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LICHEN COMMUNITY STRUCTURE ACROSS AN URBAN TO RURAL LANDSCAPE GRADIENT

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ABSTRACT

Epiphytic lichen communities are established indicators of urbanization. In urban areas, a decrease in species diversity may occur due to increased air pollution, lower moisture levels and habitat alteration. Urban areas often have greater concentrations of air pollutants, which is detrimental to the physiology and growth of some lichen species. The range of sensitivities of lichens to sulfur dioxide, nitrogen oxides, ozone and eutrophication has resulted in the development of biomonitoring programs in European countries and national parks in the western United States. Habitat alteration resulting in the fragmentation of woodlands and lower moisture levels in urban areas may inhibit the dispersal and retard growth of lichens. This report provides an assessment of lichens as bioindicators of urbanization and air pollution in southwestern Pennsylvania and discusses the application of using lichens to monitor the management of urban parks and long-term climate change.

To investigate the application of lichens as bioindicators, we measured species richness and diversity along a landscape gradient, which included two urban sites at Schenley and Frick Parks in the metropolitan area of Pittsburgh in Allegheny County and two rural sites at Mingo Creek County Park in Washington County and Roaring Runs Natural Area, Forbes State Forest in Westmoreland County. Species richness and diversity were measured at six intensive monitoring microplots on *Quercus rubra* (red oak) trees at each site, along with broader surveys of lichens in 465 m^2 plots which surrounded the intensive monitoring plots. Lichens were also transplanted from Mingo to Frick to evaluate differences in biomass growth rates, a species diversity survey was conducted at each site and bark pH and conductivity was measured at Mingo and Frick to examine differences in substrate quality.

The lichen diversity value, a statistical estimator of the environmental conditions in a site, was greater at the rural sites (20.8 ± 3.0) than the urban sites (11.3 ± 3.5), suggesting a less disturbed lichen community at Mingo and Roaring Runs (± standard error). In the intensive monitoring plots, species richness was greater at Mingo and Roaring Runs compared to Schenley and Frick, averaging 5.2 ± 0.3, 4.7 ± 0.4, 3.7 ± 0.8 and 2.0 ± 0.4, respectively. The dominant lichens across all sites were *Lepraria lobificans*, an unidentifiable sterile crustose and *Cladonia ochrochlora*. In the transplant study, more than 40% of the lichens collected from Mingo had increased in mass compared to less than 14% at Frick, almost a magnitude difference of four, suggesting better growing conditions at Mingo.

The greater species richness and diversity of lichens at the rural sites was likely due to a combination of factors that include air pollution, habitat fragmentation and atmospheric moisture. Sulfur dioxide, a leading cause for declines in lichen communities around the world, does not vary in concentration between sites, and thus, is unlikely to be the sole factor in driving changes between the urban and rural sites. Nitrogen saturation throughout the three counties surveyed is the likely cause for the dominance of nitrophilous, eutrophic-loving lichens in the study area. Air pollution defines the larger, lichen community in the study but the differences in species richness and diversity between the sites are more likely defined by atmospheric moisture and habitat quality. Lower moisture levels in urban areas caused by the “urban heat island effect” may result in the desiccation of lichen and isolated, urban parks may inhibit dispersion and genetic diversity. Future work in this region should focus on incorporating lichen monitoring in urban park management plans for use as indicators of habitat alteration and develop long-term monitoring surveys to examine responses of the lichen community to changes in temperature and moisture relating to climate change.

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COVER PAGE: *Flavoparmelia caperata* (left), *Candelaria concolor* and *Physcia millegrana* (center) and *Cladonia macilenta var. bacillaris* (right).

1. INTRODUCTION

1.1. What is a Lichen?

Lichens are the result of a symbiotic association between a fungus and alga. The fungus, which is usually of the Phylum Ascomycetes makes up most of the vegetative body of lichens, called the thallus. Within the interstices of the fungal filaments is the alga, which is either a cyanobacterium (blue-green algae) or a Chlorophyta (green algae) (Fig. 1). The fungus supplies the alga with water and minerals in addition to protecting the alga from dessication and mechanical abrasion. The alga leak soluble carbohydrate molecules to fungal tissues manufactured by photosynthesis. Lichens reproduce sexually through the release of spores from reproductive structures called Ascomycota, and some have specialized vegetative, reproductive structures called soredia and isidia that allow them to reproduce asexually. The soredia and isidia are common and widespread among lichens, allowing them to be used as important characters in identification.

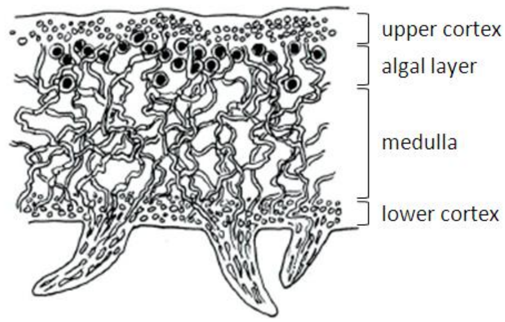

Figure 1. Cross section through the thallus of a foliose lichen (Hinds & Hinds, 2007).

Lichens come in a variety of sizes and shapes, traditionally grouped into crustose, foliose and fruticose growth forms. Crustose lichens typically lack a lower cortex, are not represented by a leafy thallus and are difficult to separate from the substrate. Foliose lichens are flat and leaf-like with root-like appendages called rhizines that anchor the lichen to the substrate. Fruticose lichens lack distinct upper and lower surfaces and include a variety of shapes such as upright, reproductive stalks called podetia, shrub-like thalli or pendant thalli. A fourth growth form sometimes distinguished is squamulose, in which the thallus is divided into segments much smaller than foliose lichens.

1.2. Biomonitoring with Lichens

The use of biomonitoring to assess pollution has increased during the last several decades. Such organisms accumulate environmental contaminants causing an unbalance between tolerant and sensitive species, which allows for a quantitative comparison between contamination levels in geographically diverse areas. More recently, biomonitoring has been adopted to examine the response of species to climate change for developing long-term conservation strategies (Ellis et al., 2007).

As early as 1866 a study was published using epiphytic lichens as bioindicators (Nylander, 1866). Since then, there have been more studies on lichens as bioindicators of air quality than on any other species (Ferry et al., 1973), and lichens have been defined as “permanent control systems” for air pollution assessment (Nimis et al., 1989). European countries and the combined efforts of the U.S. National Park, Fish and Wildlife and Forest Services have recognized the potential of lichens in biomonitoring studies, and thus, have established standardized methods using lichens for monitoring air quality (Asta, 2002; Blett et al., 2003).

The most frequently used lichen biomonitoring methods are community analysis, transplant studies and lichen tissue analysis. Community analysis is a passive monitoring method that compares the species composition of tolerant and sensitive lichens throughout a geographical region and can be used to map the extent and magnitude of air contaminants (McCune et al., 1997; van Herk, 1999; Perlmutter, 2010). These types of studies also can demonstrate the improvement of air quality over time (Showman, 1997). Transplant studies provide additional advantages such as the ability to sample in areas devoid of lichens and examine the affects of bioconcentration of air pollutants on the physiological conditions and growth of lichens (Godinho et al., 2008). Tissue analysis can reveal the bioaccumulation of air contaminants in lichens, providing an indirect measurement of the magnitude of air pollutants within a region (Lawrey, 1993).

Lichens are ideal for biomonitoring because they have extensive geographical ranges, allowing studies to examine pollution gradients over large areas. These properties make them applicable for spatial and temporal evaluation of contaminant accumulation in the environment. The diversity of lichens and their differing levels of sensitivity allow for informative community analyses. Lichen sensitivity to environmental stress are due to (1) lichens lacking a cuticle (protective layer) like those found in higher plants, (2) uptake of substances occurring primarily from the atmosphere and not a root system, (3) lichens have slow growth rates and (4) injuries cannot be quickly restored (Asta, 2002). Epiphytic lichens are better suited than lichens on soils or rock substrates to study air pollution effects because they receive greater exposure to air contaminants and do not have direct access to soil nutrient pools or rock minerals (Blett et al., 2003).

In general, air pollution sensitivity among lichens increase from crustose to foliose, and lastly to fruticose, although there are exceptions to this progression. Fruticose forms have a greater surface area exposed to the environment compared to foliose, and foliose and fruticose lichens are more susceptible to physical damage than crustose because they are more loosely bound to the substrate. Species diversity measures are most informative if all three forms of lichens are surveyed, not just the macrolichens consisting of foliose and fruticose. Some of the most sensitive lichens contain the cyanobacterium alga component and have fruticose forms, such as those from the genera *Alectoria*, *Bryoria*, *Ramalina*, *Lobaria*, *Pseudocyphellaria*, *Nephroma* and *Usnea* (Blett et al., 2003).

2. OBJECTIVES AND JUSTIFICATIONS

2.1. Lichens as Bioindicators of Urbanization

One objective of this study was to conduct community analyses of lichens from an urban to rural landscape gradient around Pittsburgh, Pennsylvania, along with transplant studies to determine the application of using lichens as bioindicators of air quality, climate change and habitat alteration. In 2009, the American Lung Association ranked Pittsburgh as No. 1 on the list of cities most polluted by short-term exposure to fine particulate matter ($PM_{2.5}$) (ALA, 2009), with the major source of $PM_{2.5}$ originating from the Ohio River Valley (Tang et al., 2004). Pittsburgh has a long industrial history, which began with the War of 1812 sparked by Pittsburgh's rich seam of bituminous coal and three navigable rivers and continued until the collapse of the steel industry in the 1980s. Based on Pittsburgh's industrial history and urban development that contributes to traffic congestion and habitat fragmentation in Allegheny County, our hypothesis was that the species diversity and physiologic condition of epiphytic lichens would be a good indicator of poorer air quality in urban parks compared to rural parks in neighboring counties of southwestern Pennsylvania. To accomplish these objectives, two urban parks in the metropolitan region of Pittsburgh in Allegheny County and two rural parks in the neighboring counties of Washington and Westmoreland Counties were intensively surveyed for epiphytic lichens.

