CHAPTER 6.
Lichen Species to Bioindicate Air Quality in Eastern United States from Elemental Composition: Lessons from the Midwest

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INTRODUCTION

Elemental concentration in lichens is a popular and cost-effective tool to bioindicate pollution load at plots (Donovan and others 2016, Paoli and others 2014, Root and others 2015) and to complement costly instrumented monitoring to help assess environmental health. From recent development of lichen elemental bioindicators for air pollution in the U.S. upper Midwest (Will-Wolf and others 2017a, 2017b, In press) for the U.S. Department of Agriculture, Forest Service, Forest Inventory and Analysis Program (FIA), we learned important lessons about suitability of lichen species in large-scale monitoring programs for the Eastern United States. Five macrolichen species common in Eastern North America (E NA) (Brodo and others 2001) were evaluated in that study: Evernia mesomorpha (code Evemes; small/medium size fruticose growth form), Flavoparmelia caperata (code Flacap; large foliose), Parmelia sulcata (code Parsul; medium foliose), Physcia aipolia and P. stellaris combined (code Phyaip; small foliose, tightly appressed), and Punctelia rudecta (code Punrud; large foliose). Elemental data and multi-element Pollution Indices derived from them clearly represented relative site pollution load better than did regionally modeled pollutant deposition (Will-Wolf and others 2017a), as has also been found in other studies (e.g., Bari and others 2001, Boquete and others 2009, Geiser and Neitlich 2007, Root and others 2015). Evemes, Flacap, and Phyaip were recommended as bioindicator species for the study region (Will-Wolf and others 2017a, In press).

Based on the upper Midwest studies and 1994–2005 species frequencies in the FIA National Lichen Database (table 6.1) Will-Wolf and others (In press) recommended bioindicator species for Northeastern (NE) region States (Connecticut, Maine, Massachusetts, New Hampshire, New York, Rhode Island, and Vermont), Mid-Atlantic (MidA) region States (Delaware, Maryland, New Jersey, Ohio, Pennsylvania, Virginia, and West Virginia), and Southeastern (SE) region States (Alabama, Georgia, North Carolina, South Carolina, and Virginia). Virginia was included in both the MidA and the SE regions to assist ecological evaluation of region boundaries.

For this study, our objective was to evaluate the recommendations of Will-Wolf and others (in press) using data from other studies. We evaluated 1994–2005 distribution patterns of all five tested macrolichen bioindicator species with respect to pollution load and nearby forest cover in the NE and MidA regions using lichen community data for subsets of FIA plots.
Table 6.1—Percentage of unique 1994–2005 FIA plots in eastern U.S. lichen regions with five target lichen elemental indicator species (names in bold) plus an additional speciesa

<table>
<thead>
<tr>
<th>Lichen species</th>
<th>FIA lichen regionc</th>
<th>NC (204p)</th>
<th>NE (625p)</th>
<th>MidA (779p)</th>
<th>SE (357p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any target species</td>
<td>96.6%</td>
<td>97.0%</td>
<td>91.9%</td>
<td>88.2%</td>
<td></td>
</tr>
<tr>
<td>Any of the four most common target species</td>
<td>95.6%</td>
<td>95.8%</td>
<td>91.9%</td>
<td>88.2%</td>
<td></td>
</tr>
<tr>
<td>Any of the three most common target species</td>
<td>95.1%</td>
<td>95.4%</td>
<td>91.3%</td>
<td>88.2%</td>
<td></td>
</tr>
<tr>
<td>Either of the two most common target species</td>
<td>90.7%</td>
<td>93.9%</td>
<td>85.1%</td>
<td>86.3%</td>
<td></td>
</tr>
<tr>
<td><em>Evernia mesomorpha</em> Nyl.</td>
<td>56.4%</td>
<td>57.8%</td>
<td>3.3%</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>Flavoparmelia caperata</em> (L.) Hale</td>
<td>69.1%</td>
<td>63.0%</td>
<td>76.9%</td>
<td>60.2%</td>
<td></td>
</tr>
<tr>
<td><em>Parmelia sulcata</em> Taylor</td>
<td>71.6%</td>
<td>85.6%</td>
<td>52.4%</td>
<td>4.2%</td>
<td></td>
</tr>
<tr>
<td><em>Physcia aipolia</em> (Ehrh. ex Humb.) Fürn. and <em>P. stellaris</em> (L.) Nyl. (no other studies)</td>
<td>78.4%</td>
<td>40.5%</td>
<td>23.1%</td>
<td>32.8%</td>
<td></td>
</tr>
<tr>
<td><em>Punctelia rudecta</em> (Ach.) Krog</td>
<td>39.2%</td>
<td>64.0%</td>
<td>58.0%</td>
<td>78.7%</td>
<td></td>
</tr>
<tr>
<td><em>Punctelia missouriensis</em> G. Wilh. &amp; Ladd (possible future target species; no usage)</td>
<td>6.4%</td>
<td>0</td>
<td>10.8%</td>
<td>5.0%</td>
<td></td>
</tr>
</tbody>
</table>

