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Highway pollution effects on microhabitat community structure of corticolous lichens

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Abstract. We studied lichen communities on bole and base tree trunk segments along forest edge-to-interior gradients on opposite sides of a major highway and a control site in central North Carolina, U.S.A., to investigate if these two communities differ and if so, do they differ in response to highway pollution. At each site we measured various environmental parameters including ambient air NO_2 concentrations, and sampled lichens on 5–7 trees along each of five parallel transects established at the forest edge and at 25, 60, 100 and 150 m into the forest. We compared lichen communities between the two trunk segments via species richness and composition by habit, photobiont type, and reproductive strategy. We then ran dual (bole and base) NMS ordinations with subsequent correlation/regression analyses to explore/test relationships of lichen parameters with environmental variables among the 15 sample transects combined. Species richness was similar between trunk segments at transect and site levels as well as overall. Bole and base communities were more compositionally similar to each other at the highway sites than they were at the control site, based on Bray-Curtis similarity indices (*BC*). Tree base communities differed in terms of functional groupings, with greater proportions of squamulose, cyanolichen and sterile species than found in tree bole communities, but varyingly so among sites. Patterns of bole-base *BC* values with distance from the forest edge were not apparent in any of the sites. Ordination analyses resulted in Axis 1 representing most of the variation for each trunk segment. Along this axis, correlations were similar between boles and bases, with the strongest ones involving lichen species richness (negative) and NO_2 concentrations (positive); notably weak correlations involved tree species number, canopy cover and DBH. Similar patterns were found when lichen species number was correlated with environmental parameters directly, with NO_2 concentration correlating strongest at each trunk segment. Among functional groups, % crustose and % fertile species on bases correlated significantly with NO_2. Lichen species–NO_2 relationships on boles and bases were both found to be highly significant quadratic relationships with base lichen richness being stronger.

Keywords. Lichen biodiversity, NO_2, NMS ordination, tree base, tree bole.

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Lichens are well known to be highly sensitive to atmospheric pollution, with simpler communities found in more adversely affected environments (Nimis et al. 2002). Recent focus has been on effects of nitrogenous air pollution on lichens, including effects of oxidized (e.g., NO_x) forms that are largely

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emitted from fuel combustion (although some NH_3 is emitted from vehicles with catalytic converters designed to reduce NO_x emissions) (NADP 2000). Differential tolerances of lichen species to nitrogenous pollutants lead to a general decrease in species richness, a loss of sensitive species ("acidophytes" or "nitrophobes"), and an increase in species that are adapted to high nitrogen environments ("nitrophytes" or "nitrophiles") (e.g., Davies et al. 2007; Johansson et al. 2012). Observational studies of forest nitrification using lichens as indicators have focused on the western United States (Fenn et al. 2008; Geiser et al. 2010; Jovan et al. 2012) and more recently on the northeast (Will-Wolf et al. 2015). Experimental studies include that of Johansson et al. (2012), who found differential responses to N-solution treatments representing different ambient pollution scenarios, with some species responding more adversely than others.

In our previous report (Perlmutter et al. 2017a) we described lichen communities on tree trunks from the ground to 1.5 m up and how they changed with proximity to a highway, suggesting a negative relationship with highway pollution. However, vertical distribution studies have shown that lichen communities can vary by relative position on the trunk, e.g., in comparisons of tree bases and bole sections at breast height (Fritz 2009; Hale 1965; Komposch & Hafellner 2000; Pirintsos et al. 1993). In particular, cyanolichens, which are known to be pollution-sensitive (Richardson & Cameron 2004), are especially adapted to shaded, moist conditions (Belnap 2001) like those of tree bases. In two North Carolina studies of cyanolichen diversity, most species were found on tree bases, where adequate moisture and stable substrate conditions appeared to be critical for their establishment and growth (Becker 1980; Becker et al. 1977). Yet most air quality effects studies are performed on trunks at breast height (Asta et al. 2002). This report focuses on characterizing response of lichen communities to highway pollution, represented by ambient air NO_2 concentrations reported herein, on both tree boles and bases. This report aims to answer the following two questions: do tree bole lichen communities differ from tree base communities? If so, do bole and base lichen communities respond differently to roadside conditions, and if so, how?

Methods and Materials

Study area. Our study was conducted in western Wake County, North Carolina, in the Triassic Basins Level IV Ecoregion within the Piedmont Level III Ecoregion (Griffith et al. 2002). The Triassic Basins are characterized by metamorphosed sedimentary rocks that are distinct from the granitic geology that is more typical of the Piedmont (Griffith et al. 2002; Omernik & Griffith 2008). Soils in all sites were sandy loams derived from Triassic-age sandstone, shale and mudstone (Cawthorn 1970). The forest structure in all sites consisted of a mixture of hardwoods and conifers, including oaks (*Quercus* spp.), hickories (*Carya* spp.), maples (*Acer* spp.), loblolly pine (*Pinus taeda*), eastern red cedar (*Juniperus virginiana*), tulip tree (*Liriodendron tulipifera*), sweetgum (*Liquidambar styraciflua*) and American holly (*Ilex americana*).

We selected three forested sites in western Wake County for study. Two were along a major highway in a conurban setting, and a third was in a remote area to serve as a background control (**Fig. 1A**). The highway-exposed sites are on opposite sides of Interstate 40 (I-40), an eight-lane highway that has among the highest traffic volume in the county, averaging 162,000 vehicles per day (NCDOT 2014) (**Fig. 1B**). Ambient NO_x concentrations, measured by the Triple Oaks roadside monitor located 3.4 km NW from the sites (**Fig. 1A**), averaged 19.1 ppb in 2015.

The highway sites are in the Raleigh metropolitan area between the City of Raleigh and Research Triangle Park just south of the Raleigh-Durham International Airport (RDU). One site (LC) is on the SW side of I-40 in Lake Crabtree County Park, while the other (RA) is on the NE side of the highway on property owned and managed by the RDU Airport Authority. Both sites include sections of the I-40 right-of-way corridor, managed by the NC Department of Transportation and demarcated with a deer fence at about 30 m into the forest. An intermittent stream flows adjacent to RA and through LC. The control site (HL) is located away from major roads, facing the shore of Harris Lake in Harris Lake County Park (**Fig. 1C**). It is located 13 km away from the nearest urban center in Fuquay-Varina. Outside of the park and neighboring power plant, the area of southeastern Wake County is largely mixed hardwood-pine forest with no nearby agriculture. Ambient NO_x concentrations, measured by

