sciendo

Plant and Fungal Systematics 64(2): 137–147, 2019
DOI: 10.2478/pfs-2019-0015

ISSN 2544-7459 (print)
ISSN 2657-5000 (online)

Lichen species as element bioindicators for air pollution in the eastern United States of America

Susan Will-Wolf[1*] & Sarah Jovan[2]

Article info

Received: 25 Apr. 2019
Revision received: 13 Aug. 2019
Accepted: 14 Aug. 2019
Published: 2 Dec. 2019

Associate Editor

Robert Lücking

Abstract. Lichen element (N, S, metals) indicators of local air pollution load (a widely used technique) are recommended for five predefined regions covering central and southern parts of the eastern United States. The final recommendations integrate the advice of regional lichenologists, information from regional floras, and species abundance data from a United States Forest Service Forest Inventory and Analysis Program (FIA) lichen database for 11 of the 21 covered eastern states. Recommended species were frequent in their region, easy for nonspecialists to distinguish in the field after training, and easy to handle using clean protocols. Regression models of species abundance in FIA plots from five southeastern states vs. climate, air pollution (both from a regional lichen response model) and type of nearby landcover (from the National Land Cover Database) identified species' environmental limitations. *Punctelia rudecta* is recommended for cooler forested uplands of all regions, with three *Physcia* species combined and *Punctelia missouriensis* for isolated woodlands or urban areas of three regions. *Parmotrema hypotropum* and *P. hypoleucinum* combined (weak environmental limitation) or *P. perforatum*. and *P. subrigidum* combined (limited in more polluted areas) are recommended for warmer Coastal Plains in two regions each. Additional species are recommended for single regions. Each species must be quantitatively evaluated in each region, to demonstrate indication reliability in practice and to calculate element data conversions between species for region-wide bioindication.

Key words: air quality, Florida, lichen element indicator, Mid-Atlantic, nitrogen, Ohio Valley, South Central USA, Southeast USA, sulfur

Introduction

We propose lichen species for element-based bioindication of air pollution in central and southern parts of the eastern United States of America, to support broad assessments of environmental health in those regions. Element concentration in lichens is a long-tested tool for bioindication of pollution (Ferry et al. 1973; Lawrey 1984) that is popular and cost-effective (Lawrey 2011; Paoli et al. 2014; Root et al. 2015; Donovan et al. 2016); it complements costly instrumented monitoring to represent the local pollution load more accurately than regional pollution models do (Bari et al. 2001; Geiser & Neitlich 2007; Boquete et al. 2009; Will-Wolf et al. 2015b, 2017a, 2018b). The original emphasis on the impact of SO_2 and heavy metals pollution on lichens (Ferry et al. 1973) has recently shifted in developed countries to the impact of N pollution (Jovan et al. 2012; Fenn et al. 2003). However, the use of S, Al, Fe and other metals as well as N as biomonitors of pollution load (not just impacts on sensitive lichens) remains important in modern studies (e.g., Glavich & Geiser 2008; Will-Wolf et al. 2015a; Donovan et al. 2016). Epiphytic lichens have been the primary focus of element bioindication (e.g., Bargagli & Nimis 2002); saxicolous lichens have also been used (e.g., Lawrey 1993; Zschau et al. 2003).

Element bioindicator species should be moderately pollution-tolerant (element concentration reflects the environmental load more than internal metabolic response does: Bargagli & Mikhailova 2002; Wolterbeek 2002; Yemets et al. 2014; Will-Wolf et al. 2017a), common and widespread (broad coverage of many sites across regions) and easy to distinguish in the field to support collection of 1.5–2 g multi-individual samples (e.g., Puckett 1988; Conti & Cecchetti 2001; Wolterbeek 2002). Species easy to collect and prepare for measurement, combined with efficient laboratory protocols, support economic feasibility for a large study (Glavich & Geiser 2008; Will-Wolf

[1] Department of Botany, University of Wisconsin-Madison, 430 Lincoln Drive, Madison, Wisconsin 53706-1381, USA.
[2] USDA Forest Service, Portland Forestry Sciences Lab, 620 SW Main, Suite 400, Portland, Oregon 97205, USA. e-mail: sjovan@fs.fed.us
[*] Corresponding author e-mail: swwolf@wisc.edu

et al. 2017b, 2019). The cost factor means that mostly macrolichens (foliose and fruticose growth forms) are used. Bioindication from a single species is preferred, while full coverage of sites across large and ecologically variable regions often requires multiple species (Bargagli & Mikhailova 2002; Wolterbeek 2002). Element accumulation rates often differ by species; data conversion between species provides equivalent element data across such large regions (e.g., Sloof & Wolterbeek 1993; Karakas & Tuncel 2004; Root et al. 2015; Will-Wolf et al. 2017a, 2018b). Rigorous field protocols help ensure the quality of measured element data (Bargagli & Nimis 2002). Collection of large pieces of mature specimens reduces the variation of element content and facilitates species confirmation later (important for samples from nonspecialists: Will-Wolf et al. 2017a). Immature thalli can be more pollution-sensitive than mature thalli (maturity affects element accumulation rates: Lawrey & Hale 1977, 1979); element concentration can differ by the physical location within the thallus (Bargagli & Mikhailova 2002). Composite samples from multiple locations within a narrow range of substrate and habitat conditions (trees or rocks only, narrow range of canopy cover, etc.) reduce and level out within-site variation, increasing the reliability of site-level representations of the pollution load (Wolterbeek 2000; Garty 2002; Will-Wolf et al. 2017b).

Three of five epiphytic lichen species collected by trained nonspecialist field staff (Will-Wolf et al. 2017a, b, 2019) for element (N, S, many metals) bioindication in the Forest Inventory and Analysis Program (FIA; United States Department of Agriculture, Forest Service) for the North Central FIA lichen region (Fig. 1) are relevant to this study. Wide coverage of that study area was achieved by combining data from *Flavoparmelia caperata* (linked with higher proportion of nearby forested landcover) and *Physcia aipolia* plus *P. stellaris* (linked with lower proportion of nearby forested landcover). *Physcia* species (see Table 1) grouped for multi-individual samples had been found to be only ~80% distinguishable by a lichen specialist without chemical spot tests in the field (Will-Wolf, pers. comm.). *Punctelia rudecta* was not reliably distinguished in the field from other gray foliose species, leading to poor data quality. *P. rudecta* and other gray foliose species were indeed reliable element bioindicators in studies of eastern states when collected by lichen specialists (Olmez et al. 1985; Lawrey 1993; Bennett & Wetmore 1999; Cleavitt et al. 2015; Will-Wolf et al.

