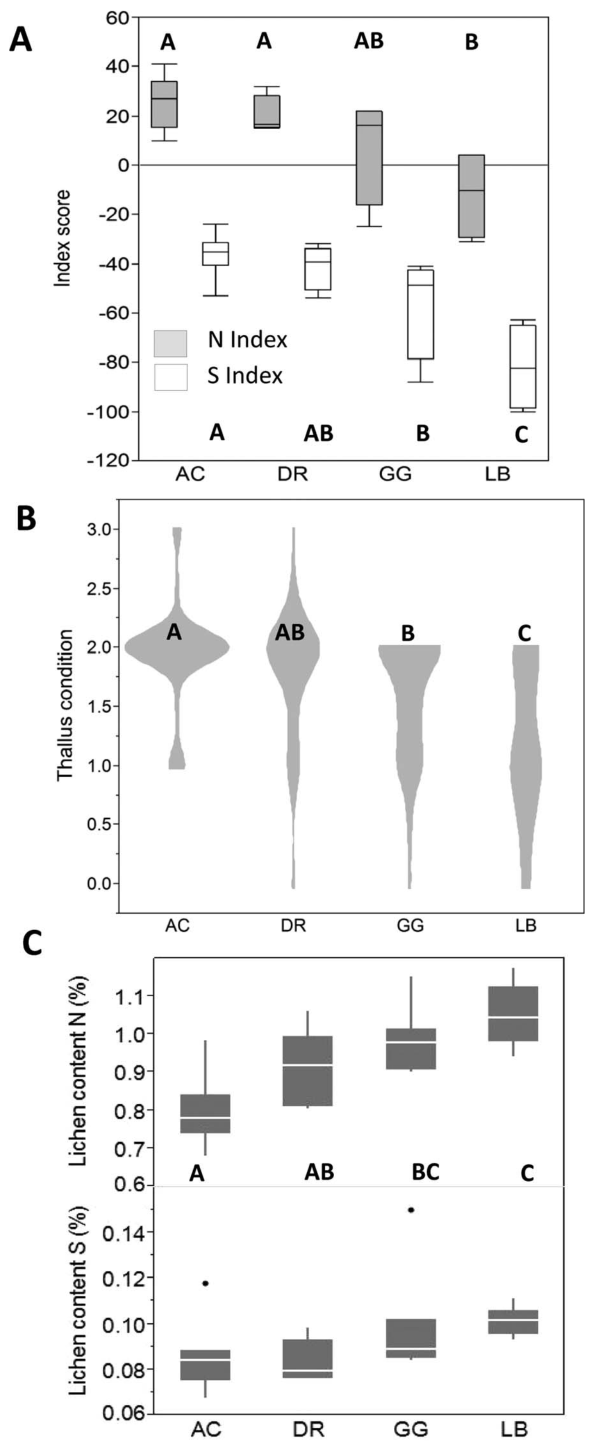

Figure 6. Lichen metrics for 24 plots in four Class I areas of the northeastern U.S.: Acadia National Park (AC), Presidential Range-Dry River Wilderness (DR), Great Gulf Wilderness (GG) and Lye Brook Wilderness (LB). **A.** Box plots of N and S Indices based on sensitivity ratings for lichens collected in the four areas. **B.** Contour maps of thallus condition depicting the change in the distribution of thallus condition. The condition scores are: 0) poor, 1) fair, 2) good and 3) robust. Refer to Methods and Fig. 2 for further details on thallus condition scoring. **C.** Box plots of percent N and S content in lichen thalli collected in the four areas. Letters denote significantly different means by Tukey HSD ($\alpha = 0.05$).

Table 2. Lichen tissue percent nitrogen content compared across four areas of the northeastern U.S., Whiteface Mountain in the Adirondacks, NY (ADKS), Lye Brook Wilderness, Green Mountains, VT (GMNF), the Presidential Range-Dry River Wilderness, Great Gulf Wilderness and Crawford Path in the White Mountains, NH (WMNF) and several stands in Acadia National Park (ACAD). Note that only GMNF is equivalent to LB. All other areas have non-lichen plot locations included as detailed in the Methods.

Lichen species	Area	Number of samples	N content (%) Mean (1SE)
Evernia mesomorpha	ADKS	4	1.217 (0.150)
	GMNF	4	1.054 (0.032)
	WMNF	7	0.890 (0.042)
Platismatia glauca	ADKS	5	1.024 (0.082)
	GMNF	2	0.977 (0.021)
	WMNF	9	0.813 (0.030)
	ACAD	7	0.798 (0.037)

significantly less common outside of AC (**Fig. 5**). Refer to Cleavitt et al. (2009) and Dibble et al. (2015) for plot species lists and rare species information.

The number of sensitive and tolerant species detected at the sites were strongly correlated ($p < 0.0001$) to total species richness in all cases [N sensitive ($r = 0.931$); S sensitive ($r = 0.886$); S tolerant ($r = 0.773$)] except species tolerant of high N deposition ($r = 0.313$; $p > 0.05$). Nitrogen-tolerant (13 species) and S-sensitive (20 species) lichens were the least frequent classifications. The richness of both S- and N-sensitive species was significantly higher at AC (**Fig. 5; Supplementary Table S1**).