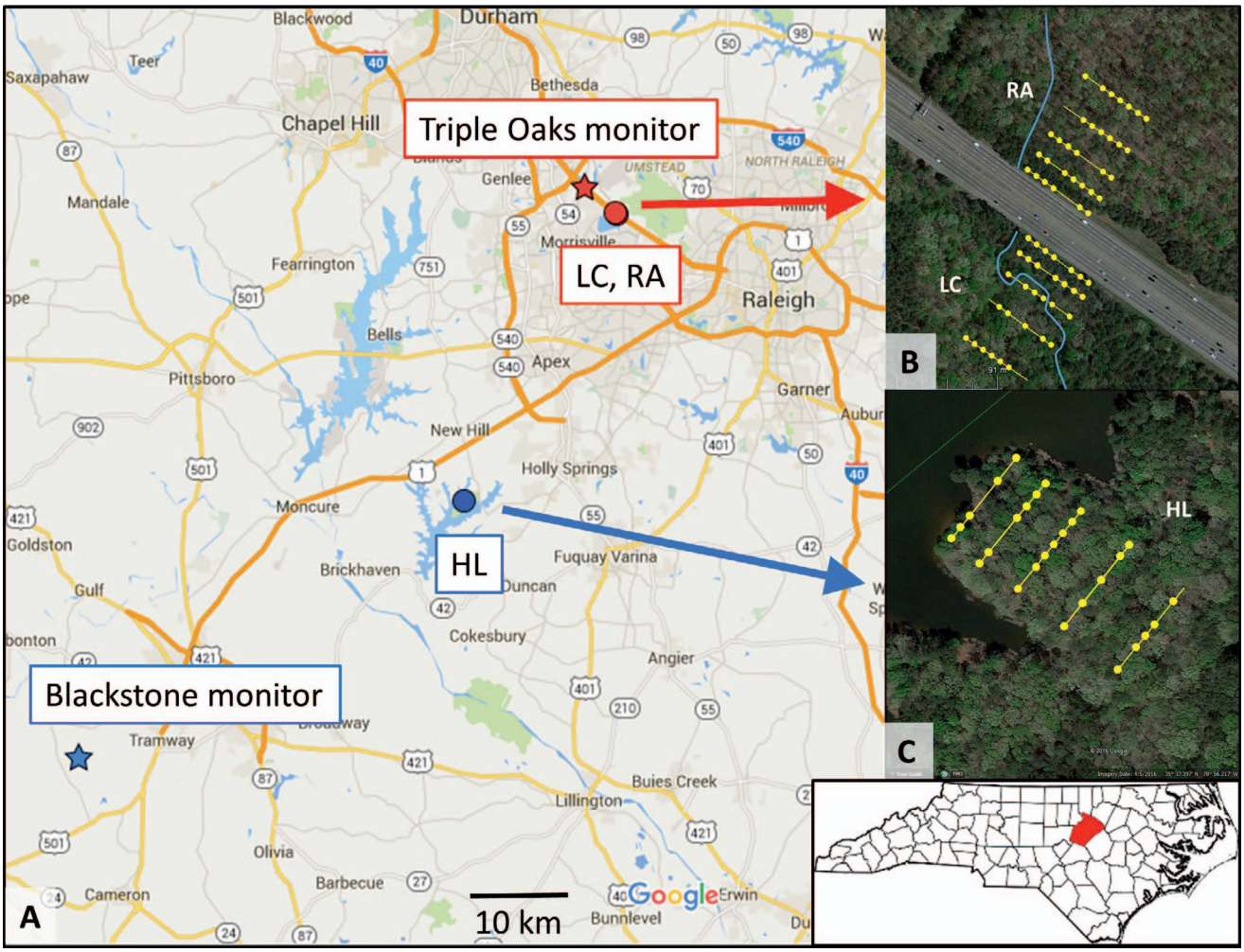


Figure 1. Study sites in Wake County, North Carolina, U.S.A. **A.** Map depicting locations of study sites (•) and ambient air monitoring stations (★). **B.** Highway-exposed sites showing parallel transect layout with sampled trees (light gray dots, yellow in online pdf) and intermittent stream (pale curving line, blue in online pdf). **C.** Control site showing parallel transect layout with sampled trees (dots). LC: Lake Crabtree County Park, RA: RDU Airport Authority property, HL: Harris Lake County Park.

the Blackstone background monitor located 38 km SW from the site in rural Lee County (**Fig. 1A**), averaged 1.7 ppb in 2015. Traffic volume of Blackstone Road, near which the monitor resides, averages 240 vehicles per day (NCDOT 2014).

Sampling design and methods. At each site a series of five 70 m-long line transects was established parallel to the forest edge at the forest edge and at 25, 60, 100 and 150 m distant for a total site area of 1.05 ha. The third transect was originally planned to be at 50 m, but a stream bisects the LC site at that distance, so we moved it to 60 m to sample trees. For consistency we established the third transect at 60 m in the RA and HL sites. In the control site we laid transects on a small peninsula between two minor inlets, because this location provided the best example of mixed forest with an edge exposed to background air quality. To correct for the higher proportion of near-edge microhabitats in this site and to provide comparable edge-to-interior distances to those at the highway sites, we measured distances of all sampled trees to the nearest shoreline and averaged these by transect.

Within each transect we selected one representative tree for trunk lichen community sampling at every 10 meters, if a suitable tree was present. We did not limit tree selection based on trunk diameter or tree species in order to maximize lichen species capture, and to better represent the forest lichen biota of a given transect. Tree selection followed Asta et al. (2001), in which a suitable live tree was free-standing with little or no undergrowth, no obvious

damage or markings, and standing at no more than 10° from the vertical. We recorded each sample tree's species, measured its canopy cover with a spherical crown densiometer, Convex Model A (Forestry Suppliers, Jackson, Mississippi) following Lemmon (1956), and measured the trunk diameter at breast height (DBH). We then split the trunk sampling area into two segments: bole (from 0.5–1.5 m height) and base (from the ground to 0.5 m height). In each trunk segment we sampled the cryptogamic community by recording the presence of bryophytes and collecting voucher specimens of each lichen species encountered. We sampled the three sites in June (LC), July (HL), and October 2014 (RA).

Lichen specimens were identified to species (a few specimens could only be identified to genus, but all taxa were considered "species" for analytical purposes) using keys such as those in Brodo et al. (2001), Harris & Ladd (2005) and Lendemer et al. (2013). Species determinations included laboratory techniques including examination of morphological features, microscopic examination of reproductive structures and chemical spot tests. Specimens were deposited as vouchers in the University of North Carolina, Chapel Hill Herbarium (NCU) with a partial duplicate set in the North Carolina Museum of Natural Sciences–Nature Research Center in Raleigh, North Carolina. We entered specimen records into the Consortium of North American Lichen Herbaria online database (CNALH, www.lichenportal.org) for the NCU vouchers.

Ambient air NO_2 measurements. To directly assess the air pollution environment of the study transects, nitrogen dioxide (NO_2) concentrations were sampled at the midpoint of each transect. We affixed duplicate passive NO_2 samplers (DI-FRAM100 Rapid Air Monitor, Gradko Environmental, UK, here after "RAM samplers") in a shelter that was strapped to a tree at 2 m height for an exposure period of 72 hours from 27–30 April 2015. With a detection limit of 0.037 $\mu g/m^3$ (0.16 ppb), the RAM samplers contain 20% triethanolamine in deionized water absorbent, designed for an uptake rate of 26.8 mL/min at 20°C and thus shorter exposure periods (one hour to one week) than other passive samplers, which are designed for 1–2 week exposure periods (Gradko 2012). We chose a 72-hr exposure period to minimize risk of sampler loss through vandalism in the public-accessible site locations.

At the end of the exposure period, the samplers were removed and shipped with a set of field blanks (i.e., unexposed samplers) to Ormantine U.S.A. (Palm Bay, Florida) for analysis. Samplers were analyzed for NO_2 by UV/visible spectrophotometry of nitrate ions that chemically adsorbed onto the filters during field exposure (Gradko 2012). The resulting NO_2 concentrations were time-weight averaged to represent the exposure period.

Trunk segment comparisons. Species lists were compiled for each site and evaluated for differences in species richness and composition between the two trunk segments. We tested for differences in mean species number per transect among the three sites using two sample Student's *t*-tests. Normality of data sets was tested using the D'Agostino-Pearson test as recommended by Motulsky (2017) for these and other statistical testing described below.