a Reprinted with permission from Will-Wolf and others (In press); data from Jovan and others (see footnote 2).
b The three species most frequently found in each region have values in bold. Past elemental indicator use in Eastern United States and Western Europe indicated in parentheses after name.
c FIA lichen regions are North Central (NC), Northeastern (NE), MidAtlantic (MidA), and Southeastern (SE); number of plots (p) in parentheses after lichen region code.

Comparisons of our results with those of the upper Midwest study and its recommendations for these regions support implementation of lichen elemental bioindicators in those regions. None of the NE, MidA, or SE regions currently has extensive lichen elemental data; government network instrumented monitor sites are scattered unevenly and often measure different pollutants, and onsite instrument monitoring is very expensive (Will-Wolf and others 2017a). A Pollution Index from lichen elemental

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analyses would greatly improve monitoring of environmental health across eastern FIA plots in most subregions.

METHODS

For the upper Midwest study (fig. 6.1), trained non-specialists (FIA field staff) collected single-species composite samples for all five target species under 20–50 percent tree canopy near permanent FIA plots using rigorous protocols [details in Will-Wolf and others (2017a, footnote 3)]. An expert collected samples of the same species from temporary plots using both rigorous protocols and several variants of relaxed (and more economical) protocols. Elemental data from combustion and ICP-OES (inductively coupled plasma optical emission spectroscopy) were validated, and then field sample quality and subsequent elemental data quality were evaluated; only the most rigorous protocols were supported [details in Will-Wolf and others (2017b)] to provide reliable data. Data were converted between species with General Linear Models (GLM) or regression [both in SPSS (2015)], then combined for analyses; two pollution indices were developed from multiple elements [aluminum (Al), cobalt (Co), chromium (Cr), copper (Cu), and iron (Fe) for one; nitrogen (N) and sulfur (S) for the other]. Estimation of relative pollution load from lichen elemental concentrations was confirmed by comparison with monitor site data (Will-Wolf and others 2017a). Lichen elemental bioindicators and protocols were recommended for implementation in other eastern U.S. regions (Will-Wolf and others, In press).

Figure 6.1—Approximate locations of lichen sites and instrument monitor sites for the upper Midwest study. Wisconsin, marked in the U.S. inset, is in the center with Minnesota west, Iowa southwest, and Illinois south. Full names of ecoregion provinces (Cleland and others 2007, McNab and others 2007) indicated by background shading are: 212–Laurentian Mixed Forest, 222–Midwest Broadleaf Forest, and 251–Prairie Parkland (Temperate). [Adapted with permission from Will-Wolf and others (2017a).]
Frequency of one or more of the five tested bioindicator species and one additional possible indicator species with respect to pollution load and proportion of nearby land in forest cover was evaluated with scatterplots for the three project areas discussed in this study (upper Midwest: Will-Wolf and others 2017a; NE: Will-Wolf and others 2015a; MidA: footnote 3). Relationships of bioindicator species abundance to pollution load and nearby landcover were evaluated for NE and MidA datasets with Pearson and Spearman rank correlations and with linear regression using original data and log10-transformed data for pollution load and nearby forest cover (SPSS 2015). For correlations, Pearson $r^2$ or Spearman $\rho^2$, probability ($p$), and direction are reported; for regressions, adjusted $r^2$, $p$, independent variables, and interpretation are reported. To account for experiment-wide error, correlations with $0.05 > p > 0.005$ were considered weak, and only correlations with $r^2$ or $\rho^2 \geq 0.10$ ($p < 0.00001$) were considered ecologically important. Regression models with $0.05 > p > 0.01$ were considered weak.