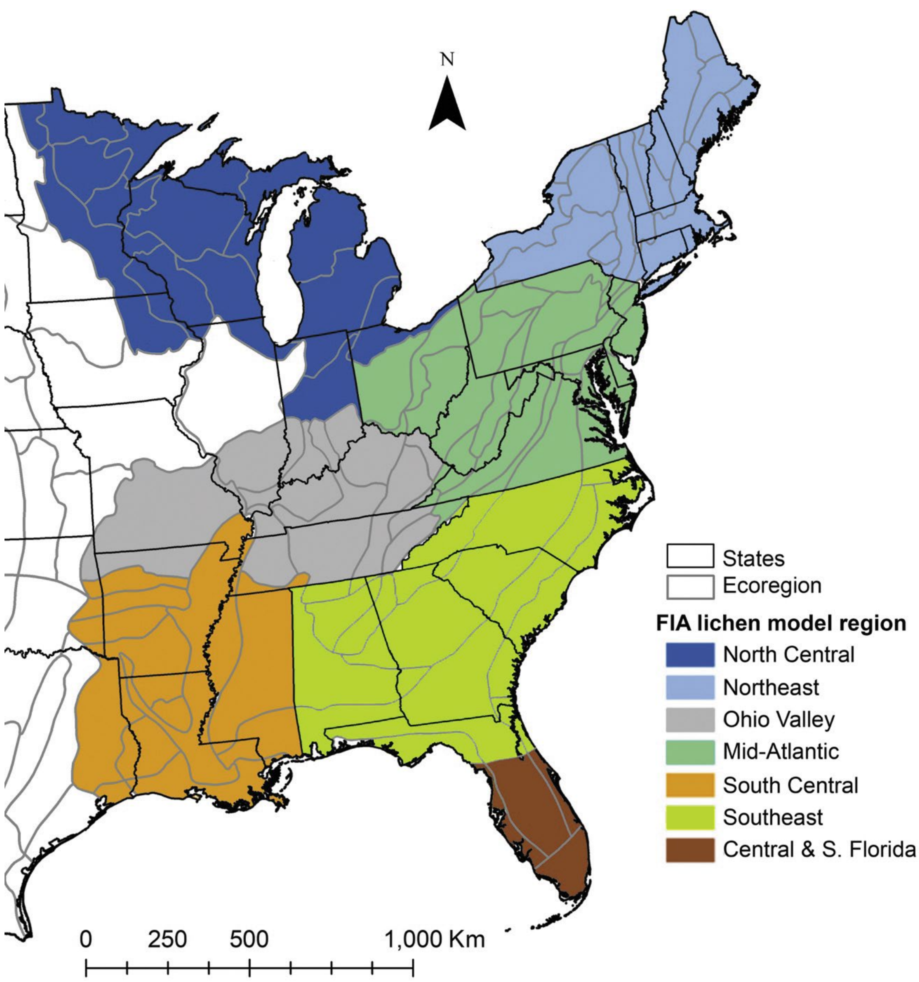

Figure 1. Eastern USA Forest Inventory and Analysis Program lichen model regions (adapted with permission from Will-Wolf & Neitlich 2010). Boundaries for the Northeast, Mid-Atlantic and Southeast regions with published lichen models (McCune et al. 1997; Will-Wolf et al. 2015a, 2018b) coincide mostly with state borders. Virginia, in the Mid-Atlantic region for this map, is also included in the Southeast region for lichen models. Boundaries for other, proposed, regions reflect ecoregion boundaries (Cleland et al. 2007; McNab et al. 2007) to a greater extent.

Table 1. Frequency in FIA plots of lichen species or species groups recommended as possible lichen element bioindicators for the Mid-Atlantic and southern FIA regions from Figure 1 (high numbers in bold). Older synonyms for lichen species in brackets. Abbreviations: N = number of plots; p = plots. [1] From Will-Wolf et al. (2018a, 2019)

Lichen species or group	Frequency in plots (% of total)		
	Mid-Atlantic region	Virginia	Alabama, Georgia, North Carolina, South Carolina
	N=779 p	N=81 p	N=269 p
Flavoparmelia caperata (L.) Hale [= *Pseudoparmelia caperata*]	**76.9**[1]	**95.1**	48.7
Punctelia rudecta (Ach.) Krog.	58.0[1]	95.1	73.2
Punctelia missouriensis G. Wilh & Ladd	10.8[1]	14.8	1.5
Physcia aipolia/pumilior/stellaris	23.1[1]	**46.9**	27.9
Physcia aipolia (Ehrh. ex Humb.) Fürnr. var. *aipolia* and/or *P. pumilior* R. C. Harris	10.3	23.5	19.7
Physcia stellaris (L.) Nyl.	15.6	32.1	12.6
Canoparmelia caroliniana (Nyl.) Elix & Hale	–	19.8	**58.0**
Parmotrema hypotropum (Nyl.) Hale and/or *P. hypoleucinum* (Steiner) Hale	–	**76.5**	39.4
Parmotrema perforatum/subrigidum	–	16.0	**46.1**
Parmotrema perforatum (Jacq.) A. Massal.	–	12.3	**45.4**
Parmotrema subrigidum Egan [= *P. rigidum*]	–	3.7	17.1
Parmotrema reticulatum (Taylor) M. Choisy	–	**50.6**	**57.6**
Parmotrema tinctorum (Delise *ex* Nyl.) Hale	–	3.7	21.6
Usnea strigosa (Ach.) Eaton	–	**49.4**	**62.5**

2015b, 2018b). *F. caperata* has been the most frequently used species (Schutte 1977; Olmez et al. 1985; Lawrey 1985, 2011; Showman & Hendricks 1989; Glavich & Geiser 2008; review in Will-Wolf et al. 2017b).

Lichen species were recommended by Will-Wolf et al. (2018a) for element bioindication in the Mid-Atlantic and Southeast FIA lichen regions, as delineated in Figure 1, based on the above study and Will-Wolf et al. (2015b, 2018b), as well as the relative frequency of studied species from FIA lichen data from eastern states (Jovan et al. 2020a, b). Proportion of nearby forested landcover helped explain geographic coverage by multiple species in the Mid-Atlantic but not the Southeast FIA region. Recommendations for the Mid-Atlantic FIA region included adding other species to *Flavoparmelia caperata* and *Punctelia rudecta* previously used there (Will-Wolf et al. 2018b). These two species were also recommended for the Southeast region, noting that additional species are needed for the Coastal Plain area. The reliable presence of trees across most of the eastern United States underlies our focus on corticolous lichen species for this study. We also considered one saxicolous species: *F. baltimorensis* was a successful element bioindicator of pollution load (Lawrey 1985, 1993; Lawrey & Hale 1977, 1979, 1981, 1988: Pb featured; N, S, and metals included in some studies) in the Washington, DC area straddling the Mid-Atlantic and Southeast FIA regions. This species, closely related to *F. caperata*, has the same range in eastern states (Brodo et al. 2001). Earlier lichen element bioindication studies in the Southeast, Florida, Ohio Valley and South Central FIA regions (Fig. 1) were in small areas (Bosserman & Hagner 1981, *Usnea* and *Parmelia* genus-only; Walther et al. 1990; Pyatt et al. 1999) or in scattered National Park system units (Wetmore 1983, 1992; Wetmore & Bennett 1997: some large); they did report validated data for many elements.

We had three objectives for this study: (i) to supplement recommendations of Will-Wolf et al. (2018a) for the Mid-Atlantic FIA region, (ii) to revise recommendations (ibid.) for the Southeast FIA region, and (iii) to newly recommend species for testing as element bioindicators for the Florida, Ohio Valley and South Central FIA regions (Fig. 1: none of the latter three regions currently has much lichen element data). The government network's instrumented monitoring sites are few and are unevenly scattered in all regions; lichen element bioindication is an alternative to very expensive on-site instrumented monitoring (Will-Wolf et al. 2017a, 2018a). New or revised recommendations are based on field observations by region experts, published studies and quantitative evaluation of FIA data on the distribution and abundance of lichen species in the Southeast FIA region. Based on our evaluation of the response of lichens to the environment, we selected species groups likely to cover each broad region.

Methods

We used several approaches to identify and evaluate additional lichen species to recommend for element bioindication in FIA lichen regions. In addition to the general characteristics for suitable element bioindicator species, and the protocol guidelines (noted in Introduction), we followed two constraints for recommending species for use in FIA and similarly large-scale and cost-conscious programs: (1) A suite of no more than four species should together achieve ≥90% coverage across an FIA lichen region; and (2) the species or species group should be reliably distinguishable in the field by nonspecialists after one or two days of training. For a designated group of two lookalike species, a multi-individual sample could include either each species alone or both together. Protocol elements used for the FIA and suggested for evaluation of recommended species include: (i) clean sample collection and handling protocols (reduce off-site contamination), (ii) ~0.5 hr average for field collection of 1–2 species, 2 samples each, 6+ individuals/sample from across site, with samples kept cool and dry, (iii) species confirmation (evaluate training success) with a dissecting microscope, UV lamp and chemical spot tests only, with species groups not separated, (iv) ~0.75 hr/sample for all preparation, including removal of substrate, (v) measure (pre-screened good-quality samples) many elements (N, S, Al, Fe, other metals) in a certified batch-processing facility, (vi) validate data for each element (internal laboratory standards, data repeatability from site replicate samples, external lichen standards with each batch), and (vii) post-screen data for validated elements (Conti & Cecchetti 2001; Donovan et al. 2016; Will-Wolf et al. 2017b). The goal for protocol elements is to efficiently generate reliable data within cost constraints. With published lichen element studies covering only small parts of the Southeast, Florida, Ohio Valley and South Central FIA regions, we consulted regional lichen experts for species recommendations. We analyzed the available FIA lichen abundance data to identify the environmental limitations of recommended species; this was made possible by prior modeling of regional lichen community responses to general air quality (NOx and SOx combined) and climate (McCune et al. 1997). Expert recommendations and analyses, ease of field recognition by nonspecialist personnel, and ease of laboratory handling supported the final species recommendations.