We also compared lichen species frequencies by site via relative occurrences of each species per tree per site (i.e., frequency of lichen species x/no. trees per site by segment), and determined the most common species on each trunk segment in each site. We measured trunk segment lichen community similarity among sites using a pairwise Bray-Curtis Index (*BC*), in which we compared species relative frequencies using the formula described in Perlmutter et al. (2017a). Calculated *BC* values range from 0 (no species shared by the two sampled micro-communities) to 1 (the two micro-communities shared all species in the same proportions).

Site lichen biotas were sorted by growth form (i.e., habit), photobiont type (i.e., chlorolichen or cyanolichen) and reproductive strategy (i.e., sexual or vegetative). Habit types included crustose, foliose, fruticose and squamulose. Cyanolichens are lichens that have cyanobacteria as a primary photobiont and/or nitrogen fixer; chlorolichens are lichens that lack cyanobacteria. Sexual lichens include species that predominantly bear ascomata, whereas vegetative lichens are those that predominantly lack ascomata, instead reproducing via diaspores (i.e., isidia, soredia) or fragmentation. Tree bole and base community compositions were visually compared and lichen relative frequencies were tested for differences using paired Student's *t*-tests.

Multivariate analysis. To explore relationships between lichen communities of the two trunk segments and associated environmental parameters including air NO_2 concentrations (**Table 1**), we

Table 1. Measured parameters in five parallel transects in two highway and one control site that were used in NMS Ordination.

Transect	Distance from edge (m)[a]	Elevation (m)	No. trees (*n*)	No. tree spp.	% Canopy cover[a]	DBH (cm)[a]	Rel. bryophyte freq.		No. lichen spp.		NO_2 conc. (ppb)[b]
							Bole	Base	Bole	Base	
Highway Sites											
LC 1	10	99	7	4	70.1 (±2.5)	26.0 (±3.4)	0.00	0.00	3	2	3.20 (2.88, 3.53)
LC 2	25	94	7	5	87.4 (±0.9)	31.3 (±4.2)	0.14	0.57	6	10	2.13 (1.92, 2.34)
LC 3	60	93	6	5	87.0 (±1.0)	34.8 (±6.9)	0.50	1.00	15	10	1.95 (1.95, 1.95)
LC 4	100	100	5	3	83.9 (±1.5)	13.1 (±3.4)	1.00	1.00	13	9	1.92 (1.89, 1.95)
LC 5	150	98	6	3	83.4 (±1.8)	10.1 (±2.8)	0.67	0.83	16	13	1.82 (1.62, 2.01)
RA 1	10	97	6	2	72.3 (±2.7)	36.0 (±5.6)	0.00	0.00	3	3	3.82 (2.53, 5.11)
RA 2	25	96	7	6	85.8 (±0.7)	27.4 (±4.9)	0.71	0.86	7	11	2.49 (1.66, 3.32)
RA 3	60	97	6	4	84.6 (±0.4)	28.9 (±5.8)	0.67	1.00	8	12	1.36 (1.34, 1.38)
RA 4	100	100	6	5	83.7 (±1.3)	28.1 (±6.2)	0.67	1.00	8	16	1.45 (1.28, 1.62)
RA 5	150	100	7	5	89.0 (±0.7)	16.6 (±2.0)	0.57	1.00	22	19	1.14 (1.09, 1.18)
Control Site											
HL 1	2.0 (±2.0)	67	5	5	71.5 (±2.7)	23.8 (±6.2)	0.40	1.00	30	26	0.63 (0.54, 0.72)
HL 2	19.2 (±4.0)	68	5	5	76.2 (±2.2)	25.7 (±7.4)	0.67	1.00	25	19	0.70 (0.59, 0.80)
HL 3	28.6 (±4.8)	69	7	5	77.2 (±1.4)	28.7 (±8.7)	0.57	0.86	29	28	0.58 (0.58, 0.59)
HL 4	36.0 (±6.0)	70	5	5	80.7 (±1.4)	19.7 (±7.7)	1.00	1.00	15	15	0.55 (0.52, 0.59)
HL 5	73.0 (±3.7)	70	5	4	77.6 (±3.0)	16.8 (±3.6)	1.00	1.00	23	17	0.90 (0.62, 1.19)

[a] Mean (±SE), n = number trees sampled in previous column. [b] Mean (duplicate measures).

conducted a Nonmetric Multidimensional Scaling (NMS) analysis using Sørensen (Bray-Curtis) distance measure in PC-ORD version 6.0 (McCune & Mefford 2011) for each segment. Transect occurrences of each lichen species (i.e., number of trees observed with a given lichen species per transect) were relativized into proportion frequencies for use in analysis. We first evaluated for groupings by cluster analysis using Sørensen (Bray-Curtis) distance measure and Flexible Beta group linkage methods, with beta set to –0.25 as recommended by McCune & Grace (2002). The resulting dendrogram was evaluated by percent chaining and interpretability, and groupings were incorporated into the second matrix of environmental variables for ordination with the first axis consisting of relativized lichen species frequencies.

In each NMS ordination we used the autopilot mode with the "slow and thorough" option selected, which conducts 250 runs with real data and 250 runs with randomized data with 500 maximum iterations in up to six dimensions or axes. We created ordination graphs by plotting each sample transect in the ordination space, with axes scaled as proportion to the maximum. Pearson correlations were calculated between environmental variables and ordination scores, and between lichen frequencies and ordination scores. To evaluate the quality of ordination results, coefficients of determination (r^2) were calculated in PC-ORD for each ordination axis. The resulting r^2 values represent the amount of variation that is represented by each axis. The two trunk segment ordination results were compared to check for apparent differences.

Lichen community relationships with environmental parameters. To further explore specific relationships between lichen biotic parameters and environmental variables of all 15 sample transects, and assess for differences between the two trunk segments, we tested for correlation and if apparent (i.e., $r > 0.5$ or < -0.5), tested by regression for significance and to determine the best fit model for a given relationship. Two sets of tests were run: one for each trunk segment. Lichen variable categories included richness (total species number), habit (% crustose spp.), photobiont (% cyanolichen spp.), and reproductive mode (% sexually reproducing spp.). Environmental parameters included distance from forest edge (m), elevation (m), tree species number, mean DBH (cm), mean % canopy cover, bryophyte abundance (relativized frequency), and mean NO_2 concentration (ppb). Tests were run using R (R Core Team 2013) and Microsoft Excel 2013.

Table 2. Number of lichen species on tree boles and bases and their Bray-Curtis (*BC*) similarities in three forested sites (two highway-exposed and one background control) in central North Carolina, U.S.A.

	Total species		
Site	Bole	Base	*BC*
LC (Highway)	32	24	0.68
RA (Highway)	27	25	0.59
HL (Control)	59	56	0.67

Table 3. Mean (±SE) lichen species richness per tree trunk segment and t-test results of segment differences in three sites in western Wake County, North Carolina.

Site	No. trees (*n*)	Bole	Base	*t*-statistic	*p*-value
LC (Highway)	31	2.4 (± 0.4)	2.4 (± 0.3)	–1.2	0.26
RA (Highway)	32	2.3 (± 0.4)	2.3 (± 0.3)	1.1	0.34
HL (Control)	28	6.3 (± 0.7)	5.9 (± 0.6)	–2.6	0.05

Results

Among 632 collections, we found a total of 103 lichen species growing on sampled tree trunks. Excluding allied fungi and supplemental collections, we included 95 lichen species in the analysis. We found 76 lichen species on tree boles and 72 on tree bases among the three sites, with an overall similarity of $BC = 0.70$ between the two trunk levels. Species checklists, including placement on trunks (i.e., bole and base) are reported in Perlmutter et al. (2017b). As we reported previously (Perlmutter et al. 2017a), sample tree diversity was similar across sites with the exception of pines being sampled in the highway sites but not in the control site (**Supplementary Table S1**).