Lichen indices for S and N. Mean N Index scores were significantly lower (less sensitive and more tolerant species) at LB compared to AC and DR plots (**Fig. 6A**). Differences in S Index scores across areas were larger than differences in N Index scores. S Index scores were higher (more sensitive and less tolerant species) at AC than GG and LB. At LB, S Index scores were lower and N index scores were much lower than all other areas (**Fig. 6A**).

Thallus condition scores. Thallus condition declined from AC>DR>GG>LB; ($F_{3,20} = 15.49$; $p < 0.0001$; **Fig. 6B**). Overall, the contour maps show that most lichen thalli at AC plots were in good condition with a few robust and poor specimens (**Fig. 6B**). The DR "hour glass" narrows indicating some specimens fall farther below good condition. In contrast, no GG and LB specimens were robust (no scores above 2) and most thalli were in poor condition (**Fig. 6B**).

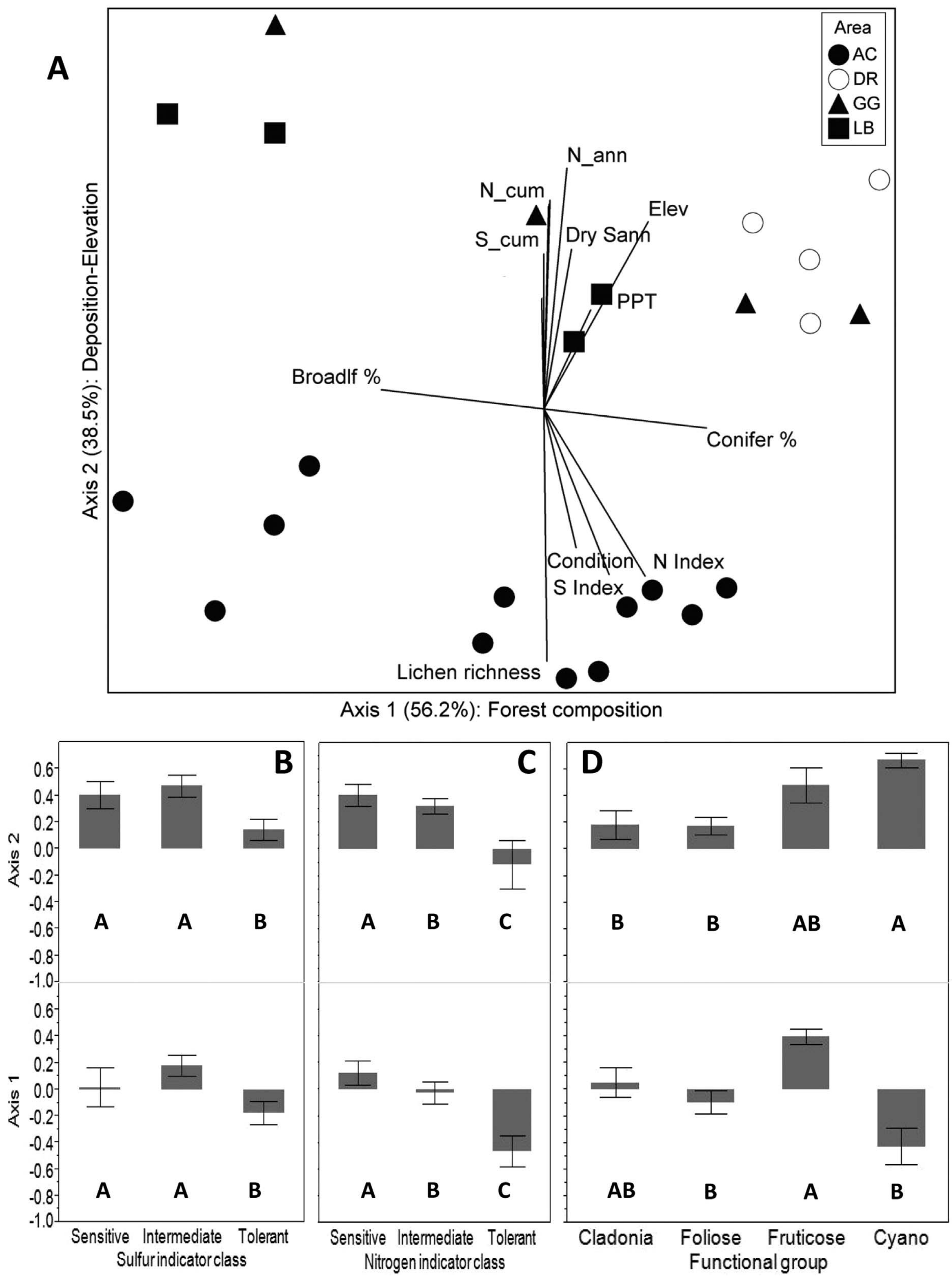

Figure 7. A. NMS ordination of 24 plots in four Class I areas by lichen composition with joint bi-plot of variables with 0.30 or higher r^2 to one of the ordination axes. Plots are coded by study area. Bi-plot vector variable abbreviations are: elevation (Elev), S cumulative deposition 2000–2013 (Scum), annual dry S deposition (Dry Sann), annual total N deposition (Nann), N cumulative deposition 2000–2013 (Ncum), percent broadleaf density (Broadlf%), percent conifer density (Conifer%), and thallus condition score (Condition). **B.** Relationships of S indicator species to the ordination axes by

To assess deposition patterns associated with poor thallus condition, comparisons by lichen frequency groups (common, uncommon) were made across the three areas with low thallus condition scores (DR, GG and LB). For the uncommon species, thallus condition was significantly better at DR than at GG and LB ($F_{2,69} = 14.05$; $p < 0.0001$). Of the 14 common species (occurring in at least two plots per area), those from LB had significantly lower scores than those from DR and GG ($F_{2,94} = 11.36$; $p < 0.0001$).