Five lichenologists who work regularly in our target regions were consulted for this project. The characteristics of the recommended species, the most likely lookalike species for each, and the likelihood that nonspecialists could be trained to reliably distinguish recommended species from others were also evaluated, using details given in taxonomic treatments for the regions (Harris 1990; Brodo et al. 2001; DeBolt et al. 2007; Lendemer et al. 2013; Brodo 2016; Lendemer & Noell 2018; Harris & Ladd 2019). James Lendemer (ibid.) is a professional lichenologist at the New York Botanical Garden with extensive experience, collections, and publications on lichens in the Mid-Atlantic and Southeast FIA regions. Malcolm Hodges (Lücking et al. 2011; Buck 2016; Seabrook 2018) and Sean Q. Beeching (Hill et al. 2007; Lücking et al. 2011; Beeching 2016; Buck 2016) conduct county lichen surveys in the state of Georgia, USA. They regularly consult with lichen experts (especially James Lendemer), attend Tuckerman Lichen Workshops (Buck 2016) and deposit vouchers in public herbaria. They are considered by American professional lichenologists to be among the most knowledgeable (though technically amateur) local lichenologists for this region. Roger Rosentreter, a professional lichenologist focused on the western USA (Rosentreter et al. 2016; McCune et al. 2018), has also worked in the eastern United States (Will-Wolf et al. 2015a), and has for several years documented Florida macrolichens (DeBolt et al. 2007). Professional lichenologist Douglas Ladd has focused on midcontinent USA (Wilhelm & Ladd 1992; Ladd 2002; Peck et al. 2004; Harris & Ladd 2019), including the South Central and western Ohio Valley FIA regions (Fig. 1).

Occurrence data were extracted from the FIA lichen database (Jovan et al. 2019b) for the Mid-Atlantic and Southeast FIA regions to support additional analyses of recommended lichen species. The data covered species newly recommended by consultants as well as those previously recommended from other studies (Will-Wolf et al. 2018a). Data from permanent FIA plots located in a stratified random manner (USDA FS 2017) give explicitly unbiased quantitative representations of species occurrence and abundance in forested areas, to complement the recommendations of consulting lichenologists. Virginia data were separated from data for other Southeast FIA region states, because earlier lichen regional community response models (McCune et al. 1997; Will-Wolf et al. 2018b) demonstrated notable differences in lichen species between the Mid-Atlantic and Southeast FIA regions. Separate analyses for Virginia (at the boundary and included in both regional models) facilitated more detailed comparisons of changes in species frequency along this north–south gradient. The frequencies of possible bioindicator species for Virginia and the other four Southeast FIA region states were compared with frequencies for the entire Mid-Atlantic FIA region (Will-Wolf et al. 2018a, 2019).

Using correlation and linear regression (SPSS 2015), we examined the relationships of possible bioindicator species for Virginia and the Southeast FIA region to climate, air quality and type of nearby landcover. The analyses used original abundance data from FIA plots and both original and log10-transformed data for environmental variables. For correlations, the stronger of Pearson r or Spearman *rho*, probability (p), and direction are reported. For regressions, the strongest model equation (data transformation encoded in variable name as needed), adjusted r^2 and p are reported. To account for experiment-wide error, correlations and regressions with $0.05 > p > 0.005$ were considered weak; only those with correlation r^2 or $rho^2 \geq 0.10$ or regression-adjusted $r^2 \geq 0.10$ (accounting for at least 10% of variation) were considered ecologically important.

Environmental variables were obtained for all plots (Table 1: N = 81 for Virginia; N = 269 for four Southeast states). The percentage of developed, agricultural and forested land in a circular 3.14 km^2 area centered on a plot (1000 m radius) represented nearby landcover (buffer size from Will-Wolf et al. 2017a, 2019). Data were extracted using ArcMap10.5.1 (ESRI 2018) from the public National Land Cover Database NLCD2011 (Wickham et al. 2014; MLRC 2019), using exact plot coordinates. Climate and air quality at each plot were represented by scores of each plot on Axes 1 and 2 of the Southeast FIA region lichen nonmetric multidimensional scaling ordination (NMS) model (McCune et al. 1997), calculated in PC-ORDv6.22 routine NMS Scores (McCune & Mefford 2019). Plot scores on each axis are calculated only from their lichen species composition. The axes represent major gradients in lichen community composition across the region, interpreted from correlations with external environmental variables. Axis 1 scores (Climate Index; correlated with both elevation and latitude) are higher for plots with species composition indicating cooler climates. Axis 2 scores (Air Quality Index; correlated with known pollution status of plots) are higher for plots with species composition indicating cleaner air.

Results and discussion

Each of the five regional lichenologists consulted for the FIA lichen regions (Fig. 1) emphasized ecological differences between the uplands (not tied to specific elevations in this study: Ozark highlands/uplands for the western Ohio Valley and South Central FIA regions; the eastern Ohio Valley region; southern Appalachians/Piedmont/other uplands for the Mid-Atlantic and Southeast FIA regions) and lowlands (often described as below the 'Fall Line' – a regional elevation-linked geographic boundary) including the Coastal Plains (along both the Atlantic and Gulf Coasts; Florida is all Coastal Plain at the scale of this study) that guided their recommendations (Table 1). Recommendations for uplands were consistent between experts. All agreed that species in the genus *Parmotrema* are important to consider for lowland and Coastal Plain areas; suggestions for particular species differed. As studies comparing element accumulation of *Parmotrema* species are lacking (many *Parmotrema* species occur in these regions), we did not consider using *Parmotrema* as a genus-level indicator (data likely too variable, given the many species) for any region. No specific recommendations were given by regional lichenologists for the eastern Ohio Valley FIA region; recommendations for uplands of adjacent regions are suggested. Occurrence models for recommended species in Virginia and the other four Southeast FIA region states (Tables 2 and 3) aided our selection of sets of species to fully cover each region. In general, the distributions of proposed species were less limited by the amount of nearby forest cover in this study than was found for the full Mid-Atlantic FIA region (Will-Wolf et al. 2018b). Some taxonomic issues were identified during this study; they are described and our solutions are explained in the next two paragraphs.

Beeching and Hodges reported that *Physcia aipolia* is not found in Georgia; their collections identified by James Lendemer are the physically and chemically similar *P. pumilior* described by Harris (1990) from Florida. Lendemer reported that *P. pumilior* rather than *P. aipolia* is found through most of the central and eastern parts of the Mid-Atlantic FIA region (Lendemer & Noell 2018). Brodo et al. (2013), citing specimens from four northern North American herbaria and all New York Botanical Garden collections (NYBG 2019), reflected that *P. aipolia* does not occur in the eastern half of the Mid-Atlantic region (sensu Fig. 1) nor in Southeast region states, where *P. pumilior* is widespread and common. The distributions of the two probably overlap in the far western Mid-Atlantic region and in the Ohio Valley region; overlap is confirmed along the western parts of the South Central and Ohio Valley FIA regions (Ladd pers. comm.). Brodo et al. (2001) reported the wide distribution of *P. pumilior* across the southeastern USA and described *P. aipolia* in updated species keys (Brodo 2016) only as generally temperate, pending re-evaluation of specimens in many

Table 2. Correlations of potential element indicator species with environmental variables. Abbreviations: N = number of plots; NS = not significant; p = probability; P = Pearson correlation; S = Spearman correlation. Table 1 species not listed here had no significant correlations with environmental variables.