Frequency of each lichen species, percent nearby forest cover, and relative pollution load were available from all three earlier studies. Nearby forest cover was the percentage of pixels with forest cover in a buffer zone around each site: ~1 km$^2$ buffer for NE, ~3 km$^2$ for upper Midwest, and ~5 km$^2$ for MidA. Pollution load was represented by the lichen-based site Pollution Index developed independently for each of the three project areas. For the NE region, bioindicator lichen species abundance at 218 plots across all States (fig. 6.2A) was available from development of statistically independent climate and pollution indices for the region (Will-Wolf and others 2015a); species abundance at 219 plots in the MidA region (including Virginia) was available from a similar study (footnote 3). Plots were spread across most MidA States, but represented only the eastern one-quarter of Ohio (fig. 6.2B).
Figure 6.2—Northeastern (A) and MidAtlantic (B) region project plots (approximate locations). For comparison, the city of Newark, NJ, is at the bottom center of the map in (A) and near the upper right corner of the map in (B). On-frame plots are permanent FIA plots; off-frame plots are temporary plots surveyed only for each project. Full names of ecoregion provinces (Cleland and others 2007; McNab and others 2007) indicated by background shading in (A) are: 221–Eastern Broadleaf Forest, 211–Northeastern Mixed Forest, and M211–Adirondack-New England Mixed Forest - Coniferous Forest - Alpine Meadow. Full names in (B) are: M211–Adirondack-New England Mixed Forest - Coniferous Forest - Alpine Meadow; M221–Central Appalachians Broadleaf Forest - Coniferous Forest - Meadow; 221–Eastern Broadleaf Forest, 211–Northeastern Mixed Forest, and 231/232–Southeastern/Outer Coastal Plain Mixed Forest (two provinces combined, abbreviated as “Southeastern” in the legend).

[(A) Adapted with permission from Will-Wolf and others (2015a); (B) adapted with permission from Will-Wolf and others (see footnote 3).]
RESULTS AND DISCUSSION

Relative success of lichen species as elemental bioindicators in the upper Midwest study generated several recommendations for both applications and limitations in three other eastern U.S. regions. Comparison with lichen community composition in two of those regions helped evaluate those recommendations.

Forest Inventory and Analysis field staff successfully distinguished Evemes, Flacap, and Phyaip from other lichen species (Will-Wolf and others 2017b). Flacap and Phyaip samples had good elemental data quality (203 samples of all five species at 83 sites after successful data conversion: Will-Wolf and others 2017a). Evemes samples had good data quality below a maximum relative pollution load (estimated from Pollution Index N + S and instrumented monitor site data) that reflected species sensitivity consistent with other U.S. studies (e.g., Will-Wolf and others 2015a); comparison of figures 6.3A and 6.3B illustrates exclusion of some Evemes samples from more polluted sites to achieve successful data conversion (via GLM: Will-Wolf and others 2017a) for equivalence with Flacap. Original Evemes N values (fig. 6.3A) were higher than Flacap values at the same less polluted sites. After conversion and exclusion of sites (fig. 6.3B), Evemes and Flacap values for the same sites overlap. Thus Evemes would be a reliable elemental bioindicator, but only for sites below that maximum relative pollution load, as estimated from the site Pollution Index calculated only from less sensitive lichen species. The tight correspondences between N values and the Lichen Pollution Index in figures 6.3B, 6.4A, and 6.4B reflect that converted N data for all lichen species were incorporated into the Lichen Pollution Index. Flacap (often used for lichen elemental analysis, e.g., Will-Wolf and others 2015b, 2017a, footnote 3) and Phyaip (not used before but successful in the upper Midwest study) between them covered the study area (fig. 6.4C); partitioning of sites between the species was in Will-Wolf and others (2017a) qualitatively linked as much to percentage of nearby forest cover as to site air pollution. Presence of each species was more strongly correlated with nearby forest cover than with pollution load, and logistic regression on presence of each species found nearby
Figure 6.4—(A, C) Flacap and Phyai or (B, D) Flacap and Punrud pairs compared for the upper Midwest study. In A and B, species pairs are compared for converted nitrogen (N) concentrations vs. Pollution Index. In C and D, presence of each species is plotted by site Pollution Index and nearby forest cover. Many symbols overlap, from both species at the same site and from sites with similar values. [Data from Will-Wolf and others (In press).]
forest cover to be the most significant predictor variable [Will-Wolf and others (in press)], an unexpected result.