Lichen elemental analysis. Consistent with deposition, S and N concentrations in lichen thalli increased from AC<DR<GG<LB (**Fig. 6C**). However, these differences were not significant for S ($F_{3,26} = 2.89$; $p > 0.05$) and there was some overlap for N ($F_{3,26} = 11.62$; $p < 0.0001$; post hoc subsets; **Fig. 6C**). Nitrogen concentrations in *Platismatia* were above the background range (0.55–0.80%) associated with healthy lichen communities (**Table 2**; Dillman et al. 2007; Glavich & Geiser 2008) in all areas except AC. Like deposition, S and N concentrations in lichen thalli were strongly correlated to each other ($r^2 = 0.838$; $F_{1,22} = 108.62$; $p < 0.0001$).

Community composition. Lichen species compositions differed greatly across plots and the ordination captured 94.7% of the variation in the data set (final stress for two axes: 7.712; final instability < 0.00001). Axis 1, related to forest composition (% broadleaf; $r^2 = 0.507$, the highest correlation to this Axis), accounted for 56.2% of the variation in species composition (**Table 3**; **Fig. 7A**). Axis 2 explained an additional 38.5% of the variation and was highly correlated to deposition, lichen richness, both lichen sensitivity indices and to a lesser extent elevation (**Table 3**; **Fig. 7A**).

Species ordination scores and S- and N-sensitive species richness varied along both axes: Axis 1 (forest composition; S: $F_{2,373} = 30.02$; $p < 0.0001$; N: $F_{2,373} = 33.35$; $p < 0.0001$) and Axis 2 (deposition-elevation; S: $F_{2,373} = 11.83$; $p < 0.0001$; N: $F_{2,373} = 23.78$; $p < 0.0001$; **Fig. 7B & C**). Both S- and N-sensitive species richness was greatest at lowest deposition sites (**Fig. 7B & C**). Species of intermediate tolerance were closer in ordination space to the sensitive than to the tolerant species along Axis 2 (**Fig. 7B & C**; subsets). For S indicator groups, species did not separate along Axis 1 (forest composition); however, for N indicator groups, N-tolerant species were more likely to be found on plots with a higher percentage of broadleaf trees (**Fig. 7A & C**).

Lichen functional groups separated strongly on both axes (Axis 1: $F_{3,67} = 5.98$; $p = 0.0011$; Axis 2: $F_{3,67} = 4.41$; $p = 0.0068$; **Fig. 7D**). Cyanolichen species were the most restricted functional group on the deposition axis (Axis 2: more positive scores indicate occurrence on plots with lowest deposition). Association of fruticose lichens for conifer-dominated plots, and cyanolichens for broadleaf-dominated plots was apparent along Axis 1 (Axis 1 scores **Fig. 7D**: positive scores indicate conifer occurrence, while negative scores indicate broadleaf occurrence).

Curve fitting of lichen metrics to deposition values. Lichen species richness, thallus condition, the number of sensitive species, and S and N Index scores were compared for fit to deposition estimates using linear, exponential and logistic curves (**Table 4**). Metrics that were based on the number of species per plot (total species richness, sensitive species richness) were typically best fit to negative exponential relationships (**Table 4**). One exception was the relationship between dry S deposition estimates and S-sensitive species, which had a sharper decline as deposition increased and was described best by a logistic function (**Table 4**). Metrics that were unitless (thallus condition and sensitivity indices) were best described by negative linear relationships (**Table 4**). In general, dry S_{cum} and oxidized N_{cum} deposition were very good predictors of patterns in lichen metrics (**Table 5**). Lichen richness fitted more closely to the N deposition estimates than to S estimates (**Table 5**). Thallus condition related equally well to S and N, but significantly better to cumulative versus annual deposition estimates (**Table 5**). Dry S_{cum} was a better fit to both S-sensitive species and S-Index. For the N deposition estimates, model differentiation was less certain (all Δ RMSE < 10%; **Table 5**).

←
mean score (± 1SE). **C.** Relationships of N indicator species to the ordination axes by mean score (± 1SE). For both B and C, sensitive species had median deposition at <5 kg ha^{-1} yr^{-1}, intermediate species at 5–10 kg ha^{-1} yr^{-1}, and tolerant species at >10 kg ha^{-1} yr^{-1} (**Supplementary Table S1**). **D.** Relationship of lichen functional groups to the ordination axes by mean ordination score of all species in that group (± 1SE). For all graphs, bars with different letters are significantly different at $\alpha = 0.05$ by Tukey's HSD.