Lichen patterns: among sites. Each site had slightly more species on boles than on bases (**Table 2**). Mean lichen species richness per trunk tested higher on boles in HL, but differences were not detected in LC or in RA (**Table 3**). Tree bole lichen species richness by transect ($n_i = 5$ per site) differed between LC and HL ($t = –3.7$, $p = 0.006$) as well as between RA and HL ($t = –3.4$, $p = 0.009$), but not between LC and RA ($t = 0.19$, $p = 0.86$). Tree base lichen species richness by transect ($n_i = 5$ per site) shared a similar pattern (LC v. HL: $t = –3.4$, $p = 0.01$; RA v. HL: $t = –2.1$, $p = 0.06$; LC v. RA; $t = –1.0$, $p = 0.37$).

Trunk segment *BC* indices ranged from 0.59–0.68 among sites (**Table 2**). LC had 15 lichen species found only on boles (39% of the total biota) and seven species (18%) only on bases; RA had 13 (27%) only on boles and 14 (29%) only on bases; HL had 17 only on boles (22%) and 13 (17%) only on bases. Frequencies of species found only on either boles or bases were low, with a maximum of 8%. Species found on tree boles at ≥0.10 relative frequency (i.e., occurrences per total trees sampled) included *Flavoparmelia caperata, Graphis scripta, Nadvornikia sorediata, Pseudosagedia cestrensis*, *Punctelia rudecta* and *Variolaria pustulata*. Species found on tree bases at ≥0.10 relative frequency included *Canoparmelia caroliniana, Cladonia ravenelii, F. caperata, Myelochroa aurulenta, P. cestrensis, P. rudecta* and *V. pustulata*. Among species found exclusively on tree bases were four cyanolichens: *Coccocarpia palmicola, Enchylium conglomeratum, Leptogium austroamericanum* and *Lobatria quercizans*. All but *L. austroamericanum* were found only in the HL site. In addition, three supplemental species of cyanolichens in genera *Fuscopannaria* and *Peltigera* were found on tree bases in HL (Perlmutter et al. 2017b).

In each site tree base communities had greater proportions of squamulose, cyanolichen, and vegetative species than did tree bole communities (**Figs. 2, 3 & 4**). Squamulose proportions tested higher on tree bases in RA ($t = –3.96$; $p = 0.008$; $n = 32$) and HL ($t = –2.35$; $p = 0.039$; $n = 28$), but not different in LC ($t = –1.10$; $p = 0.332$; $n = 31$). Cyanolichen proportions appeared greater on trunk bases in each site, but were not tested between trunk segments because the data were either found to be non-normal by the D'Agostino-Pearson (*DP*) test (LC: $DP = 12.25$, $p < 0.05$ for boles and bases; HL: $DP = 12.25$, $p < 0.05$ for boles only); or lacking (no cyanolichens on RA boles). Vegetative species proportions tested higher on tree bases in HL ($t = –3.02$; $p = 0.019$) and in LC ($t = –2.38$; $p = 0.038$), but not different in RA ($t = –1.87$; $p = 0.134$). Within sites tree bole and base lichen species richness per transect increased with distance from 2–3 at the forest edge in each site to 13–15 at 150 m distant in LC, and to 19–22 at 150 m in RA (**Table 1**).

Ambient air NO_2. The passive air samplers captured concentrations ranging 0.5 to 5.1 ppb NO_2 with duplicate averages showing clear patterns of distance decay in the highway-exposed sites (**Table 1**). The highway-exposed sites each had higher average NO_2 concentrations than did the control site; mean concentrations tested lower in the control site than in the highway-exposed sites (single factor

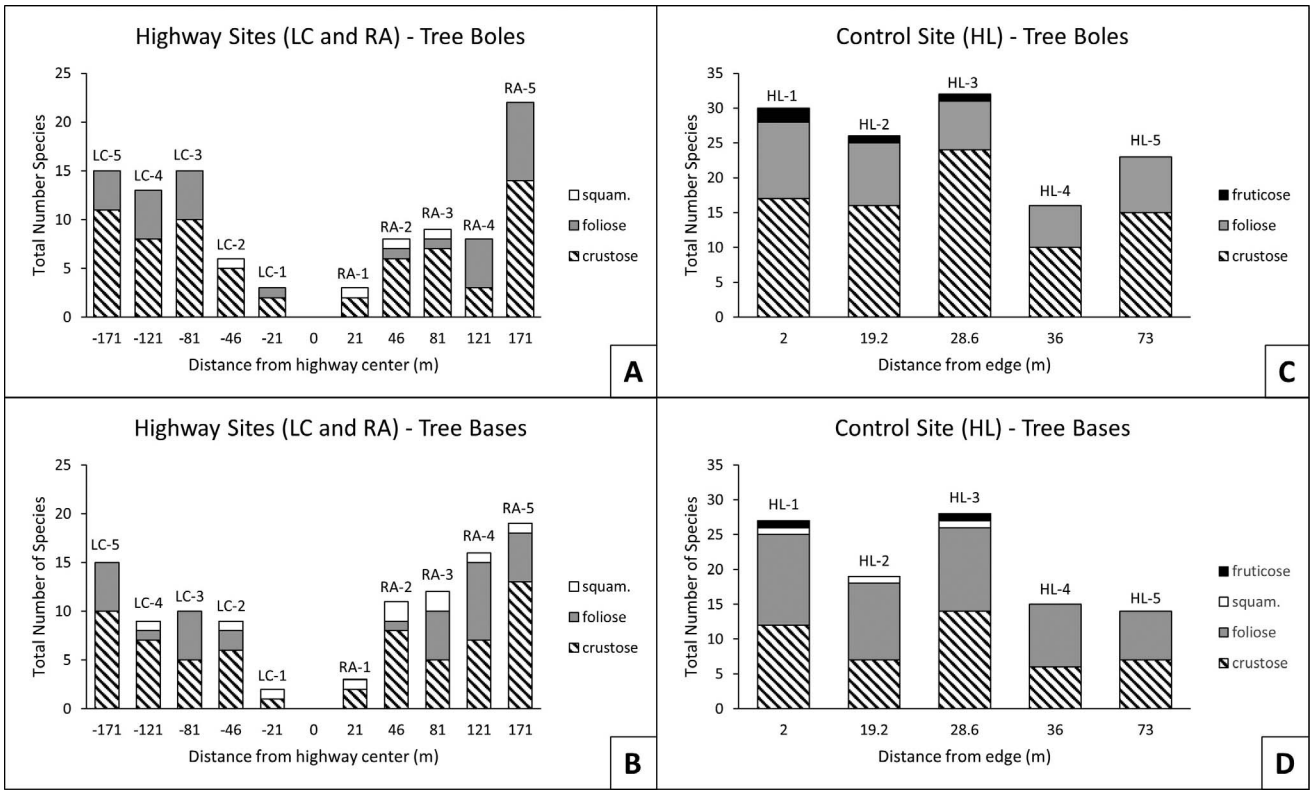


Figure 2. Lichen community patterns compared by habit on tree boles and bases across a forest edge-interior gradient in two highway-exposed sites (panels A, B) and one site away from road influence (panels C, D) in western Wake County, North Carolina, 2014. Data are total species per sample transect.