Non-specialist field staff had difficulty distinguishing Parsul and Punrud [both successful in expert studies, e.g., Olmez and others 1985; reviewed in Will-Wolf and others (2017b)] from other lichen species; this led to few samples collected and poor elemental data quality in ~10 percent of samples. Parsul and Punrud identification difficulties for non-specialists were an unexpected outcome of interest particularly to large monitoring programs; the human context of a study is as important to bioindicator choice as is the scientific context. Scatterplots of Flacap and Punrud (figs. 6.4B and 6.4D) illustrate that while converted data for Punrud samples overlapped Flacap samples along most of the pollution range, they were much less frequent; Parsul had a similar pattern.

Based on these earlier studies, Flacap (in areas with less isolated forests) and Phyaip (in areas with more isolated forests) were the primary recommended lichen elemental bioindicators for the full North Central region, with Evemes a secondary recommended bioindicator in northern areas and at less polluted sites because it is so cost-effective to handle (Will-Wolf and others 2017a, in press). Based on the upper Midwest studies and on frequency of species in FIA plots (table 6.1), Will-Wolf and others (in press) recommended Flacap (widespread) and Evemes (northern and mountainous areas with less air pollution) as primary bioindicator species for the NE region, with Phyaip as a secondary species in areas (usually with more isolated forests) where samples of a primary species were not found. For the MidA region Flacap and Evemes (both widespread) were recommended as primary bioindicators (Punrud requires intensive identification training for non-specialist field staff), with Phyaip as a secondary species at plots in less forested landscapes.

Comparisons of 1994–2005 bioindicator species distributions with pollution indices and nearby forest cover for the NE and MidA data subsets helped better predict success of recommended bioindicators in these regions. The NE dataset is ~35 percent of the available FIA plots with lichen data in those States (table 6.1); the MidA dataset is ~28 percent of the available FIA plots with lichen data. Plot distribution for the NE and MidA region projects (fig. 6.2) was more even than for the upper Midwest study (fig. 6.1), though all ecoregions were well-represented in all three studies. In the NE and MidA datasets, full lichen community composition varied strongly with both climate and air pollution represented by a Pollution Index statistically independent of climate (Will-Wolf and others 2015a, footnote 3). In each dataset, lichen composition was more weakly linked to nearby forest cover than to climate or pollution, and, in the same pattern as the upper Midwest study, Pollution Index was negatively correlated with nearby forest cover. For both NE and MidA, correlation between Pollution Index and nearby land in forest cover, while statistically strong ($p < 0.0005$), was small
enough ($r^2 = 0.28$ for NE, $0.36$ for MidA) to enter both variables into the same regression model. In both these datasets, Physcia stellaris was more frequent at plots than was Physcia aipolia (from laboratory identifications; not reliably distinguishable in the field), so Phyaip represented the former species more, in contrast to the upper Midwest study where Physcia aipolia was the more frequent species in Phyaip based on laboratory identifications. Comparisons of indicator species pairs over the subset of plots in each dataset where at least one of them occurred helped evaluate how well suites of indicator species would represent the region. In addition, the MidA project illustrated differences between lichen species abundance at FIA plots and ability to collect a bulk lichen elemental sample within a narrow canopy range in the vicinity of an FIA plot. Flacap was recorded at $93\%$ of $219$ MidA plots (footnote 3), but adequate elemental samples were collected at only $85\%$ of $26$ temporary sites spanning the pollution gradient. Thus these analyses of lichen species abundances can at best be very general estimators for the likelihood of collecting adequate samples for elemental analyses. Indicator species not mentioned in the next two paragraphs were too uncommon in a region to consider further.