Table 3. Correlation of bi-plot variables to first and second ordination axes derived by non-metric multi-dimensional scaling (NMS). Plot N = 24; significance of the Pearson's correlation coefficients are given as: *p < 0.05; **p < 0.01; ***p < 0.001; and ****p < 0.0001.

Variable	Axis 1	Axis 2
Environmental variables:		
Elevation	0.327	0.584**
Conifer %	0.507*	0.061
Broadlf %	0.507*	0.061
N_{ann}	0.072	0.752****
S_{ann}	0.012	0.538**
N_{cum}	0.018	0.639***
Lichen metrics:		
Lichen richness	0.011	0.786****
Fruticose spp.	0.191	0.480*
Cyanolichen spp.	0.166	0.409*
N Index score	0.318	0.521**
S Index score	0.203	0.517**
Thallus condition	0.055	0.531**

Comparison of lichen elemental values and deposition. Nitrogen concentrations were higher in fruticose *Evernia mesomorpha* than in the foliose species, *Platismatia glauca* and *Hypogymnia physodes* (**Table 2**; note *H. physodes* data consistent with *P. glauca*, but not shown in table) and the two types of thalli had S and N contents correlated to one another (**Supplementary Table S2**). The only significant fit between lichen thallus S and N and estimated deposition values was between foliose N (% of dry wt) and N_{cum} ($N = 14$; $r^2 = 0.399$; $F = 7.30$; $p = 0.021$). Foliose N also had significant linear relationships to three out of four lichen metrics, while all other thallus S and N relationships were non-significant (**Supplementary Table S2**). In contrast, *Evernia* N correlated better to aerosol $PM_{2.5}$ NO_3 (Pearson's $r = 0.754$) and total N ($r = 0.845$) than *Platismatia* N (NO_3: $r = 0.632$; total N: $r = 0.416$), but the strength of correlation for *Evernia* may have resulted from inclusion of full deposition gradient from ACAD to ADKS for this species (**Table 2**; **Supplementary Table S2**).

Summary. Estimates of N deposition were better predictors of patterns in the lichen metrics than those of S deposition in three out of four cases (**Table 6**). For both S and N, the majority of relationships were tighter using cumulative deposition estimates (**Table 6**). Only the relationship to species composition was closer for annual values, and for N there were several models for which cumulative deposition was a better fit, yet still equivalent to annual deposition (**Table 6**; **Fig. 7A**). For S, dry deposition estimates represented the lichen metric differences better than total S in four out of five instances (**Table 6**). For N, there was weak support for the greater relevance of oxidized N (3 of 6 comparisons), but again clear model differentiation for N deposition was often lacking (**Table 6**).

Discussion

Deposition environment. In general, the expected pattern of increased deposition from east to west was seen in modeled deposition values specific to our plots (**Figs. 1 & 3**). The main surprises were: 1) the mismatch between S_{ann} and S_{cum}, and 2) the greater S than N deposition at AC (**Fig. 3A**). The mismatch in S_{ann} is explainable, in part, because AC plots were surveyed in (2005–2006) when S deposition at AC was higher and closer to 2011–2013 deposition at all other sites. However, at AC dry deposition contributions to S_{ann} were lower than all other sites, despite the earlier sample years at AC (**Fig. 3B**).

The second point may be explained by a steeper east to west gradient in N deposition than S deposition across our study areas. Relatively shorter regional transport distances for N than for S result partly from the lower release heights of upwind N emissions. Oxidized N from motor vehicles and reduced N from agricultural sources are released at ground level, while upwind S emissions have historically come from point sources with tall smokestacks. Gaseous ammonia and nitric acid are more chemically reactive and dry deposit more rapidly than sulfur dioxide. Aerosol ammonium nitrate is chemically unstable and can dissociate into reactive gaseous precursors (NH_3 and HNO_3), while aerosol sulfate compounds are chemically more stable and persist longer in the atmosphere. The observation of relatively higher S than N at AC is likely due to these differences in transformation, transport and deposition of S and N from sources upwind of the region, rather than to effects of (minimal) sources within the region.

We also sought to understand the relatively poor correlations between estimated deposition and lichen thallus concentrations of S and N at the plot level. Between the areas, a lack of significant differences in S content may reflect the convergence in present day S inputs, but these values did not correspond well with any S deposition estimates. For N, the differences between the two WMNF areas, DR

Table 4. Comparison of curve fitting for relationship of lichen metrics (dependent) to deposition environment (independent). The best curve fit had the lowest AICc and the evidence ratio offers an indication of how much more likely the "best" model is than the compared model based on differences in AICc. N = 24 plots for all fits. Note S_{ann} was tested, but had no fits with $R^2 > 0.25$, and is not included here. Deposition variables are abbreviated: S_{ann} and N_{ann} for annual deposition as the mean of the year prior to and year of plot sampling, S_{cum} and N_{cum} are the cumulative deposition for the years 2000–2013. For the curve types: "P" refers to the number of parameters.