The Pennsylvania Department of Environmental Protection air monitoring stations closest to the sampling sites are the Pittsburgh-Carnegie Science Center 10 km west of the urban parks, Schenley and Frick; Charleroi 14 km southeast of the rural site, Mingo Creek Park in Washington County and Greensburg 33 km northwest of the rural site at Roaring Runs Natural Area in Forbes State Forest in Westmoreland County (PADEP, 2006). Figure 2 shows air quality data for the three air monitoring stations, which includes $PM_{2.5}$, ozone (O_3), sulfur dioxide (SO_2) and nitrogen dioxide (NO_2) from 1996 to 2006. During the 10-year period, there was a general improvement in air quality, with SO_2 and NO_2 concentrations well below the U.S. Environmental Protection Agency's (EPA) National Ambient Air Quality Standards (NAAQS). Since 1995, significant reductions in SO_2 and nitrogen oxides (NO_x) have occurred due to the 1990 Clean Air Act Amendments and Acid Rain Program (EPA, 2010a). In the eastern United States, SO_2 concentrations declined by 48% and total N deposition declined by 19% between 1990 and 2008 (EPA, 2010a).

Nitrogen dioxide is the only pollutant in which concentrations were greater at the Pittsburgh monitoring station than Charleroi and Greensburg. The primary source for NO_2 is transportation, which causes greater concentrations of NO_2 in the Pittsburgh metropolitan area. The lack of differences in concentration of air contaminants among the monitoring stations, excluding NO_2, is due to dispersion of local and non-local air pollutants in the atmosphere. The rural sampling sites in Washington and Westmoreland Counties are southwest of Pittsburgh, which is downwind from any point sources in Pittsburgh. More than 80% of SO_2 emissions from Allegheny County's point sources are generated by the Reliant Cheswick Power Station in Springdale, Pennsylvania (Kelly & Besselman, 2009). The coal-fired power station also emits the county's majority of hydrochloric and hydrofluoric acids (Kelly & Besselman, 2009).

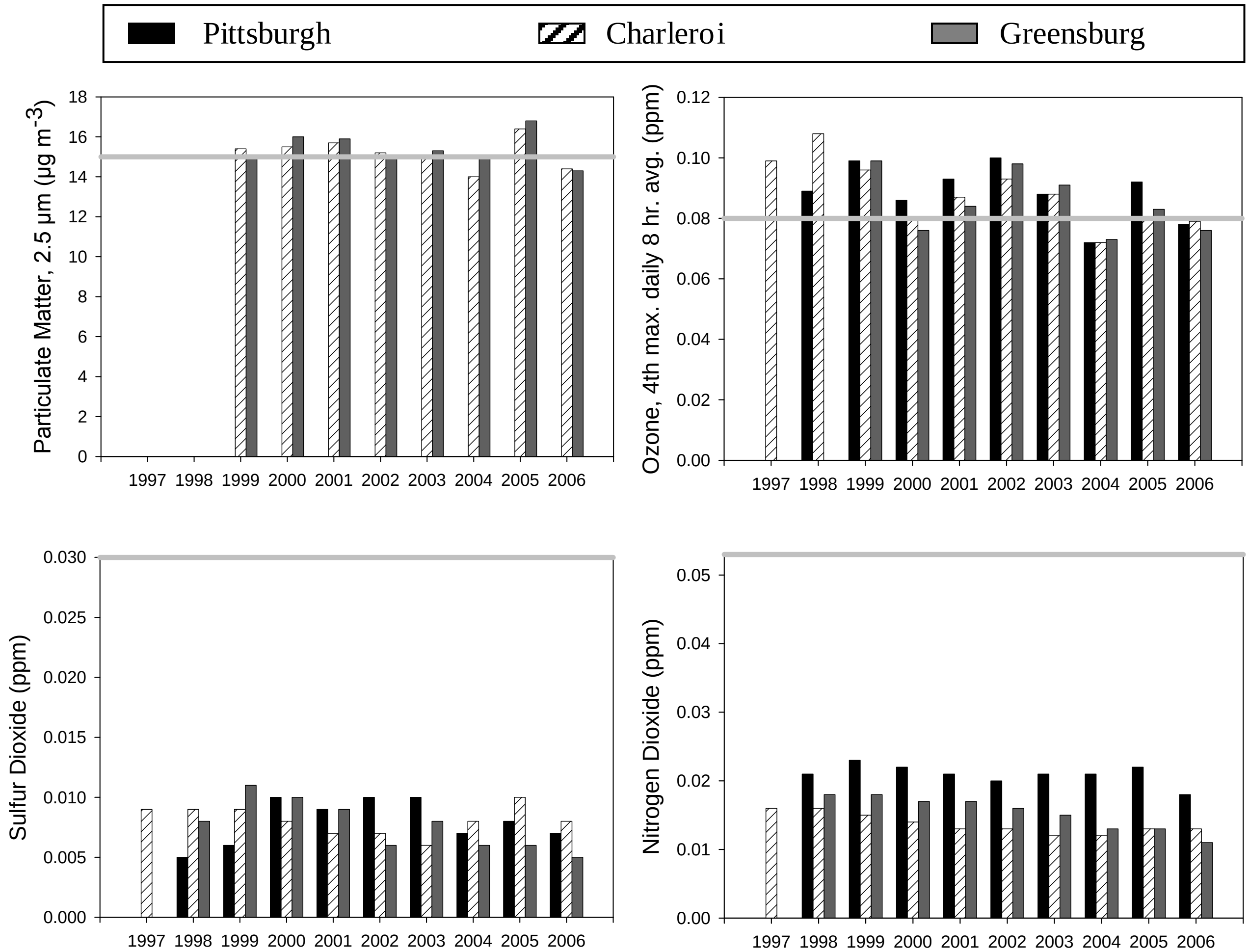

Figure 2. Air quality data from 1997 to 2006 from the Pennsylvania Department of Environmental Protection air monitoring stations: Pittsburgh-Carnegie Science Center, Charleroi-Borough Waste Treatment Plant and Greensburg-Pennsylvania Department of Transportation (PADEP, 2006). Gray horizontal line is the U.S. Environmental Protection Agency National Ambient Air Quality Standards. No data was collected from Pittsburgh for particulate matter or during years without bars.

2.2. Historical Comparisons of Lichen Communities

A second objective of this study was to compare historical records of lichens to the results of our species diversity survey to assess changes in species assemblages over time. Only through long-term comparisons of lichen communities in a geographical area can we also examine the impact of climate change on lichens. Our hypothesis was that the species assemblages of lichens have changed over time due to an increase in urbanization and a decrease in industry within the last half century.

One of the earliest publications studying the "city effect" on epiphytic lichens concluded that city-induced drought was the primary influencing factor on pollution-tolerant lichens in a city, whereas, air pollution was the most influential factor describing lichen diversity over a larger area (Brodo, 1966). Brodo (1966) was studying lichen distribution and effects on transplanted lichens on Long Island in New York City, in which he determined that the city climate was an important factor in restricting the growth of city lichens, as well as, air pollutants such as SO_2 and particulate matter. Washburn and Culley (2006) studied the effect of the greater Cincinnati metropolitan area on macrolichens and found that annual vehicle traffic negatively affected lichen abundance, suggesting that mobile source emissions of NO_2 may be an important factor. A review of studies examining the "city effect" across North America concluded that some of the most tolerant lichens of urbanization are *Candelaria concolor*, *Parmelia sulcata*, *Physcia stellaris*, *Punctelia rudecta* and overwhelmingly, *Physcia millegrana* (Washburn & Culley, 2006).

Several studies in the Ohio River Valley (Wetmore, 1989; Showman, 1997) and western Pennsylvania (McClenahen et al., 2007) have investigated the recolonization of lichens over a period of time. Showman (1997) and McClenahen et al. (2007) observed an increase in the species richness of lichens of 0.56 species per year from 1997 to 2003 and 0.25 species per year. from 1973 to 1996, respectively. These increases were likely a result of improved air quality, mainly SO_2 and NO_2 within the past few decades. Wetmore (1989) compares recent collections in Cuyahoga Valley National Recreation Area further back to between 1895 and 1917, finding that today's poorer air quality has caused 79% of the historical lichen flora to disappear from the park. It should be noted that continuous, long-term monitoring of the lichen community may provide more substantial conclusions on the impact of air pollution to lichen diversity and abundance than a single study. In a single study within an area of improving air quality conditions, the species diversity and abundance may reflect site characteristics more than pollution gradients. To assess the application of lichens as bioindicators of air pollution more comprehensively, this study compared the species of lichens identified in this study to checklists of lichens collected from Allegheny, Washington and Westmoreland Counties published in 1948 (Mozingo, 1948).

3. MATERIALS AND METHODS

3.1. Study Area

Lichen communities were sampled at four sites in southwestern Pennsylvania in Allegheny, Washington and Westmoreland Counties (Fig. 3). The study area lies in the Allegheny Plateaus Physiographic Province that is characterized by mixed topography on residuum. Mean climatic data (1971-2000) for Pittsburgh included winter minimum and summer maximum temperatures of -30 °C in January and 39.4 °C in July, respectively (NCDC, 2004). Annual mean precipitation and snowfall was 961 and 1024 mm, respectively (NCDC, 2004).

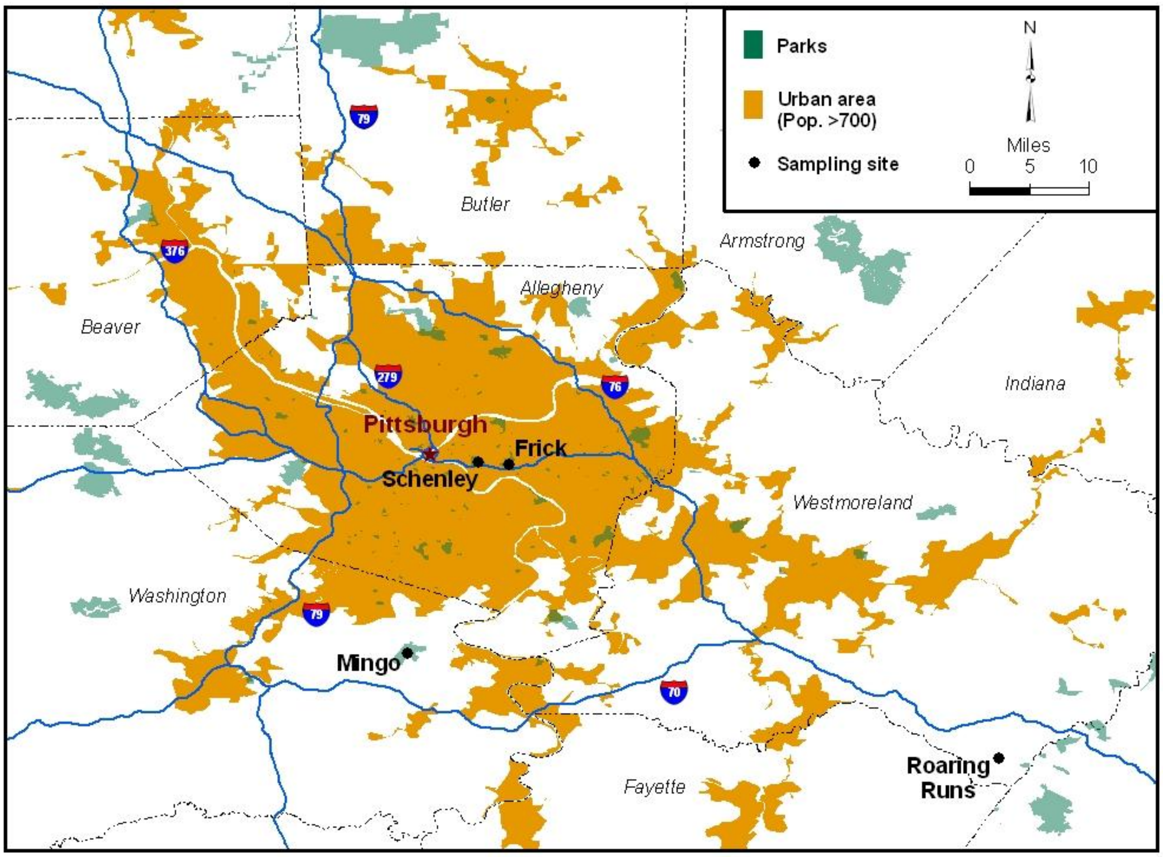

Figure 3. Location of sampling sites labeled as Schenley and Frick in Allegheny County, Mingo in Washington County and Roaring Runs in Westmoreland County in southwestern Pennsylvania.