ANOVA: $F_{2,12} = 6.7$, $p = 0.01$). Within-site patterns of NO_2 concentrations were tested by linear and logarithmic regression, with the better fit associated with a logarithmic regression in the two highway sites (LC: $F_{1,3} = 31.5$, $p = 0.01$ with an r^2 of 0.91; RA: $F_{1,3} = 53.6$, $p = 0.005$ with an $r^2 = 0.95$). No relationship was found between NO_2 and distance from the forest edge in the control site (HL, linear: $F_{1,3} = 1.2$, $p = 0.28$ with an $r^2 = 0.36$).

Multivariate analysis. The cluster analysis resulted in similar lichen species groupings for the two trunk segments, each consisting of three groups. One group consisted of transects closest to the highway (0–25 m from the forest edge) on either side of I-40; a second group included transects at 25–150 m from the edge in the highway sites, and the third group comprised of transects furthest from I-40 in the highway sites plus all transects in the control site. Chaining in the dendrograms was 16.67% for bole communities and 23.30% for base communities.

The PC-ORD autopilot procedure determined the best solution with three dimensions for the tree bole data and one with two dimensions for the tree base data (**Table 4**). In both ordinations Axis 1 represented the majority of the data variation with that of the tree bases representing more. Ordination results of both boles and bases (**Fig. 5A & B**) showed most lichen species and sample transects to the left of the centroid along Axis 1, and broadly distributed along Axis 2. Among variables, NO_2 had the strongest positive correlations with Axis 1, while lichen species richness had the strongest negative correlations with Axis 1.

Correlation and regression analyses. Lichen species number had the strongest negative correlations with NO_2 at both trunk segments, with that involving tree bases slightly stronger (**Table 5**). Lichens species number also had strong negative correlations with elevation, but this appeared to be an artifact because the HL site is lower in elevation than the highway sites. Trunk base lichen species number had a high positive correlation with bryophyte frequency, and bryophyte frequency correlated negatively with NO_2. Among lichen functional groupings, base % crustose spp. correlated positively with NO_2, base % cyanolichen spp.

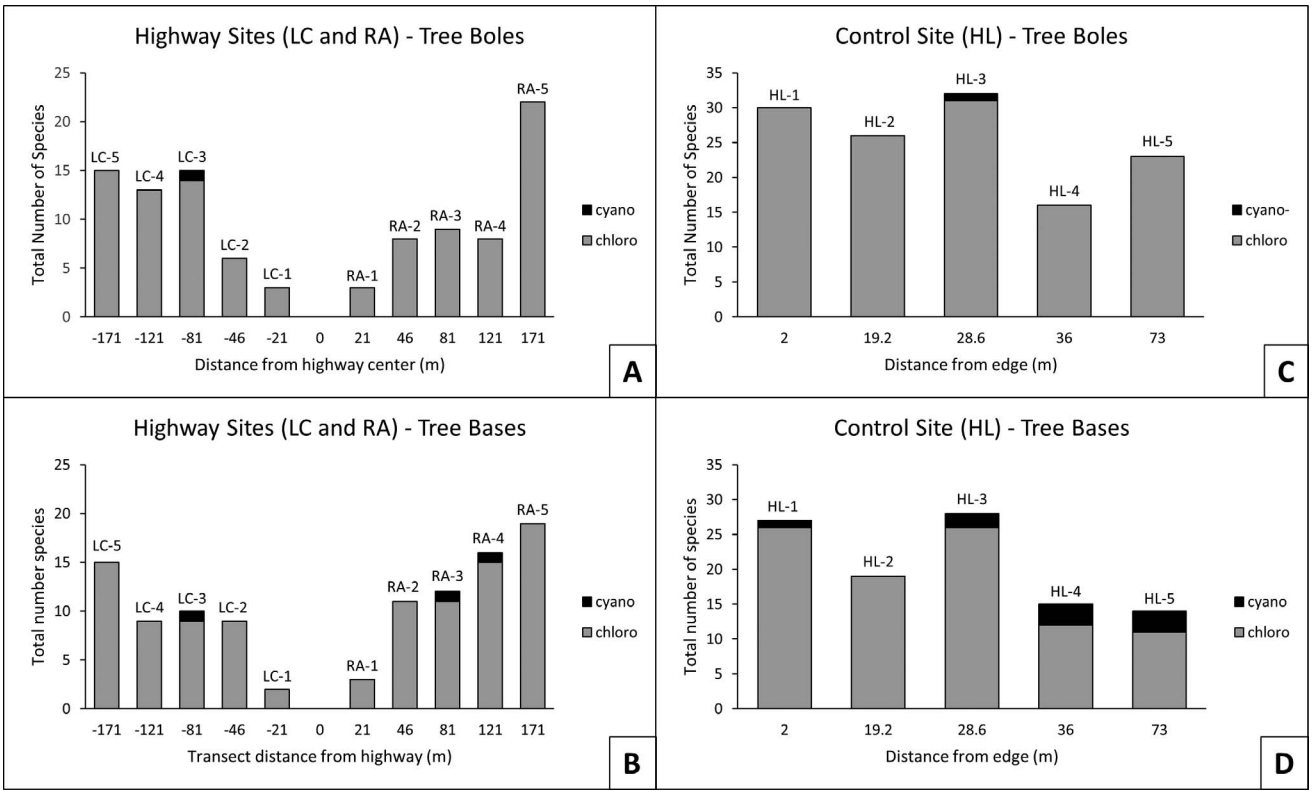


Figure 3. Lichen community patterns compared by photobiont on tree boles and bases across a forest edge-interior gradient in two highway-exposed sites (panels A, B) and one site away from road influence (panels C, D) in western Wake County, North Carolina, 2014. Data are total species per sample transect.

Table 4. NMS Ordination results of lichen species and environmental parameters across 15 sample transects in three sites in western Wake County, North Carolina, U.S.A., sampled in 2014.

NMS statistic	Bole	Base
No. Axes	3	2
Final Stress	5.31	12.84
Final Instability	0.00	0.00
No. Iterations	78	69
Axis 1 r^2	0.660	0.705
Axis 2 r^2	0.128	0.089
Axis 3 r^2	0.114	—
Axis 1 Correlations		
Distance from edge	–0.264	–0.167
Elevation	0.638	0.731
Canopy Cover	–0.032	–0.050
Tree spp.	–0.381	–0.352
DBH	0.514	0.482
Bryophyte freq.	–0.678	–0.767
Lichen spp.	–0.850	–0.799
NO_2 conc.	0.886	0.949

nearly correlated negatively with NO_2, and base % sexual spp. correlated positively with NO_2 (**Table 5**). No functional groupings on tree boles appeared to correlate with NO_2. Among all transects, a quadratic regression was found the best fit between NO_2 concentrations and lichen species richness at both trunk segments (bole: $r^2 = 0.71$, $p < 0.001$; base: $r^2 = 0.75$, $p < 0.001$; $n = 15$ transects) (**Fig. 6**).