Independent	Dependent	Best curve fit	R^2	Curve compared	Evidence ratio
Dry S_{ann}	Lichen richness	Linear	0.427	Exponential 2P	1.17
	Thallus condition	Linear	0.441	Exponential 2P	1.16
	S-sensitive spp.	Logistic 3P	0.706	Linear	2.62
	S Index	Linear	0.328	Exponential 3P	1.92
S_{cum}	Lichen richness	Exponential 2P	0.493	Linear	1.62
	Thallus condition	Linear	0.537	Exponential 2P	1.04
	S-sensitive spp.	Logistic 3P	0.734	Exponential 3P	1.88
	S Index	Linear	0.476	Exponential 3P	3.53
Dry S_{cum}	Lichen richness	Exponential 3P	0.559	Exponential 2P	1.23
	Thallus condition	Exponential 2P	0.604	Linear	1.55
	S-sensitive spp.	Logistic 3P	0.781	Exponential 2P	16.1
	S Index	Linear	0.541	Logistic 3P	3.89
N_{ann}	Lichen richness	Exponential 2P	0.550	Linear	1.12
	Thallus condition	Linear	0.468	Exponential 2P	1.00
	N-sensitive spp.	Exponential 2P	0.706	Linear	1.96
	N Index	Linear	0.264	Exponential 3P	2.88
N_{cum}	Lichen richness	Exponential 2P	0.598	Linear	1.33
	Thallus condition	Linear	0.573	Exponential 2P	1.27
	N-sensitive spp.	Exponential 2P	0.735	Exponential 3P	3.86
	N Index	Linear	0.366	Exponential 3P	3.26
Reduced N_{cum}	Lichen richness	Exponential 2P	0.596	Linear	1.28
	Thallus condition	Linear	0.562	Exponential 2P	1.27
	N-sensitive spp.	Exponential 2P	0.739	Exponential 3P	3.70
	N Index	Linear	0.360	Exponential 3P	3.45
Oxidized N_{cum}	Lichen richness	Exponential 2P	0.599	Linear	1.37
	Thallus condition	Linear	0.579	Exponential 2P	1.26
	N-sensitive spp.	Exponential 2P	0.732	Exponential 3P	3.95
	N Index	Linear	0.369	Exponential 3P	3.18

and GG, were reversed for TDEP estimates and lichen elemental content (**Figs. 3C & 6C**). Recent work from the northwest U.S. have demonstrated the integrative nature of lichen elemental concentrations (Root et al. 2013). The elevation and topographic complexity of the DR and GG plots would increase the importance of cloud water inputs; the CMAQ model does not account for cloud water. The strong correspondence of all other independent lichen metrics with the pattern of DR being slightly cleaner than GG argues that the lichen N contents were more representative of lichen exposures than the TDEP data in this instance, and that lichen elemental analyses can serve as a valuable tool to calibrate deposition modeling efforts in complex terrain.

The relationship between lichen elemental content and aerosol N was difficult to assess with only four monitoring stations. However, the relationship between lichen N and aerosol N for the northeastern states does not seem to be as tight as the relationship reported by Geiser et al. (2010) or Root et al. (2015) for the northwestern states. This difference may be, in part, because particulate NO_3 and total particulate N are a small fraction (typically 5–10% at our northeastern sites), but it may represent a better surrogate of total N deposition in the Northwest than in the Northeast, where sources contributing to particle formation and deposition are more variable in space and time.

While lichen elemental analysis seems a good companion to estimated deposition values, the sampling is time (cleaning samples) and money (analyzing samples) intensive. Perhaps more importantly, we were often limited by the availability of lichens and could not match species across sites. All

Table 5. Comparison between deposition values for the best curve fits to the lichen metrics. The fit with the lowest root mean squared error (RMSE) is regarded as the best fit (**Best**). Models with an increase in RSME (ΔRMSE) greater than 10% are regarded as poorer fits, while those with ΔRMSE less than 10% are considered equivalent fits for the data. Deposition variables are abbreviated: S_{ann} and N_{ann} for annual deposition as the mean of the year prior to and year of plot sampling, S_{cum} and N_{cum} are the cumulative deposition for the years 2000–2013. For the curve types "P" refers to the number of parameters.

Dependent (Curve type):	Lichen richness (Exponential 2P)	Thallus condition (Linear)	Sensitive spp. (S: Logistic 3P) (N: Exponential 2P)	Index score (Linear)
Independent:				
Dry S_{ann}	R^2: 0.419 ΔRMSE: 20.3%	R^2: 0.441 ΔRMSE: 16.6%	R^2: 0.706 ΔRMSE: 15.7%	R^2: 0.328 ΔRMSE: 21.0%
S_{cum}	R^2: 0.493 ΔRMSE: 12.4%	R^2: 0.537 ΔRMSE: 6.1%	R^2: 0.734 ΔRMSE: 10.2%	R^2: 0.476 ΔRMSE: 6.9%
Dry S_{cum}	R^2: 0.494 ΔRMSE: 12.3%	R^2: 0.589 ΔRMSE: **BEST**	R^2: 0.781 ΔRMSE: **BEST**	R^2: 0.541 ΔRMSE: **BEST**
N_{ann}	R^2: 0.550 ΔRMSE: 5.6%	R^2: 0.468 ΔRMSE: 13.4%	R^2: 0.706 ΔRMSE: 5.8%	R^2: 0.264 ΔRMSE: 7.4%
N_{cum}	R^2: 0.598 ΔRMSE: <1%	R^2: 0.573 ΔRMSE: 1.9%	R^2: 0.735 ΔRMSE: <1%	R^2: 0.366 ΔRMSE: <1%
Reduced N_{cum}	R^2: 0.596 ΔRMSE: <1%	R^2: 0.562 ΔRMSE: 3.2%	R^2: 0.739 ΔRMSE: **BEST**	R^2: 0.360 ΔRMSE: <1%
Oxidized N_{cum}	R^2: 0.599 ΔRMSE: **BEST**	R^2: 0.579 ΔRMSE: 1.3%	R^2: 0.732 ΔRMSE: 1.4%	R^2: 0.369 ΔRMSE: **BEST**