Schenley Park (Schenley) and Frick Park (Frick) are within 8 km of downtown Pittsburgh in Allegheny County. Mingo Creek Park (Mingo) is in rural, Washington County approximately 23 km south of Pittsburgh and Roaring Runs Natural Area (Roaring Runs) is part of Forbes State Forest in rural, Westmoreland County approximately 61 km southeast of Pittsburgh. All sites consist of mesophytic woodlands in the mid-successional stage. Schenley and Frick are more urban parks, with a greater percentage of open canopy to support recreational activities. Roaring Runs is the least disturbed site, which was last logged in the 1960s and is unmanaged in its succession toward a mixed, mesophytic climax forest type. The four sites were chosen because of their similarities in forest type, accessibility and location in southwestern Pennsylvania.

Table 1. Location of sample sites in Allegheny, Washington and Westmoreland Counties, Pennsylvania with associated surface areas, elevation ranges above sea level, population density estimates for 2000 (USCB, 2002) and annual traffic estimates for 2008 (PennDOT, 2009).

Site	Latitude	Longitude	Area (km^2)	Elevation Range (m)	Population (persons/km^2)*	Annual Traffic (10^6 km/yr.)
Schenley	40° 26’13”N	79° 56’40”W	1.85	232-335	3,944	320
Frick	40° 26’01”N	79° 54’19”W	2.27	238-335	3,944	270
Mingo	40° 12’06”N	80° 01’42”W	10.5	280-378	80	14
Roaring Runs	40° 03’47”N	79° 18’26”W	14.5	561-890	34	10

*Sites in areas with densities >386 are urban; <386 non-urban.

3.2. Urban Parameters

Population and annual traffic densities were used as surrogate variables for urban development and mobile source emissions of NO_2 (Table 1). Road vehicles accounted for 34% of NO_x emissions in Allegheny, Washington and Westmoreland Counties in 2005 (EPA, 2010b). Population estimates for 2000 were determined from the U.S. Census Bureau for the zip codes in which the sites occur (USCB, 2002). The average population density was determined where multiple zip codes overlap a given site. Schenley and Frick are classified by the U.S. Census Bureau as urban areas, with >386 persons/km^2, with Mingo and Roaring Runs being non-urban areas.

Annual traffic data were obtained from the Pennsylvania Department of Transportation, annual average daily traffic maps for 2008 (PennDOT, 2009). Traffic volume data within a 3 km radius of each site were multiplied by the road lengths and by 365 d/yr to determine the annual vehicle distance traveled within a designated impact area (Washburn & Culley, 2006).

3.3. Lichen Community Analyses

The epiphytic lichen community was sampled through intensive monitoring and forest inventory plots, transplants and species diversity surveys between 2008 and 2010. The intensive monitoring plots were chosen based on criteria to minimize differences in lichen communities between sites caused by atmospheric moisture, sunlight exposure and substrate type. At each site, six plots were randomly chosen in the interior of the parks avoiding locations along roadways and in wet environments such as along streams, lakes or wetlands (Table 2). For the intensive monitoring plots, a healthy *Quercus rubra* (red oak) tree having the most coverage of lichens was chosen as the plot after searching in an area of favorable lichen habitat. *Q. rubra* was found to support a wide variety and abundance of lichens compared to other species of trees and are endemic to upland, mature woodlands, thus, ensuring that all plots were in the interior of forests and not in edge habitat. To further maintain consistency among plots, all plot trees were required to have a diameter at breast height (DBH) exceeding 25 cm and a surrounding canopy cover averaging 80-90%, which was measured using hemispherical photography.

Table 2. Latitude and longitude coordinates for the location of the intensive monitoring plots and the diameter at breast height (DBH) of plot trees at Schenley, Frick, Mingo and Roaring Runs sampling sites.

	Schenley		Frick		Mingo		Roaring Runs	
Plot	**Coordinates**	**DBH (cm)**	**Coordinates**	**DBH (cm)**	**Coordinates**	**DBH (cm)**	**Coordinates**	**DBH (cm)**
1	40° 25'51"N 79° 56'35"W	28	40° 25'05"N 79° 54'32"W	95	40° 11'17"N 80° 02'53"W	52	40° 03'19"N 79° 16'10"W	48
2	40° 25'54"N 79° 56'17"W	59	40° 26'15"N 79° 54'09"W	75	40° 11'51"N 80° 02'03"W	58	40° 02'54"N 79° 15'45"W	51
3	40° 25'58"N 79° 56'53"W	73	40° 25'45"N 79° 54'17"W	75	40° 11'57"N 80° 00'58"W	76	40° 03'25"N 79° 17'39"W	53
4	40° 26'05"N 79° 56'18"W	79	40° 25'54"N 79° 54'07"W	45	40° 12'02"N 80° 02'34"W	93	40° 03'04"N 79° 18'28"W	49
5	40° 26'14"N 79° 56'44"W	57	40° 26'10"N 79° 53'54"W	64	40° 12'39"N 80° 01'21"W	67	40° 03'19"N 79° 19'40"W	32
6	40° 26'11"N 79° 56'49"W	80	40° 25'47"N 79° 53'59"W	89	40° 12'37"N 80° 01'36"W	87	40° 02'06"N 79° 16'20"W	49

On each plot tree, the percent cover of lichens was measured using a 20 x 50 cm wooden grid microplot subdivided with nylon string into 2 x 2 cm squares, similar to methods developed by Garcia et al. (2000). Eight locations were sampled on a plot tree 0.5 and 1.5 m above the ground in the north, east, south and west directions. Additionally, ground litter was searched within a 3.0 m radius of the plot and all epiphytic lichens loose or attached to fallen stems or stumps were collected for identification.

Any voucher specimens collected from the intensive monitoring plots were carefully scraped from trees to prevent damage. Lichens were identified in the field whenever possible to avoid collection, or if collected, returned to the laboratory for identification using Lichens of North America by Brodo et al. (2001). The identification of specimens was confirmed by running thin layer chromatography of lichen thalli following methods published by Bungartz (2001) and Orange et al. (2001). Lichen specimens were run in toluene:acetic acid (170:30) and toluene:ethyl acetate:formic acid (139:83:8) solvents and retention (R_f) values were compared to those published in Orange et al. (2001) to identify lichen substances and ultimately, the species. Further confirmation of specimen identification was determined by sending a subset of samples to James C. Lendemer at the N.Y. Botanical Gardens. Voucher specimens are stored at Point Park University.

The intensive monitoring plots were also the center of the forest inventory plots. The corners of a 465 m^2 plot around the central tree were flagged and all living trees with a DBH

≥5 cm were surveyed for lichens. The lichens found on the trees were categorized into three groups: crustose, foliose and squamulose. The percent cover of lichens on a tree up to 3 m above the ground was recorded for each group using the following codes: 0 for <1, 1 for 1-10, 2 for 11-25, 3 for 26-50, 4 for 51-75 and 5 for 76-100%.

At all sites, a species diversity survey was conducted by searching for lichens in all habitats. The common species were identified in the field, and unknown species were collected for identification in the laboratory. The amount of time spent searching for lichens averaged one hour per square kilometer.

3.4. Transplant Study

A modified pendant method of transplanting epiphytic lichens by McCune et al. (1996) was used for examining changes in growth rates of *Flavoparmelia caperata* between the Mingo and Frick sites. *F. caperata* is a widespread foliose lichen, which is known to be sensitive to sulfur dioxide and heavy metal air pollution (Loppi et al., 2004; Godinho et al., 2008), as well as a potential indicator of climate warming (Sochting, 2004). Transplant material was collected at Mingo on May 12 and 13, 2009 from four sampling locations randomly chosen in areas of favorable lichen habitat that matched the forest conditions of our intensive monitoring plots. At each location, approximately 15 g of *F. caperata* was scraped from each of five *Prunus serotina* (black cherry) trees that were within a sampling radius of 30 m. *P. serotina* was our original plot tree for intensive monitoring, which was later changed when it became clear that a greater abundance and diversity of lichens grow on *Q. rubra*.

The collected lichens were air dried in the laboratory for one week, after which they were divided into smaller segments. The shapes of the individual segments were sketched on paper and weighed, averaging 0.3 g. Nylon monofilament fishing line with a tensile strength of about 4 kg was glued to the back of the lichen segments with 100% clear silicone sealant, adding approximately 0.2 g to the weight of lichen pendant. The prepared pendants were air dried overnight and weighed a second time. Pendants were randomly separated into three sets for transplanting at Frick and Mingo and a reference set to remain in the laboratory stored in a dry, dark plastic container.

On June 1, 2009 one set of pendants was hung at Frick near two of our intensive monitoring plots. The pendants were hung in discrete locations that were not frequented by park visitors. At the first plot (40° 25'49"N, 79° 53'57"W) five groups of lichen pendants were tied around five different *P. serotina* trees 1.5 m above the ground using fishing line. A group of pendants consisted of five pendants with each pendant tied to a different colored bead for identification. The second plot (40° 25'42"N, 79° 54'18"W) at Frick consisted of seven groups of pendants. The second set of pendants was hung at Mingo near two of our intensive monitoring plots (40° 11'02"N, 80° 02'08"W; 40° 11'16"N, 80° 02'42"W) on June 5 and 14, 2009. At both plots, groups of five pendants were tied to each of seven *P. serotina* trees.