	Southeast model Climate Index (higher value for cooler)	Southeast model Air Quality Index (higher value for cleaner air)	% developed land	% forested land
Virginia N = 81				
Canoparmelia caroliniana	–0.411S, p = 0.0001	NS	NS	–0.473P, p < 0.00005
Physcia stellaris	NS	NS	NS	0.321P, p = 0.003
Punctelia rudecta	NS	0.453P, p < 0.00005	NS	NS
Parmotrema hypotropum/ hypoleucinum	–0.339P, p = 0.002	0.355S, p = 0.0001	NS	NS
Parmotrema perforatum	–0.461P, p < 0.00005	NS	weak negative	NS
Parmotrema perforatum/ subrigidum	–0.451P, p < 0.00005	NS	–0.394S, p = 0.0003	NS
Parmotrema reticulatum	NS	0.701S, p < 0.00005	NS	NS
Usnea strigosa	–0.469S, p < 0.00005	0.430S, p = 0.0001	NS	NS
Alabama, Georgia, North Carolina, South Carolina N = 269				
Flavoparmelia caperata	0.473S, p < 0.00005	NS	NS	NS
Punctelia rudecta	0.411S, p < 0.00005	NS	NS	NS
Parmotrema hypotropum/ hypoleucinum	0.308S, p < 0.00005	NS	NS	weak negative
Parmotrema perforatum	weak negative	weak positive	NS	NS
Parmotrema perforatum/ subrigidum	weak negative	weak positive	NS	NS
Parmotrema reticulatum	weak positive	0.323P, p < 0.00005	NS	NS
Usnea strigosa	NS	0.318S, p < 0.00005	weak negative	NS

Table 3. Regression models that explain >10% of variation in lichen species abundance. Probability = < 0.0005 for all models. Ab = abundance; AirQuaInd = Southeast Air Quality Index; ClimInd = Southeast Climate Index; dvlp% = % nearby developed landcover; for% = % nearby forested landcover; L10 = logarithm base-10-transformed. [1]Constant not significant

Species	Regression equation	F	adjusted r^2
Virginia models N = 81p			
Canoparmelia caroliniana	Ab = 10.491 – 0.484 * for%L10 + 0.296 * AirQuaIndL10 – 0.232 * ClimIndL10	16.4745	0.3672
Punctelia rudecta	Ab[1] = 0.463 * AirQuaL10	21.6084	0.2048
Parmotrema hypotropum/hypoleucinum	Ab[1] = 0.427 * AirQuaIndL10 – 0.368 * ClimInd	16.4086	0.2781
Parmotrema perforatum/subrigidum	Ab = 8.012 – 0.498 * ClimIndL10 – 0.281 * dvlp%L10	17.6706	0.3195
Parmotrema reticulatum	Ab = –1.348 + 0.696 * AirQuaInd	74.1620	0.4777
Usnea strigosa	Ab = 1.429 + 0.500 * AirQuaInd – 0.383 * ClimInd	22.4462	0.3701
Southeast four-state models N = 269 p			
Flavoparmelia caperata	Ab = 0.890 + 0.489 * ClimInd – 0.282 * AirQuaInd	43.8334	0.2422
Punctelia rudecta	Ab = –1.478 + 0.380 * ClimIndL10 + 0.146 * AirQuaIndL10	28.5932	0.1762
Parmotrema perforatum/subrigidum	Ab = –1.513 + 0.358 * AirQuaInd – 0.234 * ClimInd	22.2277	0.1493
Parmotrema reticulatum	Ab = –3.184 + 0.341 * AirQuaIndL10 + 0.211 * ClimIndL10	28.7821	0.1772
Usnea strigosa	Ab = –1.308 + 0.324 * AirQuaIndL10 – 0.133 * dvlp%	22.4923	0.1391

other USA herbaria (CNALH 2019). All Mid-Atlantic and Southeast FIA region specimens (most identified in 1993–1999) are currently recorded as *P. aipolia* (Jovan et al. 2019a, b); most would now be identified as *P. pumilior*. Distinguishing between these two species and *P. stellaris* in the field for a large element bioindicator study will likely remain as impractical for both experts and nonspecialists as it was for the North Central FIA region project (Will-Wolf 2017a, b). *P. aipolia* and *P. pumilior* have been grouped for Table 1, and all three *Physcia* species have been grouped for evaluation as one composite element indicator.

A taxonomic issue regarding *Parmotrema hypotropum* and *P. perforatum* affects their use as well the use of *P. hypoleucinum* and *P. subrigidum* as element indicators. Molecular studies of the group by Lendemer et al. (2015) found that *P. hypotropum* (sterile, sorediate) and *P. perforatum* (fertile, not sorediate) probably are the same species, while genetically distinct *P. subrigidum* is closely related and the distinct *P. hypoleucinum* is more distantly related to them. *P. perforatum* and *P. subrigidum* (both fertile) are not visually distinguishable in the field even by experts, though they can be distinguished with chemical spot tests. They must be combined in multi-individual samples for efficient element bioindication. *P. hypotropum* and *P. hypoleucinum* (both sorediate) are similarly indistinguishable in the field; Lendemer has found they are also not even reliably distinguishable with chemical spot tests – usually only thin-layer chromatography was definitive. They also must be combined for element bioindication. *P. hypotropum/hypoleucinum* is the most distinctive as well as one of the largest of the many marginally sorediate *Parmotrema* taxa in generally southeastern states of the USA (Brodo et al. 2001). Thus the combinations required for element bioindication mix two distinct species while at the same time splitting what is likely a single species. Both *P. hypoleucinum* (6 records in Mid-Atlantic and Southeast FIA regions combined, 4 at sites with *P. hypotropum*; identification uncertain, not separated for Table 1) and *P. subrigidum* (identification reliable, separated for Table 1) are uncommon in FIA records.

Mid-Atlantic and Southeast FIA region recommendations

Recommendations for the Mid-Atlantic FIA region from Will-Wolf et al. (2018a) are expanded in this study. Based on recommendations by Lendemer, plus species abundance in Virginia (Table 1), we recommend *Parmotrema hypotropum/hypoleucinum* for evaluation as an additional element indicator for lowland and Coastal Plain areas in the entire region. Lendemer noted that *P. hypoleucinum* predominates near the coast, with *P. hypotropum* usually more inland. The association of the pair with warmer climates and less polluted areas (Tables 2, 3) complements the previously used indicators *Flavoparmelia caperata* (absent under 'Virginia' in Tables 2 and 3, because no significant results; this indicates its distribution there was not correlated with and thus was not limited by the tested environmental factors) and *Punctelia rudecta* (Will-Wolf et al. 2018b; not limited by climate). It would be useful to assess differences in element accumulation between *Parmotrema hypotropum* and *P. hypoleucinum*, but that would be quite difficult given that TLC could be required to distinguish them. An earlier recommendation to evaluate composite *Physcia aipolia/pumilior/stellaris* for better coverage of urban and other locations with less nearby forested landcover is supported for Virginia, where this group has no environmental limitations (absent from Tables 2, 3). The clear differences in species frequency and response to environment between Virginia and the other four Southeast region states (Tables 1–3) are consistent with a strong north–south gradient in lichen species composition for this region noted by McCune et al. (1997) and documented in greater detail by Lendemer and Noell (2018) and Lendemer et al. (2016). The frequencies (Table 1) confirm Virginia as intermediate and reasonable to include in both the Mid-Atlantic FIA region (Will-Wolf 2018b) and Southeast FIA region (McCune et al. 1997) models.