Discussion

We found moderate differences between tree bole and base lichen communities for species composition and composition of functional groups, but not for species number. Our findings agree with vertical distribution studies that have shown lichen communities on trees to vary with height (Fritz 2009; Hale 1965; Komposch & Hafellner 2000; Pirintsos et al. 1993). Pirintsos et al. (1993) found species of *Cladonia* only on tree bases of pines in Greece, in agreement with our findings of squamulose lichens, which are also *Cladonia* species, being found predominantly on bases. Cyanolichens in our

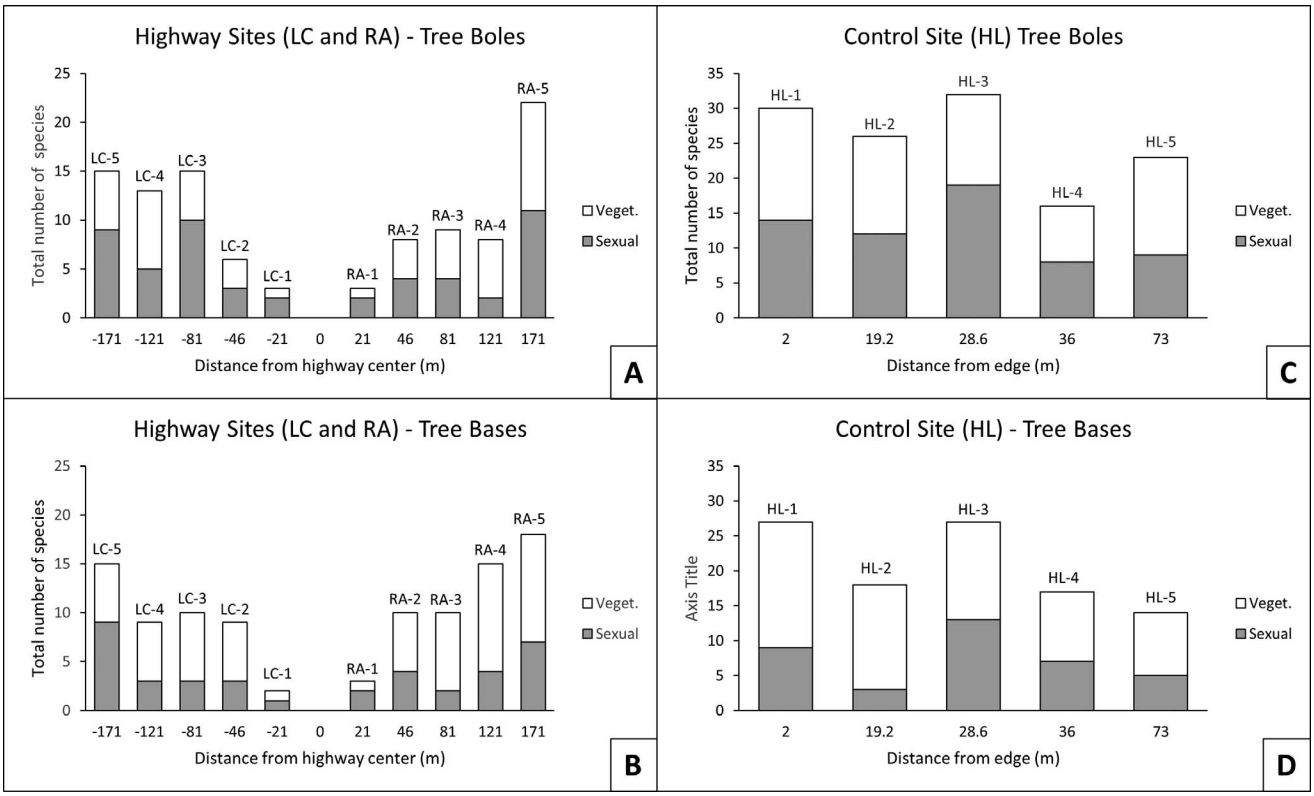


Figure 4. Lichen community patterns compared by reproductive mode on tree boles and bases across a forest edge-interior gradient in two highway-exposed sites (panels A, B) and one site away from road influence (panels C, D) in western Wake County, North Carolina, 2014. Data are total species per sample transect.

study were also found predominantly on tree bases, in agreement with cyanolichen surveys conducted in the western Piedmont and southern Appalachians of North Carolina, wherein nearly all species were found within one meter of the ground (Becker 1980; Becker e al. 1977). Cyanolichens are adapted to moist, shaded conditions of tree bases, since cyanobacteria are only physiologically active when wet and grow in low light intensities (Belnap 2001).

Most studies of lichen community response to pollution have focused on tree boles (e.g., Asta et al. 2002; Llop et al. 2012; Perlmutter 2010), while few have examined lichens on both tree boles and bases (e.g., Bates et al. 2001; Garcia et al. 2001; Mikhailova 2007). Results across studies comparing community responses (including both micro- and macrolichen forms) between the two heights were mixed. For example, tree base communities were more depauperate than their bole counterparts in a site exposed to Mexico City's urban air, more so than in a site upwind of the city (Garcia et al. 2001). However, the opposite pattern was found on pines in a similar control-impact study in Greece, wherein tree bole communities were more depauperate overall, and more so in a polluted site than were base communities (Pirintsos et al. 1993). Lichen richness patterns observed in our study (i.e., in relation to distance from the forest edge in the highway sites, and to NO_2 concentration across all sites) were similar between the two trunk segments despite the variation in species composition, suggesting that pollution exposure affected lichen community structure as well as microhabitat. However, interpretation of our results need to be treated with caution due to the small sample size (n=5 transects per site for within-site comparisons, and n=15 transects overall).

Could the two trunk heights vary in pollution concentrations affecting lichen community response? Our study did not determine whether the pollution environment is different between ground level and 1.5 m above. Information on air pollution differences between height strata on tree trunks is scant, but vertical distribution across wider scales is better known. Ozone concentrations at ground level (0.5–3 m) were lower than in the canopy (>3 m) (Neufeld et al. 1992). Pilergaard et al. (2003) found