After one year, the pendants were collected from Frick on May 27 and Mingo on May 29, 2010. The field pendants were weighed on May 30, 2010 and a subset of 24 laboratory pendants were weighed on June 1, 2010. The following equations were used to determine the biomass growth rates of pendants corrected for moisture content (McCune et al., 1996):

$$\Delta B\% = \Delta A\% - \Delta R\% \tag{1}$$

The component terms are found by

$$\Delta A\% = \frac{100\,(A_{1+x} - A_1)}{A_1 - [A_1 - A_0 R_1/R_0]} \tag{2}$$

and

$$\Delta R\% = \frac{100\,(R_{1+x} - R_1)}{R_1} \tag{3}$$

where, the actual percentage change in biomass of the transplanted lichen pendant (B) is calculated as the difference in the apparent change in biomass of the lichen pendant (A) and the change in biomass of the reference pendant (R). The equation symbols are: A_0 and R_0 = biomass of pendant before gluing on the fishing line; A_1 and R_1 = biomass of pendant after gluing on the fishing line; A_{1+x} and R_{1+x} = biomass of pendant after one year. The equation for $\Delta A\%$ removes the mass of the fishing line and silicone and corrects for moisture content using the term R_1/R_0 as a correction factor for the difference in moisture of the test sample between A_1 and A_0.

3.5. Bark pH and Conductivity

Measurements of bark pH and conductivity provide insight into the quality of substrate available for epiphytic lichens. Bark samples were collected from *Acer saccharum* (sugar maple), *P. serotina* and *Q. rubra* from Frick and Mingo between May and September 2009. These tree species were chosen based on their dominance at both sites. Approximately, three individual trees per species were sampled around each intensive monitoring and half of the transplant plots. Sampling methods were modified from Santamaria & Martin (1997), which included cleaning the bark surface with a wire brush to remove moss and lichens and then collecting bark slivers <3 mm thick from the north, east, south and west sides of the trunk at a height of 1.5 m above the ground. Bark slivers collected from all cardinal directions of a tree were combined into one sample and air dried prior to grinding. Samples were ground with a grinder and then passed through a sieve with a mesh size of 1.0 mm. Two grams of each sample were suspended in 25 ml deionized water. The suspensions were shaken for six hours and analyzed for pH and electrical conductivity (EC) using a Hannah pH/EC meter.

3.6. Data Analysis

Site comparisons of the intensive monitoring plots were analyzed by species richness, Shannon-Weiner diversity index, relative dominance and frequency and lichen diversity value (LDV). Significance testing of species richness and percent dominance was completed using one-way Analysis of Variance (ANOVA; Statext v1.2) with a significance level of $\alpha = 0.05$. To determine the Shannon-Weiner diversity index (H), the proportion of species i relative to the total number of species (p_i) is calculated, and then multiplied by the natural logarithm of this proportion ($\ln p_i$). The resulting product is summed across species and multiplied by -1.

$$H = -\Sigma p_i \ln p_i$$

Mean percent dominance per species is the mean percent number of grids that a species occurred in the microplots of all six plots, either separately for 0.5 and 1.5 m sampling heights or sampling heights combined. Relative dominance is the proportion of mean percent dominance of a species to the total percent dominance of all species and multiplied by 100. Frequency is the proportion of the number of occurrences of a species in a microplot to the total number of microplots per site. Relative frequency is the proportion of the frequency of a species to the total frequency of all species and multiplied by 100.

The LDV is a statistical estimator of the environmental conditions in a site (Asta, 2002). The first step in calculating the LDV of a site (j) is to sum the frequencies of all lichen species found on each tree (i) within the site. Substantial differences in lichen growth may occur on different sides of the trunks, thus, the frequencies have to be summed separately for each aspect (N, E, S, W) to obtain four Sums of Frequencies (SF) at each tree (SF_{iN}, SF_{iE}, SF_{iS}, SF_{iW}). For each aspect, the arithmetic mean of the Sums of Frequencies (MSF) for each site is calculated:

$$MSF_{Ni} = (SF_{1Nj} + SF_{2Nj} + SF_{3Nj} + \ldots + SF_{nNj})/n \qquad (1)$$

The LDV of a site is the sum of the MSFs of each aspect:

$$LDV_j = MSF_{Nj} + MSF_{Ej} + MSF_{Sj} + MSF_{Wj} \qquad (2)$$

The dominant trees in the forest inventory plots were determined using importance values, which is sum of relative values for dominance, frequency and density. Relative dominance is the proportion of basal area for a species to area sampled, which is then divided by the total dominance for all species and multiplied by 100. Relative frequency is the proportion of the number of plots in which a species occurs to the total number of plots sampled, which is then divided by the total frequency for all species and multiplied by 100. Relative density is the proportion of the total number of individuals of a species to the area sampled, which is then divided by the total density for all species and multiplied by 100.

Statistical significance between sites for canopy cover and percent lichen cover by type (crustose, foliose and squamulose) was determined using one-way ANOVA. One-way ANOVA was also used to compare mean percent biomass change in the transplant study and bark pH and conductivity between Frick and Mingo. Correlations of bark pH and conductivity and DBH were analyzed using a Pearson correlation.

4. RESULTS

4.1. Intensive Monitoring Plots

Mean species richness of lichens was significantly greater at Mingo and Roaring Runs than Frick, averaging 5.2 ± 0.3, 4.7 ± 0.4 and 2.0 ± 0.4, respectively (± standard error). Although Schenley had a lower mean species richness of 3.7 ± 0.8 compared to Mingo and Roaring Runs, it was not significant. The Shannon-Weiner species diversity index for lichens was 1.4, 1.1, 1.0 and 0.3 with respect to Schenley, Roaring Runs, Mingo and Frick. At all sites, moss and *Lepraria lobificans* were among the top three dominant epiphytes. Relative dominance and frequency averaged 45 and 26% for moss and 20 and 25% for *L. lobificans* across all sites, respectively (Table 3). Among the remaining top three dominant epiphytes was sterile crustose at Frick and Schenley, *Cladonia ochrochlora* at Mingo and *Cladonia caespiticia* at Roaring Runs. The top three dominant epiphytes at each site accounted for >70% relative dominance and >65% relative frequency.

Moss, sterile crustose, *C. ochrochlora* and *L. lobificans* were present at all sites. *F. caperata* and *Parmelia sulcata* were absent from Frick, but otherwise found at the other sites, and *Physcia millegrana* was only found at the urban sites. Overall, dominance of epiphytes was greater at 0.5 than 1.5 m above ground, but not significantly (Table 4). Those epiphytes that had a greater dominance at 1.5 m, although not necessarily at all sites, were sterile crustose, *F. caperata*, *Parmelia sulcata*, *Parmotrema hypotropum*, *Physcia millegrana* and *Punctelia rudecta*.

The lichen diversity value (LDV) was greatest at Mingo, followed by Roaring Runs, Schenley and least at Frick (Fig. 4). The average LDV at the rural sites compared to the urban sites was 20.8 ± 3.0 and 11.3 ± 3.5, respectively.

Table 3. Relative percent dominance and relative frequency of moss and lichens in intensive monitoring plots at Schenley, Frick, Mingo and Roaring Runs.

	Relative Percent Dominance				**Relative Frequency**			
Species	**Schenley**	**Frick**	**Mingo**	**Roaring Runs**	**Schenley**	**Frick**	**Mingo**	**Roaring Runs**
Moss	17.9	44.6	48.3	69.1	11.5	25.5	25.1	30.6
Sterile crustose	35.8	2.5	3.5	2.4	27.1	10.6	8.0	10.2
Cladonia caespiticia	-	-	-	7.7	-	-	-	17.8
Cladonia ochrochlora	17.6	0.6	36.1	0.1	17.7	6.4	24.1	1.9
Flavoparmelia caperata	1.9	-	3.2	0.4	12.5	-	12.8	8.9
Hypogymnia physodes	-	-	-	<0.1	-	-	-	1.3
Lepraria lobificans	19.4	51.9	7.3	19.1	14.6	46.8	16.6	19.7
Parmelia sulcata	0.3	-	0.1	<0.1	4.2	-	2.7	1.3
Parmelinopsis minarum	-	-	-	<0.1	-	-	-	0.6
Parmotrema hypotropum	-	-	<0.1	-	-	-	1.1	-
Phaeophyscia rubropulchra	-	-	0.2	-	-	-	2.7	-
Physcia millegrana	7.0	0.4	-	-	12.5	10.6	-	-
Punctelia caseana	-	-	0.1	1.1	-	-	1.6	7.6
Punctelia rudecta	-	-	1.1	-	-	-	5.3	-

Table 4. Mean percent dominance of moss and lichens in intensive monitoring plots at Schenley, Frick, Mingo and Roaring Runs at 0.5 and 1.5 m from the ground. The surface area of the microplot was 0.1 m^2. Standard errors in parentheses.

	mean % dominance (per 0.1 m^2)							
	Schenley		**Frick**		**Mingo**		**Roaring Runs**	
Species	**0.5 m**	**1.5 m**	**0.5 m**	**1.5 m**	**0.5 m**	**1.5 m**	**0.5 m**	**1.5 m**
Moss	13.3 (6.5)	9.4 (4.7)	22.7 (7.6)	13.7 (6.6)	77.3 (6.4)	55.9 (8.1)	68.3 (5.9)	37.8 (6.6)
Sterile crustose	23.3 (7.3)	22.1 (7.3)	1.5 (1.4)	0.6 (0.4)	1.3 (0.8)	8.3 (3.6)	1.9 (0.8)	1.9 (1.3)
Cladonia caespiticia	-	-	-	-	-	-	10.7 (3.0)	1.0 (0.5)
Cladonia ochrochlora	13.7 (5.9)	8.7 (4.6)	0.3 (0.2)	0.1 (0.1)	56.8 (7.3)	42.8 (7.5)	0.1 (0.1)	0.1 (0.1)
Flavoparmelia caperata	1.5 (0.7)	0.9 (0.5)	-	-	2.3 (1.0)	6.5 (2.6)	0.3 (0.1)	0.4 (0.2)
Hypogymnia physodes	-	-	-	-	-	-	-	<0.1 (<0.1)
Lepraria lobificans	15.1 (6.2)	9.6 (4.9)	33.7 (7.9)	8.6 (2.9)	13.5 (4.3)	6.7 (2.7)	21.1 (6.1)	8.3 (3.3)
Parmelia sulcata	0.1 (<0.1)	0.3 (0.2)	-	-	0.1 (0.1)	0.3 (0.2)	-	0.1 (0.1)
Parmelinopsis minarum	-	-	-	-	-	-	<0.1 (<0.1)	-
Parmotrema hypotropum	-	-	-	-	-	0.1 (0.1)	-	-
Phaeophyscia rubropulchra	-	-	-	-	0.5 (0.4)	<0.1 (<0.1)	-	-
Physcia millegrana	2.6 (1.7)	6.3 (4.3)	0.2 (0.2)	0.2 (0.1)	-	-	-	-
Punctelia caseana	-	-	-	-	0.2 (0.1)	<0.1 (<0.1)	1.1 (0.5)	0.5 (0.2)
Punctelia rudecta	-	-	-	-	0.6 (0.5)	2.4 (1.3)	-	-