From the NE region dataset, Phyaip appeared to be present at more of the polluted sites than did Flacap (fig. 6.5A), but the two species overlapped across the gradient of nearby forest cover in contrast to the upper Midwest sites (compare with fig. 6.4C). Phyaip abundance had weak positive correlation ($\rho h o^2 = 0.03$, $p = 0.011$) and Flacap abundance weak negative correlation ($r^2 = 0.052$, $p = 0.0007$) with the Pollution Index; both lacked significant correlation (not significant [NS]) with percent of nearby land with forest cover. The strongest significant ($p < 0.001$) regression model (though still weak; $adj r^2 = 0.07$) for Flacap predicted its abundance declined as Pollution Index (original values) increased. No regression model was significant for Phyaip. The NE Flacap and Parsul scatterplot (fig. 6.5B) showing Parsul present at more sites with high pollution and low nearby forest cover than Phyaip (fig. 6.5A) suggested Parsul might complement Flacap better than Phyaip to provide broad representation of even high-pollution FIA plots. However, Parsul abundance had weak negative correlation with pollution ($\rho h o^2 = 0.04$, $p = 0.005$; NS with nearby forest cover), and a weak regression model ($adj r^2 = 0.02$, $p = 0.02$) predicted the same pattern from log10 of Pollution Index. Thus Parsul, while present, had very low abundance at polluted sites, supporting Phyaip as the better complement to Flacap for NE elemental bioindication. The scatterplot of NE Flacap and Evemes (fig. 6.5C) presence shows Evemes was mostly restricted to plots with $> 50\%$ nearby forest cover and in the cleaner half of the Pollution Index. Evemes abundance was negatively correlated with Pollution Index ($\rho h o^2 = 0.54$, $p < 0.00001$) and positively correlated ($r^2 = 0.152$, $p < 0.00001$) with percent nearby forest cover; the strongest regression model ($adj r^2 = 0.56$, $p < 0.0000$) predicted Evemes decline only with increasing
Figure 6.5—Presence of lichen bioindicator species in 1994–2005 compared to pollution and nearby forest cover at sites in NE and MidA regions. Northeastern and MidA Pollution Indices have the same value ranges, but indicate different levels of air pollution between regions. Many symbols overlap, from both species at the same site and from sites with similar values.
[Data from Jovan and others (see footnote 2), Will-Wolf and others (2015a, footnote 3).]
log10 of Pollution Index. Evemes was clearly more limited in the NE region by pollution than by nearby forest cover, a reversal from the upper Midwest study (Will-Wolf and others 2017a, footnote 3); it was, however, the only NE region bioindicator species showing a statistical link with nearby forest cover. These patterns support the recommendations by Will-Wolf and others (in press) for Flacap and Phyaip plus secondary Evemes as bioindicator species in the NE region, though with little demonstrated influence of nearby forest cover on lichen distribution. Species distribution patterns should be evaluated with the full suite of NE FIA lichen plots to further refine bioindicator recommendations.

Both Phyaip and Punrud were present at more MidA plots with high pollution than was Flacap (figs. 6.5D, 6.5E). While all three species were present across the full range of nearby forest cover, response of species’ abundances more strongly supported the rationale by Will-Wolf and others (in press) for recommending Flacap and Punrud as primary and Phyaip as a secondary bioindicator species in the MidA region. Flacap abundance was positively correlated with forest cover ($r^2 = 0.07, p < 0.0005$) and negatively correlated ($r^2 = 0.19, p < 0.0005$) with Pollution Index. The strongest (although still weak) regression model ($r^2 = 0.09, F = 23.3, p < 0.0005$) predicted Flacap increasing only with lower pollution. Punrud abundance had weak positive correlation with percent nearby forest cover ($r^2 = 0.03, p = 0.013$) but no correlation (NS) with Pollution Index; its best but weak and ecologically unimportant regression model ($r^2 < 0.01, F = 5.8, p = 0.017$) predicted Punrud abundance increasing only with log of nearby forest cover. Phyaip abundance had weak negative correlation with Pollution Index ($r^2 = 0.02, p = 0.037$) and none (NS) with percent nearby forest cover. Thus Flacap appeared more pollution-sensitive as well as preferring high-forest landscapes, Phyaip appeared slightly sensitive to pollution but not to forest cover, and Punrud appeared to slightly prefer high-forest landscapes but be unresponsive to pollution. More sensitivity to forest cover might be noted in analyses including plots in low-forest landscapes of western Ohio (favors Phyaip) that were not in the 219-plot subset. Only 11 percent of the 219 MidA plots had < 50 percent local forest cover, as compared with 30 percent of the 83 upper Midwest study plots. Flacap abundance was negatively correlated ($r^2 = 0.19, p < 0.000001$) with Pollution Index in MidA. These analyses support recommendation of Punrud as a primary elemental bioindicator, despite its need for intensive identification training of non-specialist field staff, to complement the more environmentally sensitive Flacap and the less abundant Phyaip. Sparse MidA region lichen elemental data (footnote 3) support the recommendations and also highlight differences between species presence based on even a single individual vs. an adequate sample for elemental analysis. An adequate elemental sample is 1–2 g per species from six or more different substrates under 20–50 percent forest canopy (Will-Wolf and others 2017a, in press). For example, Flacap
was present at ~77 percent of all MidA plots (table 6.1) and 93 percent of 219 MidA subset plots, while adequate elemental samples of Flacap came from 85 percent of 26 supplemental sites (footnote 3) selected to represent the pollution gradient.