For the four Southeast FIA region states of Alabama, Georgia, North Carolina and South Carolina, Lendemer, Hodges and Beeching agreed with Will-Wolf et al. (2018a) that *Flavoparmelia caperata* and *Punctelia rudecta* could be useful element bioindicators, but only for upland areas (supported by moderate positive correlations of both species with the Southeast Climate Index: Table 2). Species occurrence (Table 1) confirms their opinion that *P. rudecta* is much more frequent in those states. Regression models (Table 3) show *F. caperata* is more restricted to uplands than *P. rudecta* but less restricted by air pollution. In contrast to the North Central FIA region project (Will-Wolf et al. 2017b), Beeching and Hodges found *Punctelia rudecta* was easy for nonspecialists (teachers in workshops) to distinguish from other similar species, while *F. caperata* was more difficult. They noted that *Physcia pumilior* and *P. stellaris* are not common (supported by species frequencies: Table 1), are mostly upland, and are mostly restricted to twigs.

Hodges, Beeching, and Lendemer (Lendemer & Ruiz 2015) recommended *Canoparmelia caroliniana* as occurring widely through the four-state region, including in cities. The species' broad distribution is also supported by its frequency in FIA data (Table 1) and lack of correlation with any environmental variable (i.e. no distribution limitations) and thus absence from the 'Southeast four states' sections of Tables 2 and 3. Below the Fall Line (i.e. in the Coastal Plain) they suggested *Parmotrema perforatum* (including *P. subrigidum*), *P. reticulatum* and *P. tinctorum* for testing as common across Coastal Plain habitats. With no current evidence on element accumulation rates for either *Parmotrema perforatum* or *P. subrigidum*, a thorough pilot study to support the validity of mixing samples of the two species across a range of habitats would be required. Since this pair of species is most critically needed for Florida (see next section), such a study should be done in that state. Species occurrences (Table 1) reflect their commonness across the four states, showing *P. tinctorum* as notably less common than the other three (therefore less useful as an indicator). *P. reticulatum* is more frequent than *P. perforatum/subrigidum* (Table 1) and less restricted to Coastal Plains and cleaner air (Tables 2, 3). Only *P. perforatum* (and with *P. subrigidum*) was supported from correlations with the Climate Index (Table 2) as preferring the warmer Coastal Plains in these states, to complement *P. rudecta* in uplands. Beeching and Hodges think it would be possible to train nonspecialists to distinguish all four species from others in the field, though *P. perforatum* (including *P. subrigidum*) is notably the most distinctive (Brodo et al. 2001). *C. caroliniana* and *P. reticulatum* probably would require more training to distinguish in the field.

Beeching, Hodges and Lendemer also recommended *Usnea strigosa* (easy to collect) for the four-state region; usually it is sterile in cities, so more training would be needed to distinguish it from other non-apotheciate *Usnea* species. It is frequent (Table 1) and not restricted by type of nearby landcover (those factors not significant; see Table 2) there. Correlations (Table 2) and regressions (Table 3) showing moderate pollution sensitivity are consistent with its observed sterility in cities. Hodges and Beeching noted that *Ramalina* species (*R. stenospora* used by Walther et al. 1990) are easy to collect but unevenly distributed and sometimes difficult to distinguish in the field. Minimally evaluated *Ramalina americana* (Will-Wolf et al. 2017b) had element concentrations a third to a quarter of those in other tested species. Such low concentrations suggest *Ramalina* might require 3–4 g of cleaned multi-individual samples to generate element values that reliably exceed measurement minima for batch procedures.

From our synthesis of all available information sources, we recommend *Punctelia rudecta*, *Parmotrema perforatum/subrigidum* (much easier to distinguish than the slightly more common *P. reticulatum*), *Canoparmelia caroliniana* (more useful than equally common P. *reticulatum* since it is reliably present in cities, as well as more widespread) and possibly *Usnea strigosa* for evaluation as element bioindicators in the four southernmost Southeast FIA region states (Fig. 1). Based on distribution modeling (previous paragraphs), these four species would fully cover the region. *C. caroliniana* and *U. strigosa* will need more intensive training than the other two for nonspecialists to reliably identify them in the field across all habitats.

Florida recommendations

For Florida, Rosentreter and Hodges agreed that *P. perforatum* and its lookalike *P. subrigidum* are likely the most widespread and common (DeBolt et al. 2007) species appropriate for element indication. The two species combined are distinctive (Brodo et al. 2001; Brodo 2016) from all other *Parmotrema* species in the state. Hodges and Rosentreter each noted they had found one of the species common and the other uncommon-to-absent at many sites (different sites for each person); each referred to a different species as the more common in their own central to northern Florida surveys. Lendemer added that, from herbarium records and his own limited collecting, *P. subrigidum* is likely the more frequent of the pair in Florida. As explained in the previous section, these two species need to be grouped for a single element indicator. A thorough comparison of element concentrations between the two species should be done in Florida across the full ranges of pollution and habitat. Rosentreter commented that *Physcia* species are uncommon in Florida, many common species are too tightly appressed to be practical for collecting without damaging trees, and many lookalike *Usnea* species are narrowly distributed across Florida. No further recommendations were made. As we lack FIA data for this region, no species distribution modeling was possible to support the expert recommendations.

South Central and Ohio Valley FIA region recommendations

For the South Central and western Ohio Valley (Missouri, adjacent southern Illinois, western quarters of Kentucky and Tennessee) FIA regions, Ladd commented that

Punctelia rudecta and *Flavoparmelia caperata* would be useful element indicators in uplands there. *P. rudecta* would be the stronger because it is more common and widely distributed (Brodo et al. 2001; Ladd 2002; Peck et al. 2004; Brodo 2016; Harris & Ladd 2019) north of the Coastal Plain; the less common *F. caperata* is a useful secondary species. Ladd reported that *Physcia pumilior* is common in area uplands; both it and *P. aipolia* occur there. The three *Physcia* species as a group extend through cities (*P. stellaris* being the most common there) and more open woodlands. *Punctelia missouriensis* in uplands, though notably less common than *P. rudecta* (Ladd 2002; Harris & Ladd 2019), was suggested as a possible secondary indicator for more open woodlands in the drier parts of the western Ohio Valley and northern South Central FIA regions, as well as eastern Kansas and Oklahoma, and northeastern Texas outside the current boundaries of FIA regions. From Ladd's comments plus results from Will-Wolf et al. (2017b), careful training would be required for nonspecialists to distinguish it from *P. rudecta* and other gray foliose species.

Ladd noted that while the congeners *Flavoparmelia caperata* (on trees) and *F. baltimorensis* (on rocks) prefer different substrates for most of their overlapping ranges (Culberson & Culberson 1982), in the Ozark uplands the two regularly occur on both trees (mostly near bases) and rocks. While trees are widespread, rocks of similar chemical composition are also widespread and plentiful in the Ozark uplands of the South Central and western Ohio Valley FIA regions; a saxicolous element indicator might be of value there. Since *F. baltimorensis* has been a reliable element indicator in the Washington, DC area (Lawrey 1993; review in Introduction), combining the two for a saxicolous indicator, or to compare saxicolous and corticolous indicators with the same pair of species, could be a useful addition to element bioindication in the South Central and Ohio Valley regions. Testing both species might show that their element data could be combined within substrate type without conversion. Ladd suggested *Parmotrema hypotropum/hypoleucinum* for the lowlands and Coastal Plain; he was confident that nonspecialists could be trained to distinguish them from other similar species in this region. Ladd noted that *P. perforatum* is often not fertile in this region (with no soredia; otherwise it would have been called *P. hypotropum*), thus removing its most distinctive character. *Parmotrema praesorediosum*, successfully used for small areas in Louisiana (Walther et al. 1990; Pyatt et al. 1999), is too narrowly distributed (Brodo et al. 2001) to be recommended in this study.

The final recommendations for the South Central and western Ohio Valley FIA regions are *Parmotrema hypotropum/hypoleucinum*, *Physcia aipolia/pumilior/stellari* and *Punctelia rudecta* to be evaluated as primary element indicators, with *F. caperata*, *F. caperata/baltimorensis* and *Punctelia missouriensis* as additional possible species to cover the region. No FIA data are available to support species distribution modeling.