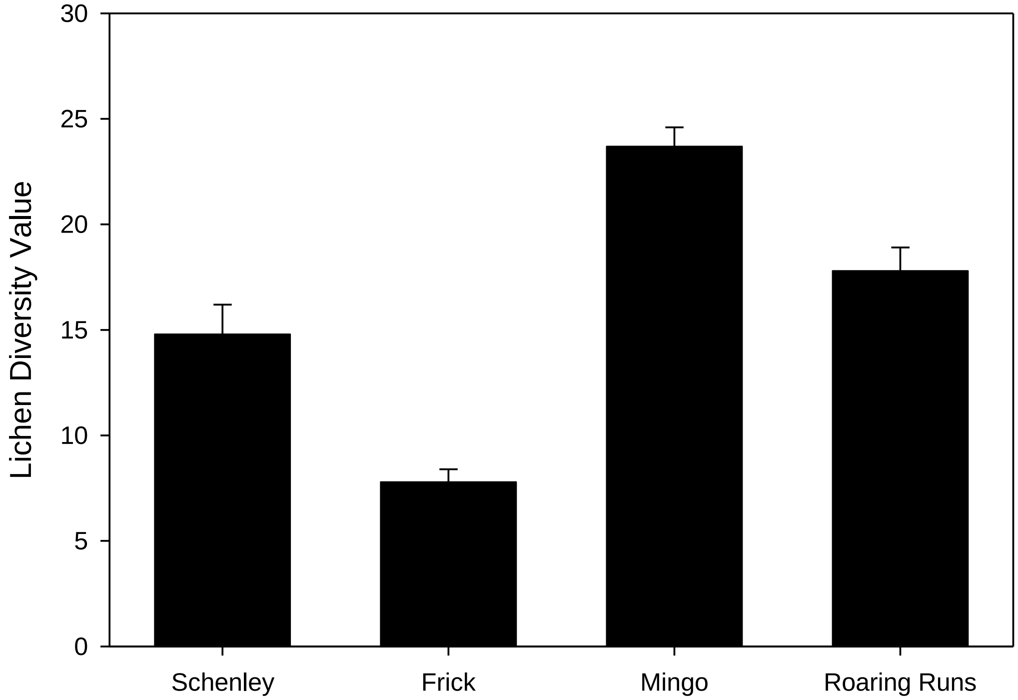

Figure 4. Lichen diversity values calculated from intensive monitoring plots. Standard error bars are shown.

4.2. Forest Inventory Plots

The species richness of trees in the forest inventory plots were similar across all sites, ranging between 6.1 per 500 m^2 at Schenley and Roaring Runs to 7.2 per 500 m^2 at Frick. However, tree density was significantly lower at Schenley (15.8 per 500 m^2) compared to Frick (35.9 per 500 m^2) and Roaring Runs (30.5 per 500 m^2), with Mingo in the middle at 25.5 per 500 m^2. There was no significant difference in percent canopy cover, which ranged between 82 and 92% across all plots and sites.

The dominant tree species at each site was determined by an importance value, which accounts for frequency, density and dominance of a tree in the plots of a given site. At Schenley, the three dominant tree species were *Q. rubra*, *Fraxinus americana* (white ash) and *A. saccharum*, with importance values of 119, 31 and 29, respectively. The three dominant tree species at Frick were *Q. rubra*, *P. serotina* and *A. saccharum*, with importance values of 68, 43 and 38, respectively. The same dominant tree species at Frick dominated at Mingo, however, the dominance order was *Q. rubra*, *A. saccharum*, and *P. serotina*, with importance values of 79, 61 and 33, respectively. At Roaring Runs, the three dominant trees were *Q. rubra*, *Acer rubrum* (red maple) and *A. saccharum*, with importance values of 73, 63 and 37, respectively.

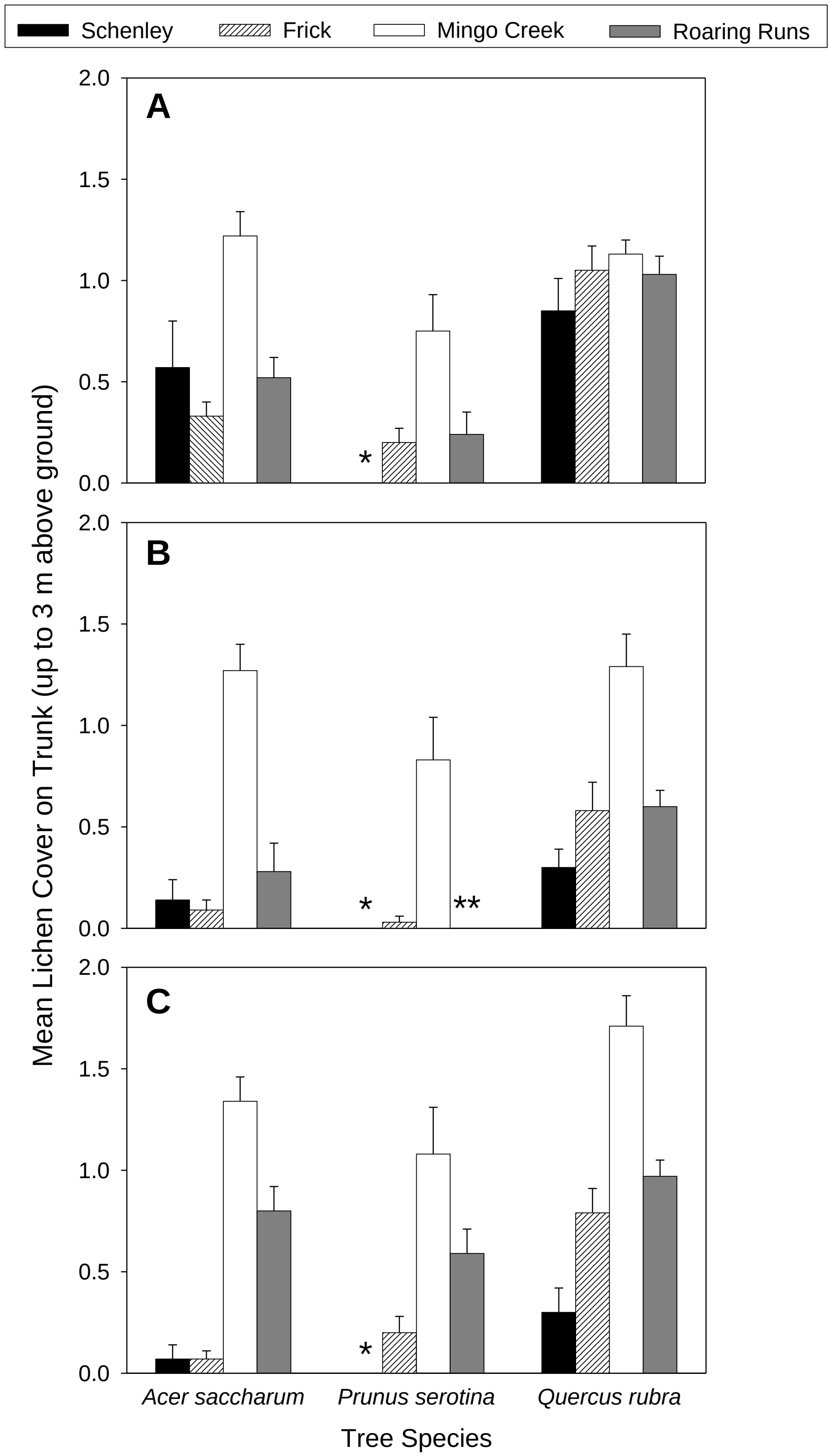

Figure 5. Mean lichen cover (0 for <1, 1 for 1-10 and 2 for 11-25%) of crustose (A), foliose (B) and squamulose (C) on *Acer saccharum*, *Prunus serotina* and *Quercus rubra* by sites at the forest inventory plots. Standard error bars are shown. (* no data; ** <1% lichen cover)

Identifying *Q. rubra* as the plot tree for the intensive monitoring plots and the center for the forest inventory plots likely resulted in its dominance in the plots. *Q. rubra*, *P. serotina* and *A. saccharum* had the greatest presence across all sites, thus, these three tree species were chosen to compare for lichen cover of crustose, foliose and squamulose (Fig. 5). The number of individual trees of a given species surveyed for lichen cover among the four sites ranged from 12 to 55 per site, excluding *P. serotina* which were not present in the plots at Schenley.

Crustose lichens, which consisted primarily of sterile crustose and *L. lobificans*, surveyed on *A. saccharum* and *P. serotina* had a significantly greater percent cover on trees at Mingo compared to the other sites by a magnitude of two to four (Fig. 5A). There was no significant difference between sites in crustose cover on *Q. rubra*. In regards to foliose cover, which consisted primarily of *F. caperata*, *Parmelia sulcata*, *Physcia millegrana*, *Punctelia caseana* and *Punctelia rudecta*, there was significantly, more than two times the coverage on all three species of trees at Mingo compared to the other sites (Fig. 5B). The squamulose coverage, which consisted of *Cladonia sp.*, was also greater on all three tree species at Mingo than the other sites but only significantly so on *A. Saccharum* and *Q. rubra*. Mingo had significantly more squamulose cover than Frick on *P. serotina* (Fig. 5C). Overall, Mingo had the greatest coverage of all three groups of lichens on each of the three species of trees, with the least difference between sites showing up on *Q. rubra* trees. Frick generally had the least coverage of all three groups of lichens on *A. saccharum* and *P. serotina*, and Schenley had the least coverage of all three groups of lichens on *Q. rubra*.