Species distribution patterns should be evaluated in the MidA region with the full suite of FIA lichen plots. Punmis (*Punctelia missouriensis*) (table 6.1), suggested by Will-Wolf and others (in press) as another possible bioindicator species for the MidA region in low forest landscapes (it is a Midwestern United States endemic: Brodo and others 2001), was present at only five of the 219 subset plots. However, even from those few sites, Punmis abundance had weak positive correlation ($r^2 = 0.02, p = 0.021$; regression NS) with the Pollution Index; such pollution tolerance could make it a useful elemental bioindicator there. The bioindicator potential of this species will become more apparent after evaluation with the full suite of MidA FIA lichen plots including the rest of Ohio (table 6.1), with likely most added sites in low-forest landscapes. Identification success by non-specialists has not been tested, but, based on its form and color, Punmis might be as difficult as Parsul or Punrud for non-specialists to distinguish from other co-occurring species.

Recommendations by Will-Wolf and others (in press) of Flacap, Phyaip, and Punrud as elemental bioindicators for the SE region are also supported to a degree from MidA analyses, based on inclusion of Virginia in both the MidA and SE regions. Evaluation of species distribution patterns for all SE plots will be particularly important to further refine bioindicator recommendations. This region is the most likely location for recommended target species to fall short of availability at 90 percent of FIA plots, based on data in table 6.1.

Distribution of elemental bioindicator species for the NE, MidA, and SE regions may have changed somewhat from that represented by the 1994–2005 FIA lichen community data discussed here. Probably because shifts from coal to natural gas for much eastern U.S. energy production accelerated starting about 2000 from expanded fracking, recent local pollution impacts on forests (Drohan and others 2012) have been offset by regional declines in air pollution (US EPA 2017). In the NE and MidA regions since 2005, nitrogen dioxide ($NO_2$) has declined ~25–30 percent, sulfur dioxide ($SO_2$) has declined ~70–75 percent, and particulates (PM2.5; correlated with pollution metals) have declined about 35–40 percent. So by 2017 the impact of air pollution on lichen species distribution may have declined in these regions, and the impact of nearby forest cover may have become relatively more important by default. It has long been known that forest fragmentation and distance from propagule source can affect lichen recolonization (Gilbert 1992), though such impacts were previously documented mostly on less common lichen species.
CONCLUSIONS

Distribution patterns of lichen elemental bioindicator species with respect to pollution and landcover in NE and MidA U.S. regions supported recommendations from an upper Midwest study, but also suggested the need for additional evaluations. One additional recommendation from this study is to evaluate Punnis as an elemental bioindicator for the MidA region. It appears from evaluation of 1994–2005 species distributions that considering nearby forest cover will be more helpful to predict bioindicator species representation at plots in the MidA region than in the NE region. Additional FIA lichen data from 1994–2005 are available for several eastern regions to conduct wider evaluations that support implementation of lichen elemental bioindicators in the NC, NE, MidA, and SE States. However, declines in air pollution since 2005 may have increased the relative importance of nearby forest cover to predicting distribution of bioindicator lichen species in all regions. Because of this, nearby forest cover should be evaluated as a factor during implementation in all eastern U.S. regions regardless of patterns from older lichen distribution data. Reliable and cost-effective plot-level bioindicators for air quality are needed to improve assessment of the environmental health of eastern U.S. forests, especially in the absence of other reliable indicators for local air quality across a wide region.

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LITERATURE CITED