The recommendations for the eastern Ohio Valley FIA region, as well as for wooded areas of less forested landscapes west and north of the Ohio Valley FIA region, are made by inference from recommendations for adjacent regions; no direct recommendations were made for these areas. *Punctelia rudecta* and *F. caperata*, successful element indicators for the adjacent Mid-Atlantic FIA region (Will-Wolf et al. 2017b, 2018b) and recommended for uplands of the Southeast, South Central and western Ohio Valley FIA regions, should be considered for landscapes with more forested landcover in the eastern Ohio Valley FIA region. Combined *Physcia aipolia/pumilior/stellaris* was successful for areas with less forested landcover in the North Central region (Will-Wolf et al. 2017a, 2019) and is recommended for the Mid-Atlantic, South Central and western Ohio Valley FIA regions (Fig. 1); *Punctelia missouriensis* was similarly recommended in all of those regions. Both taxa are recommended for less forested landscapes in the eastern Ohio Valley and outside the current boundaries of FIA lichen regions (southwestern Minnesota, Iowa, northern Missouri, eastern Kansas). As stated earlier, careful training for nonspecialists to distinguish *Punctelia missouriensis* and *P. rudecta* from each other and from other gray foliose lichen species would be needed to generate quality element data.

Conclusions

Additional lichen species recommended by experts as element indicators for the Mid-Atlantic and Southeast FIA regions are strongly supported by quantitative analyses of FIA lichen data for those regions. The weaker limitation of species distributions by type of landcover in the Southeast region, as compared with the full Mid-Atlantic FIA region (Will-Wolf et al. 2018b), simplified the recommendations to fully cover that region. The recommendations for element indicator species in the Florida, South Central and Ohio Valley FIA regions are somewhat less robust, with no support from region-wide quantitative data analysis. Thorough studies in each region should evaluate the reliability of element data for each recommended species (protocols in paragraph 1 of Methods). Measuring many elements (N, S, several metals) is recommended to document pollution from many sources across a large region. Quantitative conversion of validated data for each element between species (from replicate samples across the full air quality range) should be calculated separately for each region; Will-Wolf et al. (2018b) noted that the conversion factors for the same element in the same two lichen species differed between regions. The difficulties that seasoned nonspecialist field staff had in distinguishing some lichen species (Will-Wolf et al. 2017b) leads us to recommend overestimation of training time to distinguish the recommended species in the field, during initiation of a lichen element monitoring effort. This conservative approach will support collection of good-quality element data from the start; the training effort can be eased later as justified. The consulted lichenologists were optimistic about the ease of training nonspecialists within each region to distinguish our primary recommended species.

Acknowledgements

We thank contributing lichen experts Sean Beeching, Malcolm Hodges, Doug Ladd, James Lendemer and Roger Rosentreter for their time and thoughtful species recommendations and related comments. We thank the many FIA field staff who collected lichens in 1994–2005 in the Northern FIA region, which includes most of our Mid-Atlantic FIA lichen region, and in 1993–1999 in the Southern FIA region, which includes Virginia and our Southeast FIA lichen region; their data allowed our species distribution modeling. We thank other FIA co-authors of study-area lichen region model studies (McCune et al. 1997; Will-Wolf et al. 2018b) for their data collection and contributions to the analyses that supported this study. We thank the editors and anonymous reviewers for their comments and suggestions, which notably improved this paper.

References

Bargagli, R. & Mikhailova, I. 2002. Accumulation of inorganic contaminants. In: Nimis, P. L., Scheidegger & C., Wolseley, P. (eds) *Monitoring with Lichens – Monitoring Lichens,* pp. 65–84. NATO Science Series. Kluwer Academic Publishers, The Hague, NL.

Bargagli, R. & Nimis, P. L. 2002. Guidelines for the use of epiphytic lichens as biomonitors of atmospheric deposition of trace elements. In: Nimis, P. L., Scheidegger, C. & Wolseley, P. (eds) *Monitoring with Lichens – Monitoring Lichens*, pp. 295–300. NATO Science Series. Kluwer Academic Publishers, The Hague, NL.

Bari, A., Rosso, A., Minciardi, M. R., Troiani, F. & Piervittori, R. 2001. Analysis of heavy metals in atmospheric particulates in relation to their bioaccumulation in explanted *Pseudevernia furfuracea* thalli. *Environmental Monitoring and Assessment* 69: 205–20.

Beeching, S. Q. 2016. Reading up for the Tuckerman Workshop. *Evansia* 33: 50–53.

Bennett, J. P. & Wetmore, C. M. 1999. Changes in element contents of selected lichens over 11 years in northern Minnesota, USA. *Environmental and Experimental Botany* 41: 75–82.

Bosserman R. W. & Hagner, J. E. 1981. Elemental composition of epiphytic lichens from Okefenokee Swamp. *The Bryologist* 84: 39–47.

Boquete, M. T., Fernández, J. A., Aboal, J. R. & Real, C. 2009. Spatial structure of trace elements in extensive biomonitoring surveys with terrestrial mosses. *Science of the Total Environment* 408: 153–162.

Brodo, I. M. 2016. *Keys to lichens of North America*. Revised and expanded. Yale University Press, New Haven, Connecticut, USA.

Brodo, I. M., Freebury, C. & Alfonso, N. 2013. Notes on the lichens *Physcia aipolia* and *P. alnophila* in North America. *Evansia* 30: 110–119.

Brodo, I. M., Sharnoff, S. D. & Sharnoff, S. 2001. *Lichens of North America*. Yale University Press, New Haven, Connecticut, USA.

Buck, W. R. 2016. The Tuckerman Lichen Workshop and the Crum Bryophyte Workshop: a brief history. *Evansia* 33: 46–49.

Cleavitt, N. L., Hinds, J. W., Poirot, R. L., Geiser, L. H., Dibble, A. C., Leon, B., Perron, R. & Pardo, L. H. 2015. Epiphytic macrolichen communities correspond to patterns of sulfur and nitrogen deposition in the northeastern United States. *The Bryologist* 118: 304–325.

Cleland, D. T., Freeouf, J. A., Keys, J. E., Nowacki, G. J., Carpenter, C. & McNab, W. H. 2007. *Ecological Subregions: Sections and subsections for the conterminous United States.* Gen. Tech. Report WO-76D [Map on CD-ROM] (A.M. Sloan, cartographer) presentation scale 1:3,500,000; colored.U.S. Department of Agriculture, Forest Service, Washington, DC. https://www.fs.usda.gov/treesearch/pubs/48672 [Accessed 2 March 2019]

Consortium of North American Lichen Herbaria [CNALH]. 2019. *Physcia aipolia.* map search. https://lichenportal.org/cnalh/collections/map/index.php [Accessed 7 August 2019]

Conti, M. E. & Cecchetti, G. 2001. Biological monitoring: lichens as bioindicators of air pollution assessment – a review. *Environmental Pollution* 114: 471–492.

Culberson, W. L. & Culberson, C. F. 1982. Evolutionary modification of ecology in a common lichen species. *Systematic Botany* 7: 158–169.

DeBolt, A. M., Rosentreter, R. & Martin, E. P. 2007. Macrolichen diversity in subtropical forests of north-central Florida. *The Bryologist* 110: 254–265.

Donovan, G. H., Jovan, S. E., Gatziolis, D., Burstyn, I., Michael, Y. L., Monleon, V. J. & Amacher, M. C. 2016. Using an epiphytic moss to identify previously unknown sources of atmospheric cadmium pollution. *Science of the Total Environment* 569: 84–93.

ESR1. 2018. ArcMap 10.5.1. *ArcGIS desktop*. https://desktop.arcgis.com/en/arcmap/ [Accessed 2 March 2019]

Fenn, M. E., Baron, J. S., Allen, E. B., Rueth, H. M., Nydick, K. R., Geiser, L., Bowman, W. D., Sickman, J. O., Meixner, T., Johnson, D. W. & Neitlich, P. 2003. Ecological effects of nitrogen deposition in the western United States. *BioScience* 53: 404–420.