In the urban sites of Schenley and Frick, crustose lichens dominated the lichen community on *Q. rubra* and *A. saccharum*, and co-dominated on *P. serotina* (Table 5). Squamulose lichens dominated the lichen community on the three trees at the rural sites of Mingo and Roaring Runs, followed by crustose, and finally foliose being the least dominant on the three trees.

Table 5. Mean lichen cover (0 for <1, 1 for 1-10 and 2 for 11-25%) of crustose, foliose and squamulose on *Acer saccharum*, *Prunus serotina* and *Quercus rubra* by urban and rural sites from the forest inventory plots. Standard errors are shown in parenthesis.

		Acer saccharum	***Prunus serotina***	***Quercus rubra***
urban				
	crustose	0.38 (0.08)	0.20 (0.05)	0.91 (0.10)
	foliose	0.10 (0.05)	0.03 (0.02)	0.40 (0.08)
	squamulose	0.07 (0.03)	0.20 (0.06)	0.51 (0.09)
rural				
	crustose	0.95 (0.09)	0.45 (0.11)	1.07 (0.06)
	foliose	0.89 (0.11)	0.34 (0.11)	0.88 (0.09)
	squamulose	1.14 (0.09)	0.79 (0.13)	1.27 (0.09)

4.3. Species Diversity Survey

A total of 46 epiphytic lichen species were found throughout all of the sampling sites. The rural sites had more species, with 33 species found at Roaring Runs and 32 at Mingo compared to the urban sites, with 20 species found at Schenley and 19 at Frick (Table 6).

Table 6. Epiphytic lichen species identified at Schenley, Frick, Mingo and Roaring Runs during the intensive monitoring and species diversity surveys.

	Schenley	**Frick**	**Mingo**	**Roaring Runs**
Allocetraria oakesiana (Tuck.) Randlane & Thell				x
Amandinea polyspora (Willey) Lay & May		x	x	
Amandinea punctata (Hoffm.) Coppins & Scheid.	x			x
Arthonia caesia (Flot.) Korb.		x	x	
Buellia dialyta (Nyl.) Tuck.				x
Candelaria concolor (Dicks.) Stein	x	x	x	
Candelariella efflorescens Harris & Buck	x	x	x	
Cladonia caespiticia (Pers.) Florke		x	x	x
Cladonia cristatella Tuck.	x		x	x
Cladonia macilenta var. bacillaris Hoffm.			x	x
Cladonia ochrochlora Florke	x	x	x	x
Cladonia parasitica (Hoffm.) Hoffm.	x		x	x
Flavoparmelia caperata (L.) Hale	x	x	x	x
Graphis scripta (L.) Ach.				x
Hypogymnia physodes (L.) Nyl.			x	x
Lecania croatica (Zahlbr.) Kotlov	x			
Lecanora saligna (Schaerer) Zahlbr.	x			
Lecanora strobilina (Spreng.) Kieffer		x	x	x
Lecanora symmicta (Ach.) Ach.			x	x
Lecanora thysanophora Harris			x	
Lepraria lobificans Nyl.	x	x	x	x
Lepraria neglecta (Nyl.) Erichsen				x
Lepraria caesioalba (de Lesd.) Laundon				x
Melanelia subaurifera (Nyl.) Essl.		x	x	
Micarea peliocarpa (Anzi) Coppins & Sant.			x	x
Micarea prasina Fr.				x
Myelochroa aurulenta (Tuck.) Elix & Hale		x		
Ochrolechia arborea (Kreyer) Almb.			x	
Parmelia squarrosa Hale			x	x
Parmelia sulcata Taylor	x	x	x	x
Parmelinopsis minarum (Vainio) Elix & Hale	x		x	x
Parmotrema hypotropum (Nyl.) Hale	x	x	x	x
Phaeophyscia adiastola (Essl.) Essl.			x	
Phaeophyscia pusilloides (Zahlbr.) Essl.	x	x	x	x
Phaeophyscia rubropulchra (Degel.) Essl.	x	x	x	x
Physcia millegrana Degel.	x	x	x	x
Physcia stellaris (L.) Nyl.	x			x
Physcia subtilis Degel.		x	x	
Placynthiella dasaea (Stirton) Tonsberg				x
Placynthiella icmalea (Ach.) Coppins & James				x
Punctelia caseana Lendemer & Hodkinson	x	x	x	x
Punctelia rudecta (Ach.) Krog	x	x	x	x
Pyrrhospora varians (Ach.) Harris			x	
Ropalospora chlorantha (Tuck.) Ekman			x	x
Scoliciosporum chlorococcum (Stenh.) Vezda	x			x
Trapeliopsis flexuosa (Fr.) Coppins & James			x	x

4.4. Transplant Study

On average, 80% of the pendants hung at Mingo were collected after one year, whereas, 52% were collected at Frick (Table 7). All of the pendant trees were found, with those pendants not collected torn from the tree most likely by wind or fallen branches. The collected pendants were compared against the sketches made at the start of the transplant study, and any pendants with obvious signs of damage were not weighed. At Frick, the mean percent change in biomass (ΔB%) of the weighed pendants was -9.3 g ± 1.1, with 10% of the pendants having increased in mass. The mean percent change in biomass at Mingo was significantly greater at -1.2 g ± 0.6, with 54% of the pendants having increased in mass. Of those pendants that increased in mass, the mean increase was 1.7 g ± 0.2, with no significant difference between plots or sites.

Table 7. The percentage of pendants recovered, pendants that increased in mass and mean change in pendant biomass after one year including those pendants that decreased in mass (2009-2010) at Frick and Mingo sampling sites. Standard errors are shown in parenthesis.

	Frick		Mingo	
	Plot 1	**Plot 2**	**Plot 1**	**Plot 2**
% pendants recovered	29	84	74	86
% pendants that increased in mass	0	14	65	43
% mean change in pendant biomass	-11.1 (1.5)	-8.4 (1.5)	-0.6 (0.8)	-1.7 (0.9)

4.5. Bark pH and Conductivity

Bark pH was measured using deionized water having a mean pH of 4.26 (hydronium (H_3O^+) concentration = 8.89 x 10^{-5} *M*) and conductivity of 0 µS cm^{-1}. The H_3O^+ concentration of a neutral solution at a pH of 7.0 is an order of magnitude of approximately 1,000 times that of the deionized water used in our analyses. Thus, we can compare the bark pH of samples within our study but not against other studies that used water of a different pH to make their suspensions.

There was no significant difference in bark pH between Frick and Mingo for any of the three species of trees measured (Table 8). However, upon combining the pH values of Frick and Mingo by tree species the values were significantly different among trees, with *A. saccharum* having the highest pH and *P. serotina* having the lowest pH. The bark conductivity compared between Frick and Mingo by tree species was not significantly different on *A. saccharum* and *Q. rubra* but conductivity was greater at Frick on *P. serotina* (*P*=0.08). Upon combining the conductivity values of Frick and Mingo by tree species, *Q. rubra* had significantly lower conductivity values that both *A. saccharum* and *P. serotina* but there was no significant difference in conductivity between *A. saccharum* and *P. serotina*.

Table 8. Mean bark pH and electrical conductivity of *Acer saccharum*, *Prunus serotina* and *Quercus rubra* collected from Frick and Mingo. Standard errors are shown in parenthesis.

	Frick			Mingo		
Tree Species	***n***	**pH**	**EC (µS cm^{-1})**	***n***	**pH**	**EC (µS cm^{-1})**
Acer saccharum	4	4.8 (0.2)	223 (35)	6	4.7 (0.2)	291 (37)
Prunus serotina	16	3.4 (0.1)	330 (73)	27	3.4 (0.1)	193 (40)
Quercus rubra	12	3.9 (0.1)	144 (24)	19	3.9 (0.1)	159 (16)

A Pearson correlation was used to compare bark pH and conductivity to the DBH of the trees, with data from Frick and Mingo combined. Conductivity and DBH from *P. serotina* was the only comparison showing a significant (P<0.05; r=0.61) relationship of higher conductivity associated with increasing DBH beyond 20 cm (Fig. 6). The trees sampled from Mingo generally had smaller diameters. Mean DBH values of *A. saccharum*, *P. serotina* and *Q. rubra* at Frick were 32 cm ± 2, 30 cm ± 3 and 63 cm ± 5, respectively. At Mingo, the mean DBH values of *A. saccharum*, *P. serotina* and *Q. rubra* were 13 cm ± 2, 21 cm ± 2 and 57 cm ± 4, respectively.

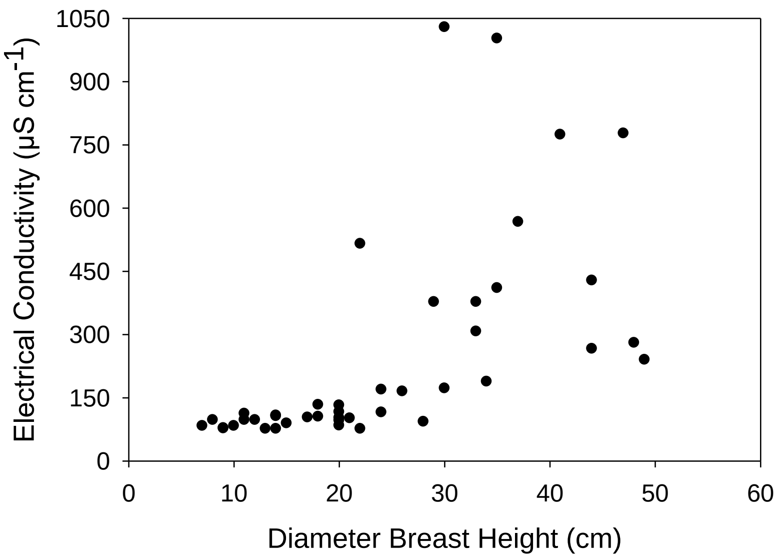

Figure 6. Comparison between bark conductivity and diameter at breast height of combined Frick and Mingo data on *Prunus serotina*. Pearson correlation was significant (P<0.05) with an r-value of 0.61.

5. DISCUSSION

5.1. Lichens as Bioindicators of Urbanization

Based on the results of this study, the lichen community at the urban sites of Schenley and Frick are less abundant and diverse, and their growth rates are slower compared to the lichens at the rural sites of Mingo and Roaring Runs. During the intensive monitoring sampling it was also evident that foliose and squamulose lichens appeared healthier, and a greater number of *Cladonia* species had erect podetia at the rural sites.