of these factors limited our sample sizes (N=10 for *Evernia*; N=14 for foliose spp.), which in turn limited our ability to detect significance of relationships between thallus elemental content and other variables (**Supplementary Table S2**).

Response to deposition. Lichen metrics were generally better correlated with cumulative deposition than annual deposition (**Table 6**). The pattern across areas was similar for N_{ann} and N_{cum} deposition making differentiation between models difficult (**Table 5**). None of the metrics related well to S_{ann}, and this is explainable in part because AC plots were surveyed in (2005–2006) when S deposition at AC was higher and closer to 2011–2013 deposition at all other sites, therefore leaving little variability in S_{ann} between areas (**Fig. 3B**). The differences in patterns of S_{ann} and S_{cum} across the areas resulted in clearer differentiation of cumulative estimates as a better fit to lichen metrics (**Table 6**). In our study, dry S deposition related more closely to patterns in lichen metrics than total or wet S deposition. Dry deposition of S may be more harmful to lichens, both because it has the potential to become highly concentrated when the thallus is rehydrated, and because it largely originates from SO_2, which has a long history of toxicity to lichens (e.g., Hawksworth & Rose 1970; Nash & Gries 2002).

De Schrijver et al. (2011) described a negative exponential relationship between cumulative N and lichen species richness in Europe based on a meta-analysis of N-addition experiments. They found much faster rates of species loss early on in the addition of N. Our pattern of negative exponential fit between N_{cum} and lichen richness across our plots probably represents the same phenomenon as a space-for-time substitution as AC plots had received much less N than plots further west. European studies demonstrating the importance of cumulative N deposition (De Schrijver et al. 2011; Johansson et al. 2010), and our results, all highlight the relevance of cumulative loads and the need to curb N inputs to prevent early rapid loss of N-sensitive species.

Another research approach to the question of effects of present versus cumulative deposition on epiphytic lichens has been to differentiate lichens collected on twigs from those on the main tree bole. Several European studies suggest that epiphytic lichens on twigs, not directly influenced by past deposition, were more representative of response to current deposition while those on the main tree trunk indicated the legacy of past acidification (Wolseley et al. 2006; Wolseley & Pryor 1999). Collection information for vouchers should therefore carefully differentiate these two substrates.

For forms of the N inputs, there was limited evidence from our study that oxidized N was more relevant than total N (**Tables 5 & 6**). In the northeastern U.S., the ratio of reduced to oxidized N has been increasing over time, but oxidized N still accounts for over half of total N deposition at all of our plots. If upwind NO_x emissions continue to decrease more rapidly than NH_x emissions, future northeastern N deposition will likely be dominated by reduced N, similar to large sections of the central and western U.S. and Europe. Low bark pH at our study plots resulting from the legacy of acidifying S inputs is reflected in bark pH values (3.80–5.98) at our cleanest Acadia plots (data from red maple and red spruce; Cleavitt et al. 2011). Jovan et al. (2012) suggest that nitrophytes (N-tolerant) responded to total N regardless of bark pH within their study range (bark pH 4.8–6.1). Even so, based on measures from AC, bark pH at our western sites are likely lower than 4.8, and at least some nitrophytes are limited by low pH and may respond faster if NH_4 contributes more to N inputs over time.

Comparison to critical values. The critical load of 4–6 kg yr^{-1} ha^{-1} of total N deposition recommended for epiphytic lichens in the Northern Forest Ecoregion (Pardo et al. 2011) was upheld here based on changes in our lichen metrics; however, N cumulative loads must also be considered. In our study, there appeared to be a threshold in the 60–80 kg N ha^{-1} range (for the 2000–2013 cumulative deposition) which differentiated AC from the other three areas. This range would correspond to annual inputs of 4.3–5.7 kg N yr^{-1} ha^{-1}, very close to the published critical load. More study is needed to understand the number of years of cumulative deposition that are biologically relevant. For example, although S and N deposition levels are currently close, historically there was much higher S than N deposition, and the actual cumulative S load is much greater than that for the 14-years of data available for our analyses.

For aerosol N, it was notable that the only area below the northwestern critical level (0.37 to 0.51 µg N m^{-3}; Geiser et al. 2010, Root et al. 2015) at the time of measurement, AC, had significantly better values for lichen metrics suggesting that the critical limits from the Northwest should be further tested in the east. Location of lichen plots near eastern IMPROVE sites are needed to more fully evaluate use of IMPROVE critical levels for lichens in the eastern U.S.