Ferry, B. W., Baddeley, M. S. & Hawksworth, D. L. 1973. *Air Pollution and Lichens*. University of Toronto Press, Ontario, Canada.

Garty, J. 2002. Biomonitoring heavy metal pollution with lichens. In: Kranner, I., Beckett, R. P., Varma, A. K. (eds), *Protocols in Lichenology. Culturing, Biochemistry, Ecophysiology and Use in Biomonitoring*, pp. 458–482. Springer-Verlag, Berlin, Germany.

Geiser, L. H. & Neitlich, P. 2007. Air pollution and climate gradients in western Oregon and Washington indicated by epiphytic macrolichens. *Environmental Pollution* 145: 203–218.

Glavich. D. A. & Geiser, L. 2008. Potential approaches to developing lichen-based critical loads and levels for nitrogen, sulfur and metal-containing atmospheric pollutants in North America. *The Bryologist* 111: 638–649.

Hale, M. E., Jr. & Lawrey, J. D. 1985. Annual rate of lead accumulation in the lichen *Pseudoparmelia baltimorensis*. *The Bryologist* 88: 5–7.

Harris, R. C. 1990. *Some Florida Lichens*. Published by the author, Bronx, NY, USA, 109 pp. Available from Recent Literature on Lichens http://www.nhm2.uio.no/botanisk/lav/RLL/PDF/R7810.pdf [Accessed 5 April 2019]

Harris, R. C. & Ladd, D. M. 2019. *Lichens of the Ozarks. Floristics and implications for biodiversity conservation*. New York Botanical Garden and The Nature Conservancy, Bronx, USA https://www.nybg.org/bsci/lichens/ozarks/ [Accessed 5 April 2019]

Hill, R., Jordan, T., Beeching, S. Q., Parks, P., Drennon, C. & Walker, W. 2007. *A guide to twelve common & conspicuous lichens of Georgia's Piedmont. [plus] lichens of Georgia mountains and coastal plains*. https://www.eealliance.org/assets/Documents/Conference_Resources/Conference_Resources2017/guide_to_12_common_lichens_of_georgia_piedmont.pdf [Accessed 5 April 2019]

Jovan, S., Riddell, J., Padgett, P. E. & Nash III, T. H. 2012. Eutrophic lichens respond to multiple forms of N: implications for critical levels and critical loads research. *Ecological Applications* 22: 1910–1922.

Jovan, S., Haldeman, M., Will-Wolf, S., Dillman, K., Geiser, L., Thompson, J. & Shaw, J. 2020a. *National Atlas of Epiphytic Lichens in Forested Habitats, U.S.A*. Accepted manuscript in preparation for publication (as of October 2019). General Technical Report. PNW-GTR-XXX. U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, OR, USA (in press).

Jovan, S., Will-Wolf, S., Geiser, L. & Dillman, K. 2020b. *User guide for the national FIA Lichen database* (beta). Accepted manuscript in preparation (as of October 2019). General Technical Report. PNW-GTR-XXX. U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station., Portland, OR, USA (in press).

Karakas, S. Y. & Tuncel, S. G. 2004. Comparison of accumulation capacities of two lichen species analyzed by instrumental neutron activation analysis. *Journal of Radioanalytical and Nuclear Chemistry* 259: 113–118.

Ladd, D. M. 2002. *Lichens of the Lower Ozark region of Missouri and Arkansas.* Missouri Ozark Forest Ecosystem Project, Missouri.

Department of Conservation, Missouri Botanical Garden, Nature Conservancy, St. Louis, USA. 140 p (revision in progress).

Lawrey, J. D. 1984. *Biology of Lichenized Fungi*. Praeger Publishers, NY, USA. 408 pp.

Lawrey, J. D. 1985. *Lichens as lead and sulfur monitors in Shenandoah National Park, Virginia.* Contract CX-0001-1-0114/PX-0001-4-1128. U.S. National Park Service, Air Quality Division, Ft. Collins, CO, USA.

Lawrey, J. D. 1993: Lichens as monitors of pollutant elements at permanent sites in Maryland and Virginia. *The Bryologist* 96: 339–341.

Lawrey, J. D. 2011. *A lichen biomonitoring program to protect resources in the National Capital Region by detecting air quality effects.* Natural Resource Technical Report NPS/NCRN/NRTR—2011/450. National Park Service, Fort Collins, CO, USA. https://irma.nps.gov/DataStore/DownloadFile/428476 [Accessed 5 April, 2019]

Lawrey, J. D. & Hale, Jr. , M. E. 1977. Natural history of Plummers Island, Maryland. XXIII. Studies on lichen growth rate at Plummers Island, Maryland. *Proceedings of the Biological Society of Washington* 90: 698–725.

Lawrey, J. D. & Hale, Jr., M. E. 1979. Lichen growth response to stress induced by automobile exhaust pollution. *Science* 204: 423–424.

Lawrey, J. D. & Hale, Jr., M. E. 1981. Retrospective study of lichen lead accumulation in the northeastern United States. *The Bryologist* 84: 449–456.

Lawrey, J. D. & Hale, Jr., M. E. 1988. Lichen evidence for changes in atmospheric pollution in Shenandoah National Park, Virginia. *The Bryologist* 91: 21–23.

Lendemer, J. C. & Noell. N. 2018. Delmarva Lichens: An illustrated manual. *Memoirs of the Torrey Botanical Society* 28: 1–386.

Lendemer, J. C. & Ruiz. A. M. 2015. Molecular data confirm morphological variability in the widespread foliose lichen *Canoparmelia carolinana* (Parmeliaceae). *Castanea* 80: 29–36.

Lendemer, J. C., Harris, R. C. & Tripp, E. A. 2013. The lichens and allied fungi of Great Smoky Mountains National Park: an annotated checklist with comprehensive keys. *Memoirs of the New York Botanical Garden* 104: 1–152.

Lendemer, J., Allen, J. & Noell. N. 2015. The P*armotrema* acid test: a look at species delineation in the *P. perforatum* group 40 y later. *Mycologia* 107: 1120–1129.

Lendemer, J. C.; Harris, R. C. & Ruiz, A. M. 2016. A review of the lichens of the Dare Regional Biodiversity Hotspot in the Mid-Atlantic Coastal Plain of North Carolina, eastern North America. *Castanea* 81: 1–77.

Lücking, R., Seavey, F., Common, R., Beeching, S. Q., Breuss, O., Buck, W. R., Crane, L. & Hodges, M. et al. 2011. The lichens of Fakahatchee Strand Preserve State Park, Florida: Proceedings from the 18th Tuckerman Workshop. *Bulletin of the Florida Museum of Natural History* 49: 127–186.

McCune, B., Dey, J. P., Peck, J. E., Heiman, K. & Will-Wolf, S. 1997. Regional gradients in lichen communities of the Southeast United States. *The Bryologist* 100: 145–158.

McCune, B. & Mefford, M. J. 2019. PC-ORDv6.22. *Multivariate analysis of ecological data.* Version 6. Wild Blueberry Media LLC, Corvallis, OR. https://www.wildblueberrymedia.net/pcord [Accessed 5 April 2019]

McCune, B., Arup, U., Breuss, O., Di Meglio. E., Di Meglio, J., Esslinger, T. L., Magain, N., Miadlikowska, J., Miller, A. E., Muggia, L., Nelson, P. R., Rosentreter, R., Schultz, M., Sheard, J. W., Tønsberg, T. & Walton, J. 2018. Biodiversity and ecology of lichens of Katmai and Lake Clark National Parks and Preserves, Alaska. *Mycosphere* 9: 859–930.