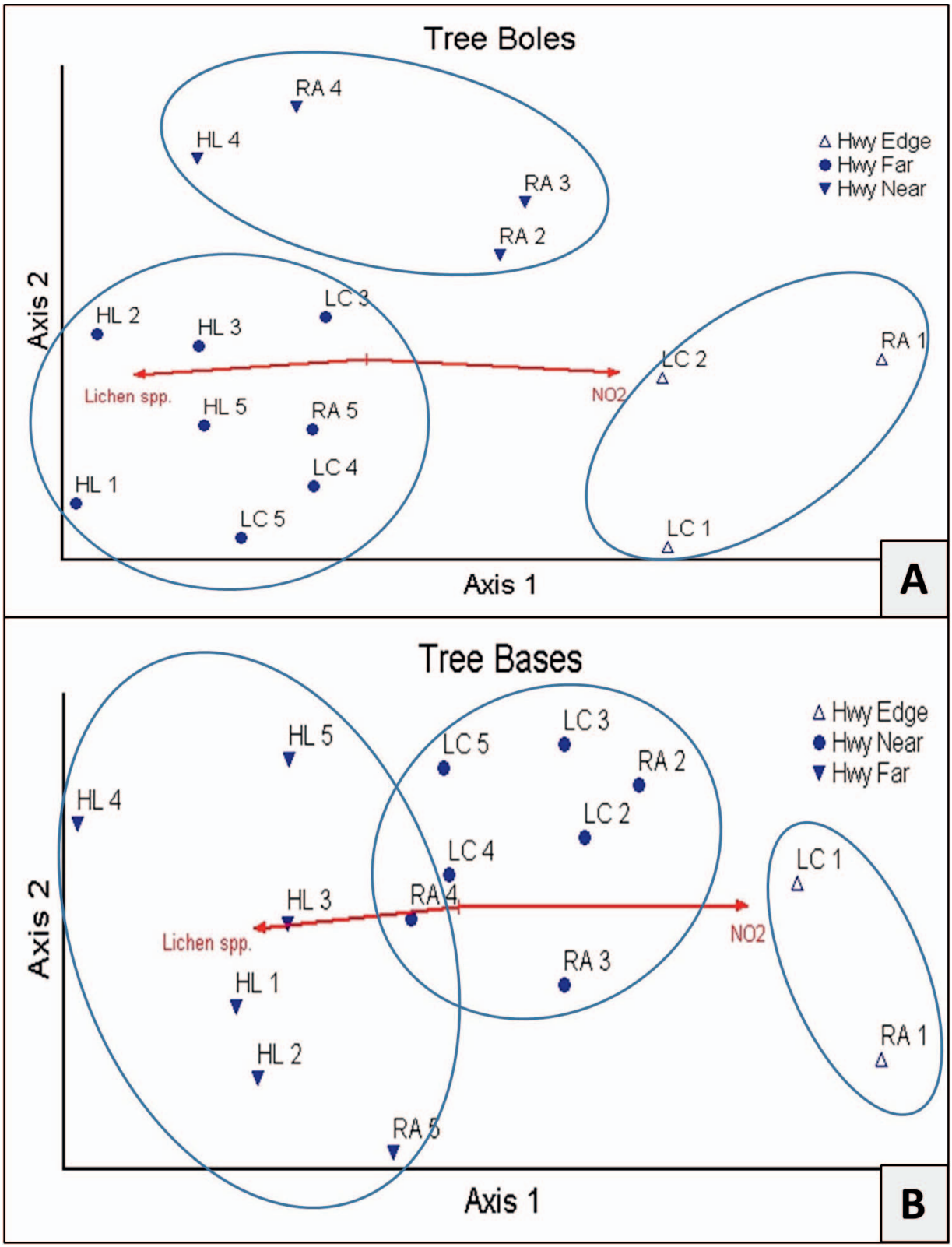


Figure 5. NMS ordination biplots of tree trunk lichen communities in sampled transects across forest edge-interior gradients in two highway polluted and one control site, Wake County, North Carolina, with groupings from cluster analysis using Sørensen (Bray-Curtis) distance measure. Sample transect groupings are related to distance from highway I-40. A = ordination of tree boles; B = ordination of tree bases. Vectors in each biplot are measured parameters that were greater than the correlation coefficient (r^2) cutoff of 0.600.

Table 5. Transect trunk segment lichen parameter correlation (r) and linear regression (F) statistics with associated environmental parameters.

	Bole		Base	
Parameter comparison	***r***	***F***	***r***	***F***
Lichen spp. v NO_2	–0.81	25.17***	–0.87	42.08***
Lichen spp. v Elevation	–0.75	17.02**	–0.69	11.89**
Lichen spp. v Distance from forest edge	0.09	0.10 ns	0.06	0.05 ns
Lichen spp. v Canopy cover	–0.16	0.34 ns	–0.03	0.01 ns
Lichen spp. v Tree spp.	0.28	1.08 ns	0.43	2.91 ns
Lichen spp. v Trunk DBH	–0.35	1.81 ns	–0.21	0.59 ns
Lichen spp. v Bryo freq.	0.39	2.30 ns	0.66	9.94**
Bryo freq. v NO_2	–0.64	9.04*	–0.82	27.61***
% Crust v NO_2	0.21	0.60 ns	0.54	5.30*
% Cyano v NO_2	–0.04	0.02 ns	–0.50	4.29 ns
% Sexual v NO_2	0.48	3.94 ns	0.53	5.21*

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = not significant.

ozone concentrations to be consistently lower at 0.1 m above ground level than at 1 m, and highest concentrations were found at 41 m. Vehicle emissions undergo considerable mixing by the movement of vehicles within 100 m of a highway in addition to natural transport and dispersion processes of wind, temperature and the physical topography of the environment (Samson 1998). Such mixing in a study of ultrafine particulates in north Raleigh, North Carolina was found to be enhanced by vertical barriers, such as structures and vegetation, (Bowker et al. 2007). Therefore, it appears that the air pollution environment in our study sites may be vertically homogeneous from the ground to 2 m above, which could explain the lack of difference in lichen community response between the two trunk segments. The differences in species composition may be better explained by microclimatic and microenvironmental differences between the bole and base trunk segments, such as with respect to shade and moisture, possible nutrient differences, and maybe even topographical differences due to changes in bark texture and/or pH.

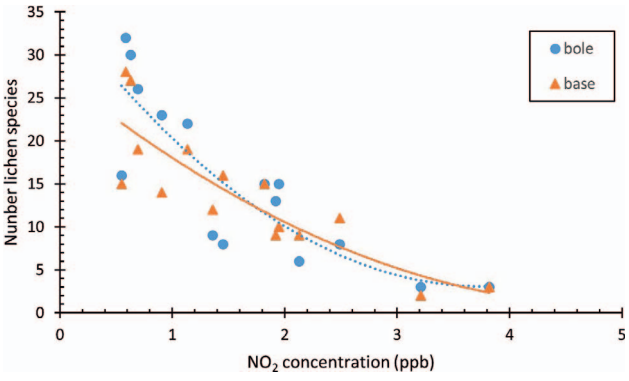


Figure 6. Combined scatterplot depicting quadratic relationships between lichen species richness and ambient air NO_2 concentrations of tree boles and bases across 15 transects sampled in three sites at varying distances from forest edges: two sites adjacent to a major highway and one adjacent to a remote lakeshore in western Wake County, North Carolina, U.S.A.

Overall, the bole and base lichen communities we examined were found to differ somewhat in species composition, but not necessarily in species richness. Lichen species richness of tree boles and bases correlated with distance from the forest edge in the highway sites but not in the control site, in agreement with patterns found for whole trunks (Perlmutter et al. 2017a). Similar patterns were found with NO_2 concentrations with lumped transects: bole and base communities correlated strongly with NO_2, but differences were not detected between trunk bole and base communities.

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Literature Cited

Asta, J., W. Ernardt, M. Ferretti, F. Fornasier, U. Kirschbaum, P. L. Nimis, O. W. Purvis, S. Pirintsos, C. Scheidegger, C. van Haluwyn & V. Wirth. 2002. Mapping lichen diversity as an indicator of environmental quality. Pages 273–279. In: P. L. Nimis, C. Scheidegger & P. A. Wosely (eds.), Monitoring with Lichens – Monitoring Lichens. Kluwer Academic Publishers, Dordrecht.

Bates, J. W., J. N. B Bell & A. C. Massara. 2001. Loss of *Lecanora conizaeoides* and other fluctuations of epiphytes on oak in S.E. England over 21 years with declining SO_2 concentrations. Atmospheric Environment 35: 2557–2568.

Becker, V. E. 1980. Nitrogen fixing lichens in forests of the southern Appalachian Mountains of North Carolina. The Bryologist 83: 29–39.

Becker, V. E., J. Reeder & R. Stetler. 1977. Biomass and habitat of nitrogen fixing lichens in an oak forest in the North Carolina Piedmont. The Bryologist 80: 93–99.