Protocol improvement by new metrics—thallus scoring. Lichen thallus condition scores were a novel and useful metric for understanding nuances of lichen response to deposition independent of forest composition. For instance, habitat suitability for functional groups, namely, cyano- and fruticose lichens, differed between broadleaf and conifer dominance. Forest composition is also partly confounded with deposition sensitivity because N-tolerant species are associated more often with broadleaf (relatively more alkaline) substrates and S-tolerant species associate with conifer (more acidic) substrates. The effect of forest composition on N-tolerant species presence has been noted previously, and can be controlled by accounting for broadleaf tree presence in analyses (Root et al. 2015).

The interdependence between thallus condition and lichen community composition was evident in the pattern of decreasing lichen health between areas. First, the number of species with robust thalli was reduced, then thallus health of the less common species decreased, and finally decreased thallus health in even the common species. If done in greater detail, thallus scoring could also allow detection of specific thallus damage, such as fungal pathogens versus gastropod grazing, both of which have both been hypothesized to increase with increasing N deposition (Asplund et al. 2010; Johansson et al. 2012; Ström 2011). Thallus condition scores could be an important and easy addition to lichen monitoring projects as they allow quantification of lichen health and scores can be reliably tallied for years after collection.

Some protocol details to consider in a study planning to implement thallus condition scores include: 1) noting the substrate for each specimen and excluding specimens collected from fallen material; 2) avoiding selecting the nicest looking example of the species from the plot, obtaining representative samples instead; and 3) scoring by a lichen expert to verify the specimen identifications in the laboratory, which makes use of their greater knowledge of "normal" range for the species and maintains consistency in scoring. As an alternative to the second point, one can evaluate all occurrences of a species collected from the plot (i.e., incidental collections) to obtain a plot average for each species.

Sensitivity indices and species groups. The sensitivity indices were in agreement with other lichen metrics in differentiating the four study areas with the

Table 6. Summary of deposition and lichen metric variables with strongest correlations in the northeastern U.S.

Lichen metric:	Source	S estimate	N estimate	S vs. N
Species composition	Table 3, Fig. 7A	Dry S_{ann}	N_{ann}	N tighter
Thallus element content	Suppl. Table S2	None	N_{cum}	N tighter
Lichen richness	Tables 4 & 5	S_{cum}	Dry N_{ann}; Oxi N_{cum}	N tighter
Thallus condition	Tables 4 & 5	Dry S_{cum}	Oxi N_{cum}	Equivalent
Sensitive species	Tables 4 & 5	Dry S_{cum}	Red N_{cum}	Not comparable
Sensitivity indices	Tables 4 & 5	Dry S_{cum}	Oxi N_{cum}	Not comparable
Summary:	Ann vs. Cum Tally	Ann: 1/5	Ann: 2/6	
		Cum: 4/5	Cum: 5/6	

highest scores at AC and the lowest scores at LB (**Fig. 6A**), and were significantly correlated to the second NMS axis (**Fig. 7A**). Some surprises in sensitivity groups (sensitive, intermediate, tolerant) were: 1) N-tolerant species richness was low compared to S-tolerant species richness even at LB; 2) sensitive and intermediate species tended to be closer in ordination space than tolerant species; and 3) plots that had higher lichen richness tended to be higher for all species groupings.

The first trend for species sensitivity groups may be due in part to the sensitivity of many N-tolerant species to acidification. Similarly, the sensitivity cut-off used to differentiate N-sensitive species of peak abundance at less than 5 kg ha^{-1} yr^{-1} was dictated by the 2002 deposition environment (available from CMAQ data for the FIA plots), and this value is essentially five times the background levels reported in "clean" areas of the western U.S., where many lichen species peak at inputs of 1.5–2.5 kg N ha^{-1} yr^{-1} (Root et al. 2015). Therefore, we may have underestimated the number of N-tolerant species.

The tolerant species for both S and N tended to have more negative scores along both ordination axes (**Fig. 7 B & C**), although this pattern is weaker for S-tolerant species. In general, S sensitivity groups (sensitive, intermediate and tolerant) appeared relatively ubiquitous on the forest composition axis, occurring equally in broadleaf, mixed and coniferous forests. The weaker patterns for S sensitivity groups most likely signal the general prevalence of acidifying inputs on lichen communities in this region, and this agrees with recent results of Will-Wolf et al. (2015).

Sensitivity groups for N were clearly separated by the NMS ordination, with sensitive species positioned in the least polluted plots in conifer forests, intermediate species in intermediate plots for pollution, and no preference for forest composition and tolerant species positioned strongly in broadleaf forests and more polluted plots (**Fig. 7C**). These patterns suggest that lichen communities in broadleaf forests may exhibit signs of community disruption by N-tolerant species earlier as N-inputs accumulate.

Functional groups. Separation of our lichen species into four functional groups highlighted the greater sensitivity of cyanolichens to deposition. The near absence of cyanolichens outside of AC suggests that presence of cyanolichens alone may be a good indicator of exceeded cumulative acid deposition in mixed and broadleaf forests that would otherwise be expected to contain such species. One caveat is that not all broadleaf tree species are equally suitable to cyanolichens and *Betula* species in particular are less suitable, therefore, differences between plots and areas may relate in part to differences in composition within broadleaf species.