McNab, W. H., Cleland, D. T., Freeouf, J. A., Keys, Jr., J. E., Nowacki, G. J., Carpenter, C. A., compilers. 2007. *Description of ecological subregions: sections of the conterminous United States* [CD-ROM]. Gen. Tech. Report WO-76B. U.S. Department of Agriculture, Forest Service, Washington, DC, USA. 80 p. https://www.fs.usda.gov/treesearch/pubs/48669 [Accessed 2 March, 2019]

Multi-Resolution Land characteristics Consortium [MRLC]. 2019. *National Land Cover Database 2011* (NLCD2011). https://www.mrlc.gov/data/nlcd-2011-land-cover-conus [Accessed 28 March, 2019]

Olmez, I., Gulovali, M. C. & Gordon, G. E. 1985. Trace element concentrations in lichens near a coal-fired power plant. *Atmospheric Environment* 19: 1663–1669.

New York Botanical Garden [NYBG]. 2019. *C. V. Starr Virtual Herbarium*. http://sweetgum.nybg.org/science/vh/ [Accessed 5 April, 2019]

Paoli, L., Guttová, A., Grassi, A., Lackovicová, A., Senko, D. & Loppi, S. 2014. Biological effects of airborne pollutants released during cement production assessed with lichens (SW Slovakia). *Ecological Indicators* 40: 127–135.

Peck, J. E., Grabner, J., Ladd, D. & Larsen, D. R. 2004. Microhabitat affinities of Missouri Ozarks lichens. *The Bryologist* 107: 47–61.

Puckett, K. J. 1988. Bryophytes and lichens as monitors of metal deposition. *Bibliotheca Lichenologica* 30: 231–267.

Pyatt, F. B., Grattan, J. P., Lacy, D., Pyatt, A. J. & Seaward, M. R. D. 1999. Comparative effectiveness of *Tillandsia usneoides* L. and *Parmotrema praesorediosum* (Nyl.) Hale as bio-indicators of atmospheric pollution in Louisiana (U.S.A.). *Water, Air, and Soil Pollution* 111: 317–326.

Root, H. T., Geiser, L. H., Jovan, S. & Neitlich, P. 2015. Epiphytic macrolichen indication of air quality and climate in interior forested mountains of the Pacific Northwest, USA. *Ecological Indicators* 53: 95–105.

Rosentreter, R., Eldridge, D., Westberg, M., Briegel-Williams, L. & Grube, M. 2016. Structure, Composition, and Function of Biocrust Lichen Communities. In Weber, B., Büdel, B., Belnap, J. (eds). *Biological Soil Crusts: An Organizing Principle in Drylands*, *Ch 7*, pp. 121–138. Springer, Berlin, Germany.

Schutte, J. A. 1977. Chromium in two corticolous lichens from Ohio and West Virginia. *The Bryologist* 80: 279–283.

Seabrook, C. 2018. A close-up look at lichens can reveal a miniature world. [Features Malcolm Hodges, a collaborator on the Georgia Lichen Project]. *Atlanta Journal-Constitution/Life*. May 11, 2018. https://www.ajc.com/lifestyles/environment/close-look-lichens-can-reveal-miniature-world/ytKSl0ABC8fdnMv4xjtG0O/ [Accessed 5 April 2019]

Showman, R. E. & Hendricks, J. C. 1989. Trace element content of *Flavoparmelia caperata* (L.) Hale due to industrial emissions. *Journal of Air and Waste Management Association* [JAPCA] 39: 317–320.

Sloof, J. E. & Wolterbeek, B, T. 1993. Interspecies comparison of lichens as biomonitors of trace-element air pollution. *Environmental Monitoring and Assessment* 25: 149–157.

SPSS. 2015. *IBM SPSS Statistics*. Version 23.0.0. 1989–2015.

United States Department of Agriculture, Forest Service [USDA FS]. 2017. *Field guides for standard* (*phase 2*) *measurements. Phase 2 measurement field guide, Version 7.1* (downloadable pdf). Forest Inventory and Analysis Program. FIA library. https://fia.fs.fed.us/library/field-guides-methods-proc/index.php [Accessed 5 April 2019]

Walther, D. A., Ramelow, G. J., Beck, J. N., Young, J. C., Callahan, J. D. & Marcon, M. 1990. Temporal changes in metal levels of the lichens *Parmotrema praesorediosum* and *Ramalina stenospora*, southwest Louisiana. *Water, Air, and Soil Pollution* 53: 189–200.

Wetmore, C. M. 1983. *Lichens of the air quality class 1 national parks. Final Report.* National Park Service – AIR, Denver, Colorado, USA. http://gis.nacse.org/lichenair/doc/Wetmore1983.pdf. [Accessed 5 April 2019]

Wetmore, C. 1992. *Lichens and Air Quality in George Washington Carver National Monument.* Report submitted to National Park Service – AIR, Denver, Colorado, USA.

Wetmore, C. & Bennett, J. 1997. *Lichens and Air Quality in Three Prairie Parks: Effigy Mounds National Monument – Iowa, Homestead National Monument – Nebraska, and Wilson's Creek National Battlefield – Missouri.* Report submitted to Biological Resources Division, U.S. Geological Survey, Washington, DC, USA.

Wilhelm, G. & Ladd, D. 1992. A new species of the lichen genus *Punctelia* from the midwestern United States. *Mycotaxon* 44: 495–504.

Wickham, J., Homer, C., Vogelman, J., McKerrow, A., Mueller, R., Herold, N. & Coulston, J. 2014. The Multi-Resolution Land Characteristics (MRLC) Consortium — 20 years of development and integration of USA national land cover data. *Remote Sensing* 6: 7424–7441.

Will-Wolf, S., Jovan, S., Neitlich, P., Peck, J. L. & Rosentreter, R. 2015a. Lichen-based Indexes evaluate responses to climate and air pollution across northeastern U.S.A. *The Bryologist* 118: 59–82.

Will-Wolf, S., Makholm, M. M., Nelsen, M. P. & Trest, M. T. 2015b. Element analysis of two common macrolichens supports bioindication of air pollution and lichen response in rural midwestern U.S.A. *The Bryologist* 118: 371–384.

Will-Wolf, S., Jovan, S. & Amacher, M. C. 2017a. Lichen elemental content bioindicators for air quality in upper Midwest, USA: a model for large-scale monitoring. *Ecological Indicators* 78: 253–263.

Will-Wolf, S., Jovan, S. & Amacher, M. C. 2017b. Lichen elements as environmental indicators: evaluation of methods for large monitoring programs. *The Lichenologist* 49: 415–424.

Will-Wolf, S., Jovan, S. & Amacher, M. C. 2018a. Lichen species to bioindicate air quality in eastern U.S. from elemental composition: lessons from the Midwest. In: Potter, K. M. & Conkling, B. L. (eds). *Forest health monitoring: national status, trends, and analysis 2017*, Ch. 6, pp 101–114. Gen. Tech. Rep. SRS-233. USDA Forest Service, Southern Research Station, Asheville, NC, USA. https://www.srs.fs.usda.gov/pubs/56285 [Accessed 5 April 2019]

Will-Wolf, S., Jovan, S., Nelsen, M. P., Trest, M. T., Rolih, K. & Reis, A. 2018b. Lichen indexes assess response to climate and air quality in the Mid-Atlantic region, USA. *The Bryologist* 121: 461–479.

Will-Wolf, S., Jovan, S., Amacher, M. C. & Patterson, P. L. 2020. *Lichen elemental indicators for air pollution in Eastern U. S. A. forests; a pilot study in the upper Midwest.* Accepted manuscript in preparation for publication (as of October 2019). General Technical Report. U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, OR, USA (in press).

Wolterbeek, B. 2002. Biomonitoring of trace element air pollution: principles, possibilities and perspectives. *Environmental Pollution* 120: 11–21.

Yemets, O. A., Solhaug, K. A. & Gauslaa, Y. 2014. Spatial dispersal of airborne pollutants and their effects on growth and viability of lichen transplants along a rural highway in Norway. *The Lichenologist* 46: 809–823.

Zschau, T., Getty, S., Gries, C., Ameron, Y., Zambrano, A. & Nash III, T. H. 2003. Historical and current atmospheric deposition to the epilithic lichen *Xanthoparmelia* in Maricopa County, Arizona. *Environmental Pollution* 125: 21–30.