The population and traffic densities surrounding the urban sites are a magnitude of 69 and 25 times greater than at the rural sites, respectively. The greater traffic density in the Pittsburgh area is a likely cause for higher concentrations of NO_2. Nitrogen dioxide emitted by road traffic can be an important pollutant affecting lichen communities in urban environments (Gombert et al., 2003). A study by Davies et al. (2007) identified a toxic effect on lichens when concentrations of NO_x exceed 70 µg m^{-3} and NO_2 exceeds 40 µg m^{-3}. Alternatively, a fumigation study by Nash (1976) found that NO_2 concentrations greater than 4×10^6 µg m^{-3} are necessary to cause chlorophyll reductions in lichens and that NO_2 is a relatively insignificant toxin to lichens because ground level concentrations rarely exceed 1×10^6 µg m^{-3}. The annual 10-year NO_2 concentration average between 1996 and 2007 measured at the Pittsburgh air monitoring station was 2.1×10^4 µg m^{-3}, compared to 1.4×10^4 µg m^{-3} at the Charleroi and Greensburg stations.

To further complicate the effects of N deposition on lichen communities, the form of N influences lichens differently. For example, ammonia pollution has been shown to increase the pH values in bark, shifting the lichen community to a dominance of nitrophilous species that are associated with eutrophic conditions (Conti & Cecchetti, 2001). Alkaline dust connected with road traffic has shown similar effects (Marmor & Randlane, 2007). The overwhelming dominance of nitrophilous, macrolichen species at all sampling sites, such as *F. caperata*, *Parmelia sulcata* and the *Phaeophyscia* and *Physcia* genera (USFS, 2010) suggest that N pollution is widespread and plays a significant role in determining the species of lichens in southwestern Pennsylvania.

The affects of SO_2 on lichens has also been extensively studied. Although SO_2 from industry and coal-fired power plants is a source of acid precipitation, the toxic nature of SO_2 is probably a more significant factor influencing lichen physiology than the acidification of bark caused by acid precipitation (Hawksworth & Hill, 1984). Lichen sensitivity to SO_2 is broad among species and can range from the tolerant species of *Hypogymnia physodes* tolerating a concentration of 3×10^9 µg m^{-3} (Conti & Cecchetti, 2001) to the sensitive species of *Usnea sp.* tolerating a concentration of only 30 µg m^{-3} (Lerond, 1978).

Community analyses can be used for monitoring air pollution but should be analyzed with caution because the results may be insensitive except for sampling in severe air pollution gradients due to other conflicting environmental factors (Showman, 1985). The annual 10-year SO_2 concentration average between 1996 and 2007 in our study are (7.9×10^3 µg m^{-3}) suggests that southwestern Pennsylvania is saturated with SO_2, in respect to lichens, and that we would expect to find only tolerant lichens across our sampling gradient. It should be noted that there was little difference in the acidity and conductivity of bark substrate between Frick and Mingo. Furthermore, most of the dominant macrolichens identified in the study, such as *Parmelia sulcata* and the *Phaeophyscia* and *Physcia* genera are considered to have an intermediate to tolerant sensitivity to SO_2 pollution (USFS, 2010). *Allocetraria oakesiana* (Lawrey, 2010) and

Parmelia squarrosa (USFS, 2010) are more sensitive to SO_2 pollution, and thus, were only found at Mingo and Roaring Runs. *Punctelia rudecta*, another species sensitive to SO_2 pollution (USFS, 2010) was found at all sampling sites but more prevalent in the rural sites, according to the intensive monitoring surveys.

Ozone (O_3) is another urban air pollutant, having shown to cause damage to the photochemical apparatus of lichens (Scheidegger & Schroeter, 1995). However, conflicting studies have suggested that it may not be possible to separate the affects of O_3 from NO_x and SO_2 on lichen communities in urban areas (McCune, 1988; Jovan & McCune, 2005). Although NO_x, SO_2 and O_3 most likely affect the lichen communities in southwestern Pennsylvania, our study area is saturated with these air pollutants, therefore, we cannot describe the differences in lichen communities between the urban and rural sites solely by the concentrations of NO_2, SO_2 and O_3. The intensive monitoring and species diversity surveys showed a complete absence of sensitive lichens consisting of those with a cyanobacteria algal component or fruticose growth form, excluding those associated with squamulose forms. Thus, confirming the widespread impact of air pollution in southwestern Pennsylvania and how it defines the lichen community on a larger geographical scale.

Yet, we found in our study a difference in the species richness and diversity of lichens between the urban and rural sites. The lichen diversity value of the urban sites (11.3 ± 3.5) was more depressed than at the rural sites (20.8 ± 3.0), suggesting a less disturbed lichen community at Mingo and Roaring Runs. In the transplant study, more than 40% of the lichens collected from Mingo had increased in mass compared to less than 14% at Frick, almost a magnitude difference of four, suggesting a healthier environment for lichens at Mingo. Furthermore, from the forest inventory study the mean ratio of percent coverage of squamulose to foliose was 0.8 to 1.0 in the urban sites and 2.0 to 1.0 in the rural sites. We believe that the heightened sensitivity of squamulose forms to foliose forms due to squamulose forms having a greater surface area exposed to the environment is a good indicator of a healthy environment for lichens.

Brodo's (1966) "city effect" is likely a dominant factor in describing the differences in the lichen communities between the urban and rural sites. Lichens are indicators of air pollution, as well as, moisture and temperature. The “urban heat island effect” describes how cities are warmer and drier compared to the countryside due to the reduction of vegetative transpiration, residual heat from automobiles and buildings and dark, asphalt surfaces that absorb solar radiation more effectively than forests. During the last century, the annual average temperature in Pennsylvania has increased by more than 0.5 ^{o}F (UCS, 2008). During the next several decades (2010-2039), the annual average temperature in Pennsylvania is expected to increase by 2.5 ^{o}F, and possibly over 4 ^{o}F by mid-century (2040-2069) (UCS, 2008). Climate change has the potential to exacerbate the “city effect” of lichens by raising temperatures, and thus, decreasing the amount of atmospheric moisture during summer drought conditions. If the climate is changing, it seems timely to document lichen distributions for future comparisons, as this study does for southwestern Pennsylvania.

Two studies linking temperature changes to lichens by Sochting (2004) and van Herk et al. (2002) suggest that increasing temperatures may increase the abundance and diversity of lichens, including *F. caperata* which was widespread throughout our sampling sites. However, a stronger factor in defining the lichen communities in our study is more likely atmospheric moisture because temperature differences between our sampling sites are not significant enough to cause notable changes. Because precipitation rates across all sites are similar, we compared the density of streams at the sampling sites and found that stream density was 0.6 km per km^2

and 1.0 km per km^2 at the urban and rural sites, respectively. At Mingo, the mean distance between sampling plots and a body of water was 0.2 km, compared to Schenley, Frick and Roaring Runs which were 0.2, 0.7 and 0.3 km, respectively. The forest inventory survey clearly showed that lichen richness was greatest at Mingo compared to any other site, and we noted throughout our study that the density of lichens was greatest in the bottomlands along streams. If moisture is a factor in managing the lichen community then the presence of moss, with its requirement for moist habitats would be greater at the rural sites than the drier, urban sites. The mean relative dominance and frequency of moss in the rural sites is 59 and 28, respectively compared to 32 and 19 in the urban sites, respectively. In a study by Perlmutter (2010), which complements our own study but was conducted around Raleigh, North Carolina, moisture was also found to play a role in lichen community structure. Thus, it is safe to conclude that moisture is an important factor that drives species richness between the urban and rural sites, and furthermore, supports Brodo's (1966) "city effect."

A final factor to consider in having an effect on lichen communities in this study is habitat quality. A study by Johansson and Ehrlen (2003) studied the influence of habitat quality on two epiphytic lichens and found that the abundance of the lichens was positively correlated with tree size and that the presence of the lichens was positively correlated with tree density and negatively correlated with the isolation of woodlands. The larger and older trees are exposed to colonization for a longer time and may provide more suitable substrate with rough bark to capture dispersing propagules as well as have different bark chemistry than younger trees (Armstrong, 1990; Gustafsson & Eriksson, 1995). A greater tree density allows for easier dispersal among neighboring trees. Finally, isolated woodlands would imply dispersal constraints, inhibiting genetic diversity among lichens in urban areas and preventing recolonization should the isolated patch become further fragmented through recreational management or environmental degradation (Johansson and Ehrlen, 2003). The habitat at Mingo and Roaring Runs is less disturbed, consisting of older growth forests that would be more suitable for lichen dispersion and propagation than the urban parks. Additionally, the size of the rural sites is a magnitude of 10 greater than the urban sites and there is a greater degree of isolation at the urban sites, being completely surrounded by development, whereas, Mingo and Roaring Runs is surrounded by woodlands or dispersed residential and agricultural lands. Therefore, habitat quality is likely a second major factor in defining the differences in the lichen communities between the urban and rural sites.

In conclusion, air pollution defines the lichen community in the larger geographical region of this study. The dominance of nitrophilous and SO_2-tolerant species throughout the sampling sites suggests that lichens are good indicators of air pollution, such as N and SO_2. However, within the study area community analyses may be too insensitive to describe the differences in lichen assemblages between sites due to NO_2 and SO_2 concentrations being similar across all sites. The differences in species richness and diversity between the sites are best defined by atmospheric moisture and habitat quality. Lower moisture levels in urban areas caused by the "urban heat island effect" result in the desiccation of lichens, and thus, do not provide as suitable a growing environment as rural sites. The more isolated urban parks may inhibit the dispersion and genetic diversity of lichens resulting in a lower diversity, as observed in the species diversity survey.

5.2. Historical Comparisons of Lichen Communities

Of the 39 species of epiphytic lichens listed as having been found in Allegheny, Washington and Westmoreland Counties in Mozingo et al. (1948), only 12 were found in this study (Table 9). Some of the discrepancies between Mozingo et al. (1948) and this study is due to the different locations of sampling sites and possible misidentification of lichens in the Mozingo et al. (1948) publication. For example, *Cladonia subulata* occurs in boreal forests and *Parmelina quercina* occurs in California, both of which was listed as having been found in this area in Mozingo et al. (1948) but neither occurs in published listings of lichens in the region (Wetmore, 1989; Flenniken & Showman, 1990; Hill, 2000; Lendemer & Macklin, 2006; McClenahen et al., 2007). Additionally, Mozingo et al. (1948) provides no records of *Physcia millegrana*, *Parmotrema hypotropum*, *Phaeophyscia pusilloides* or *Punctelia caseana*, which are easily identifiable common macrolichens throughout the region and would most likely have been present 50 years ago.