Other considerations. Response of lichens to air pollution may be complicated by climate responses (Root et al. 2015; Will-Wolf et al. 2015). Elevation did relate significantly to the second NMS axis (**Fig. 7A**). For our study plots, the variation in temperature was fairly minimal (2°C difference in monthly average temps). There was more variation in estimated annual precipitation (maximum difference 90 cm); however, precipitation was not significantly correlated to either ordination axis (**Table 3**). Differences in precipitation between plots were much smaller than in western lichen studies, where precipitation maximum differences were as much as 306 cm between sites and the driest sites limited lichen growth (Root et al. 2015). Unaccounted for climate variables, such as humidity and fog prevalence, may have important influence on epiphytic lichen occurrence.

In particular, our sites varied in distance from the Atlantic coast. Could differences in lichen communities across the areas have always existed regardless of deposition impacts? For instance, could the plots in VT simply always have lacked species regarded as sensitive to air pollution for other reasons such as distance from the coast? Examination of historical specimens in Hinds & Hinds (2007) convincingly demonstrated that many sensitive species were previously known from VT and NH. Their data lends support to our supposition that patterns reported here are largely from deposition effects. In addition, we subtracted species restricted to the coast (eight species listed under ***Species richness***) from the AC plot diversity and maintained significant differences in lichen richness between areas.

Conclusions. Lichen communities in all four Class I areas appeared adversely affected by air pollution despite significant decreases in annual deposition loading over the past 14 years. This pattern appears related to the legacy of cumulative deposition effects, which related more clearly to lichen metrics. At AC, the "cleanest" area, impacts were evident only as a negative S-Index score and high number of S-tolerant species. In addition, AC was the only area that has recently had annual deposition loadings below the published lichen N critical loads, and has lichen thallus S and N concentrations below clean-site thresholds. In contrast, the continued higher depositional loading of pollutants to LB over time have resulted in lower lichen richness, poorer thallus condition, and higher S and N concentrations in lichen thalli compared to the other three Class I areas. For all areas, the close correspondence of lichen metrics to cumulative rather than annual deposition argues for a lowering of critical loads, to prevent further loss of S- and N-sensitive species and to create conditions that would allow dispersal and establishment of more sensitive species.

Our study of 24 plots needs to be extended by a larger sample size to encompass cumulative deposition values intermediate to and less polluted than our four areas before we can set limits for cumulative deposition loads. A larger sample size would also facilitate assessment of differential vulnerability of epiphytic lichen communities by forest type; namely, domination by conifer, mixed and broadleaf trees in the northeastern U.S.

Following significant reduction in pollution inputs, there are many instances of the return of lichens to previously heavily polluted point sources (e.g., Showman & Long 1992; Gunn et al. 1995) and urban centers (e.g., Seaward 1997; Hultengren & Gralen 2004; Sparrius 2007); however, more regional recovery has yet to be documented, and return of truly sensitive species such as cyanolichens has not been documented yet. Studies such as this one form a necessary baseline for monitoring changes in lichen communities over time.

The degradation and progressive loss of lichen species from our forests are a warning of other undocumented losses and effects of pollution, while also having a direct impact on the many invertebrates, birds and small mammals that eat, hide in, or forage among lichens and bryophytes. As an important part of the northern forest ecosystem, lichens serve as sources of food and nesting material thereby affecting populations of invertebrates, birds, flying squirrels, voles and white-tailed deer (reviewed in Hinds & Hinds 2007). In addition, the impacts of pollution on lichen diversity (Cislaghi & Nimis 1997) and element concentrations (Saiki et al. 2014) have been correlated with effects on human health. Lichen species are even being evaluated for efficacy against cancer (Shrestha et al. 2015 and references cited therein), and have high conservation value for all these reasons.

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Supplementary documents online:

Supplementary Table S1. Summary of sulfur and nitrogen indicator class for the 94 lichen species (note 11 species are not rated) found on 24 plots in four Class I Areas of the northeastern U.S. We classed lichen species by the median deposition for the distribution of all eastern U.S. detections in the U.S. Forest Service FIA lichen database: 'sensitive' (<5 kg ha^{-1} yr^{-1}), intermediate (5–10 kg ha^{-1} yr^{-1}), or 'tolerant' (>10 kg ha^{-1} yr^{-1}). Functional groups are based partially on the classical growth forms; however, "Cladonia" and "Cyanolichen" were also differentiated. "Cladonia" splits out members of the genus

Cladonia, while "Cyanolichen", as used here, includes any lichen with cyanobacteria in the thallus whether as the main photobiont or in cephalodia. Species with presence in two or more plots were included in the ordination analysis and are indicated by a "1".

Supplementary Table S2. Correlation of lichen thallus S and N contents with each other, deposition estimates and other lichen metrics for plots in northern New England. Note that *Evernia* S and N do not include any plots from AC. As elsewhere in our analyses, no significant correlations were found for S_{ann} and it is not included here. For significant correlations (α = 0.05), the Pearson's r values are given, while non-significant relationships are indicated by "ns". Comparisons that were not made are indicated by "---".