Belnap, J. 2001. Chapter 19: Factors influencing Nitrogen fixation and nitrogen release in biological soil crusts. Pages 241–261. In: J. Belnap & O. L. Lange. 2001. Biological Soil Crusts: structure, function, and management. Ecological Studies 150.

Bowker, G. E., R. Baldauf, V. Isakov, A. Khlystov & W. Peterson. 2007. The effects of roadside structures on the transport and dispersion of ultrafine particles from highways. Atmospheric Environment 41: 8128–8139.

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

Cawthorn, J. W. 1970. Soil Survey of Wake County, North Carolina. United States Department of Agriculture, Soil Conservation Service. GPO, Washington, DC.

Davies, L., J. W. Bates, J. N. B. Bell, P. W. James & O. W. Purvis. 2007. Diversity and sensitivity of epiphytes to oxides of nitrogen in London. Environmental Pollution 146: 299–310.

Fenn, M. E., S. Jovan, F. Yuan, L. Geiser, T. Meixner & B. S. Gimeno. 2008. Empirical and simulated critical loads for nitrogen deposition in California mixed conifer forests. Environmental Pollution 155: 492–511.

Fritz, Ö. 2009. Vertical distribution of epiphytic bryophytes and lichens emphasizes the importance of old beeches in conservation. Biodiversity Conservation 18: 289–304. [doi:10.1007/s10531-008-9483-4.]

Garcia, A. Z., T. H. Nash III & M. A. Herrera-Campos. 2001. Lichen decline in Desierto de los Leones (Mexico City). The Bryologist 103: 428–441.

Geiser, L. H., S. E. Jovan, D. A. Glavich & M. K. Porter. 2010. Lichen-based critical loads for atmospheric nitrogen deposition in western Oregon and Washington forests, USA. Environmental Pollution 158: 2412–2421.

Gradko Environmental. 2012. Technical Data Sheet: TDS 15. DIFRAM100: Rapid Air Monitor – Nitrogen Dioxide (NO_2).

Griffith, G. E., J. M. Omernik, J. A. Comstock, M. P. Schafale, W. H. McNab, D. R. Lenat & T. F. MacPherson. 2002. Ecoregions of North Carolina. U.S. Environmental Protection Agency, Corvallis, OR, (map scale 1:1,500,000).

Hale, M. E. Jr. 1965. Vertical distribution of cryptogams in a red maple swamp in Connecticut. The Bryologist 68: 193–197.

Harris, R. C. & D. Ladd. 2005. Preliminary Draft: Ozark Lichens, Enumerating the lichens of the Ozark Highlands of Arkansas, Illinois, Kansas, Missouri, and Oklahoma. Prepared for the 14th Tuckerman Lichen Workshop, Eureka Springs, Arkansas. Published by the authors.

Johansson, O., K. Palmqvist & J. Olofsson. 2012. Nitrogen deposition drives lichen community changes through differential species responses. Global Change Biology 18: 2626–2635.

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

Komposch, H. & J. Hafellner. 2000. Diversity and vertical distributions of lichens in a Venezuelan tropical lowland rain forest. Selbyana 21(1,2): 11–24.

Lendemer, J. C., R. C. Harris & E. A. Tripp. 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. The New York Botanical Garden Press, Bronx.

Llop E., P. Pinho, P. Matos, M. J. Pereira & C. Branquinho. 2012. The use of lichen functional groups as indicators of air quality in a Mediterranean urban environment. Ecological Indicators 13: 215–221.

McCune, B. & J. B. Grace. 2002. Analysis of Ecological Communities. MjM Software Design, Gleneden Beach, Oregon, USA.

McCune, B. & M. J. Mefford. 2011. PC-ORD. Multivariate Analysis of Ecological Data. Version 6.0. MjM Software, Gleneden Beach, Oregon, USA.

Microsoft® Excel® 2013 (15.0.4963.1000) MSO (15.0.4963.1002) 32-bit. Part of Microsoft Office Professional Plus 2013.

Motulsky, H. J. "Choosing a normality test", GraphPad Statistics Guide. Available online: http://www.graphpad.com/guides/prism/7/statistics/index.htm?stat_choosing_a_normality_test.htm (Accessed September 3, 2017).

Mikhailova, I. N. 2007. Populations of epiphytic lichens under stress conditions: survival strategies. Lichenologist 39: 83–89.

National Atmospheric Deposition Program (NADP). 2000. Nitrogen in the Nation's Rain. NADP Brochure 2000-01c (revised).

Neufeld, H. S., J. R. Renfro, W. D. Hacker & D. Silsbee. 1992. Ozone in Great Smoky Mountains National Park: dynamics and effects on plants. Pages 594–617. In: R. L. Berglund (ed.), Tropospheric Ozone and the Environment II: effects, modeling and control. Air & Waste Management Association, Pittsburg, PA.

Nimis, P. L., C. Scheidegger & P. A. Wosely (eds.). 2002. Monitoring with Lichens – Monitoring Lichens. NATO Science Series IV. Earth and Environmental Sciences – Vol. 7. Kluwer Academic Publishers, Dordrecht/Boston/London.

North Carolina Department of Transportation (NCDOT). 2014. NCDOT Historical AADT Values 2013 to 2002. NCDOT, Traffic Survey Group, Transportation Planning Branch, Raleigh.

Omernik, J. & G. Griffith. 2008. Ecoregions of North Carolina and South Carolina (EPA). Retrieved from http://www.eoearth.org/view/article/152148 (8 September 2015).

Perlmutter, G. B. 2010. Bioassessing air pollution effects with epiphytic lichens in Raleigh, North Carolina, U.S.A. The Bryologist 113: 39–50.

Perlmutter, G. B., G. B. Blank, T. R. Wentworth, M. D. Lowman, H. S. Neufeld & E. Rivas Plata. 2017a. Effects of highway pollution on forest lichen community structure in western Wake County, North Carolina, USA. The Bryologist 120: 1–10.

Perlmutter, G. B., G. B. Blank & E. Rivas Plata. 2017b. Checklists of corticolous lichenized and allied fungi collected in mixed forests of western Wake County, North Carolina, USA. Evansia 34: 23–37.

Pilrgaard, K., T. N. Mikkelsen, C. Beier, N. O. Jensen, P. Ambus & H. Ro-Poulsen. 2003. Field measurements of atmosphere-biosphere interactions in a Danish beech forest. Boreal Environment Research 8: 315–333.

Pirintsos, S. A., J. Diamantopoulos & G. P. Stamou. 1993. Analysis of the vertical distribution of epiphytic lichens on *Pinus nigra* (Mount Olympos, Greece) along an altitudinal gradient. Vegetatio 109: 63–70.

Richardson, D. H. S. & R. P. Cameron. 2004. Cyanolichens: their response to pollution and possible management strategies for their conservation in northeastern North America. Northeastern Naturalist 11: 1–22.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Samson, P. J. 1988. Atmospheric transport and dispersion of air pollutants associated with vehicular emissions. Pp. 77–98. In: A. Y. Watson, R. R. Bates & D. Kennedy (eds.), Air Pollution, the

Automobile, and Public Health. National Academies Press, Washington DC.
Will-Wolf, S., S. Jovan, P. Nietlich, J. E. Peck & R. Rosentreter. 2015. Lichen-based indices to quantify responses to climate and air pollution across northeastern U.S.A. The Bryologist 118: 59–82.

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Supplementary documents online:

Supplementary Table S1. Sampled tree species in five transects along forest edge-interior gradient in two highway-adjacent and one background control site in western Wake County, North Carolina.