Mozingo et al. (1948) lists two species of *Lobaria*. Lichens from the genus *Lobaria* are difficult to mistakenly identify and have been found in Ohio (Wetmore, 1989; Flenniken & Showman, 1990) and Pennsylvania (Hill, 2000). The *Lobaria* genus are good indicators of air quality and old growth forests, which suggests that it was collected in a mature forest away from urban areas, of which there is none in Allegheny County today. It should also be noted that while our study identified five species of *Cladonia*, Mozingo et al. (1948) listed 16 species. *Cladonia* are squamulose lichens generally sensitive to air pollution, and the significant difference in the number of species suggests a decline in air quality in southwestern Pennsylvania. However, as mentioned earlier caution must be taken because Mozingo et al. (1948) does not provide the location of where the lichens were collected, which limits the ability to draw too many conclusions from historical comparisons.

This study provides the first intensive sampling of epiphytic lichens in southwestern Pennsylvania. A study by McClenahen et al. (2007) of lichens centered on Indiana County is restricted to macrolichens, which does not include the crustose or squamulose forms. Their study does however provide a temporal perspective, having surveyed macrolichens between 1997 and 2003, resulting in a linear mean rate of gain in species of 0.56 species per year (McClenahen et al., 2007). Further work is being conducted by James C. Lendemer at the New York Botanical Gardens to develop checklists of lichens in state parks and recreational areas across Pennsylvania, which will provide a species list to which future surveys can be compared against (Harris & Lendemer, 2005; Lendemer, 2005; Lendemer & Macklin, 2006a; Lendemer & Macklin, 2006b).

Table 9. Presence of lichen species published by Mozingo et al. (1948) compared against specimens identified in this study. The lichens listed are epiphytes and does not include those that predominate on rock or soil substrate. In the case of name changes or synonyms, the updated name is on the far left and the original name provided by Mozingo et al. (1948) is provided in brackets. Any subspecies listed by Mozingo et al. (1948) were not included for comparison purposes because this study did not differentiate between subspecies. The numbered codes refer to Allegheny County = 1, Washington County = 2 and Westmoreland County = 3.

	Mozingo et al., 1948	**Opdyke et al., 2010**
Amandinea punctata [*Buellia punctata*]	2	1, 3
Baeomyces roseus	3	
Caloplaca flavorubescens [*Caloplaca aurantiaca*]	2	
Cladonia bacillaris	1, 2, 3	
Cladonia borbonica	1, 3	
Cladonia caespiticia	1	1, 2, 3
Cladonia cervicornis	1, 2, 3	
Cladonia chlorophaea	1, 3	
Cladonia coniocraea	1, 2, 3	
Cladonia cristatella	1, 2, 3	1, 2, 3
Cladonia fimbriata	1	
Cladonia gracilis	2	
Cladonia grayi	1	
Cladonia macilenta	1, 2, 3	2, 3
Cladonia ochrochlora	1, 3	1, 2, 3
Cladonia parasitica [*Cladonia delicate*]	1	1, 2, 3
Cladonia ramulosa [*Cladonia pityrea*]	1	
Cladonia squamosa	1, 3	
Cladonia subulata [*Cladonia nemoxyna*]	1, 3	
Flavoparmelia caperata [*Parmelia caperata*]	1, 3	1, 2, 3
Heterodermia speciosa [*Anaptychia speciosa*]	1	
Lecanora chlarotera [*Lecanora rugosa*]	3	
Lepraria membranacea [*Amphiloma lanuginosum*]	1, 2, 3	
Lobaria amplissima [*Sticta amplissima*]	1, 3	
Lobaria pulmonaria [*Sticta pulmonaria*]	1	
Myelochroa aurulenta [*Parmelia aurulenta*]	2	1
Parmelia saxatilis	1, 3	
Parmelia sulcata	1, 2, 3	1, 2, 3
Parmelina quercina [*Parmelia quercina*]	3	
Parmotrema chinense [*Parmelia perlata*]	3	
Peltigera aphthosa	1, 2, 3	
Peltigera horizontalis	3	
Phaeophyscia ciliata [*Physcia obsura*]	2, 3	
Phaeophyscia rubropulchra [*Physcia endochrysea*]	2, 3	1, 2, 3
Physcia stellaris	3	1, 3
Punctelia borreri [*Parmelia borreri*]	1, 3	
Punctelia rudecta [*Parmelia rudecta*]	1, 2, 3	1, 2, 3
Pyxine sorediata	2, 3	
Xanthoparmelia conspersa [*Parmelia conspersa*]	1, 3	

5.3. Future Application of Research

To the best of our knowledge, this study is the first intensive monitoring of lichens in the Pittsburgh area. It provides baseline data for future studies on lichens relating to changes in communities caused by air pollution, habitat alteration and climate change. The saturation of NO_2 and SO_2 air pollutants from the Ohio River Valley and metropolitan area of Pittsburgh limit the ability to use community analyses to assess the dispersion of these pollutants. However, tissue analysis can reveal the bioaccumulation of air contaminants, providing an indirect measurement of the magnitude of air pollutants in the region. Measuring photosynthetic rates of the algal component in the lichens can also provide a measure of lichen health, as NO_2 and SO_2 has been known to cause physiological damage (Richardson, 1988). We recommend any future studies focusing on the affects of air pollutants on lichens in saturated areas to identify a subset of species throughout the study area to conduct tissue analyses and measure photosynthetic rates.

An extension of lichen biomonitoring that came out of this study is that community analyses can be used to evaluate habitat management of parks. Isolated, urban parks restrict dispersion and genetic diversity, which threatens the health of the lichen community should the park face extensive, environmental damage from a tornado or severe storm. Thus, it is important to prohibit fragmentation whenever possible, and in city environments where fragmentation is inevitable, maintain a diversity of habitats. The species richness and diversity of lichens was lowest at Frick compared to all other sites in our study, which is likely due to Frick being managed as a wooded, city park. Schenley and Mingo are managed for a greater diversity of recreational activities, having more diverse habitats and open spaces, which would allow for a greater diversity of lichens along the edges of open spaces that could then disperse into the interior of the woodlands. Since lichens are ubiquitous and community analyses can be quickly and cheaply conducted with minimal training to identify macrolichens, park managers can use lichens to monitor management plans across parks in a given region. Field methods usable by nonspecialists have already been developed through the Forest Health Monitoring program established to assess the condition and trend of forests in the United States (McCune, 2000). To promote the use of lichens in monitoring management activities, we believe that further research is needed to convince park managers and the public about the importance of lichens in their region. For example, the *Cladina* genus, better known as reindeer lichen, is a principal winter food of caribou in North America and reindeer in Europe (Brodo et al., 2001).

In addition to using lichens as biomonitors for park management, we have also shown in this study that their abundance and diversity are influenced by moisture and possibly, temperature. Sochting (2004) discusses the role of *F. caperata* as an indicator species for temperature change, being a temperate to subtropical lowland species. Sochting (2004) suggests that the apparent successful growth of *F. caperata* in Denmark, where his study took place, is attributed to an increase in mean temperatures during the last 100 years.

In the next several decades (2010-2039), annual average temperatures may increase to over 2.5 ^{o}F, and possibly over 4 ^{o}F by mid-century (2040-2069) in Pennsylvania (UCS, 2008). Annual precipitation rose from just under 965 mm in the early twentieth century to nearly 1,118 mm by its end, with a 5% expected increase through the mid-century (UCS, 2008). These projections suggest that with increasing temperature and moisture, an increase in the abundance and diversity of temperate to subtropical lichens will occur throughout Pennsylvania, assuming there is little to no increase in summer drought frequency. Conducting long-term studies of lichen communities at permanent plots throughout Pennsylvania would provide significant information on changes in temperature and atmospheric moisture, indirectly assessing precipitation and local hydrology. A future study should also focus on identifying indicator species of climate change, such as *F. caperata* for signifying increasing temperatures. According to distribution maps published by Brodo et al. (2001), *Parmelia sulcata* and *Hypogymnia physodes* are widespread throughout the east but are mainly restricted to the northern half of North America, whereas, *Parmelinopsis minarum* and *Parmotrema hypotropum* are found south of Canada. These common macrolichens could also be used as indicators of climate change. To date, indicator species have been used for air pollution based on their sensitivity level to SO_2 and N (USFS, 2010) but few have been evaluated for climate change.

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7. REFERENCES

ALA. 2009. American Lung Association State of the Air 2009. American Lung Association, Washington, D.C.

Armstrong, R.A. 1990. Dispersal, establishment and survival of soredia and fragments of the lichen *Hypogymnia physodes*. New Phytologist 114:239-245.

Asta, J. W. Erhardt, M. Ferretti, F. Fornasier, U. Kirschbaum, P.L. Nimis, O.W. Purvis, S. Pirintsos, C. Scheidegger, C. van Haluwyn, and V. Wirth. 2002. Mapping lichen diversity as an indicator of environmental quality. In: Nimis, P.L. et al. (eds.): Monitoring with Lichens - Monitoring with Lichens. NATO Science Series, IV, vol. 7. Kluwer, Dordecht, pp. 273-279.

Blett, T., L. Geiser, and E. Porter. 2003. Air pollution-related lichen monitoring in national parks, forests and refuges: Guidelines for studies intended for regulatory and management purposes. National Park Service, U.S. Fish and Wildlife Service and U.S. Forest Service.

Brodo, I.M. 1966. Lichen growth and cities: A study on Long Island, New York. The Bryologist 69:427-449.

Brodo, I.M., S.D. Sharnoff, and S. Sharnoff. 2001. Lichens of North America. Yale University Press, London, England.

Bungartz, F. 2001. Analysis of lichen substances. Lichen Herbarium, Arizona State University. Available online: http://nhc.asu.edu/herbarium/lichen_info/tlc.php.

Conti, M.E., and G. Ceccheti. 2001. Biological monitoring: lichens as bioindicators of air pollution assessment-a review. Environmental Pollution 114:471-492.

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

Ellis, C.J., B.J. Coppins, T.P. Dawson, and M.R.D. Seaward. 2007. Response of British lichens to climate change scenarios: Trends and uncertainties in the projected impact for contrasting biogeographic groups. Biological Conservation 140:217-235.

EPA. 2010a. Clean Air Status and Trends Network (CASTNET) 2008 Annual Report. U.S. Environmental Protection Agency, Washington, D.C.

EPA. 2010b. Nitrogen oxides emissions by source sector, 2005. U.S. Environmental Protection Agency, Washington, D.C. Available online: http://www.epa.gov/air/emissions/nox.htm.

Ferry, B.W., M.S. Baddeley, and D.L. Hawksworth. 1973. Air pollution and lichens. The Athlone Press, London.

Flenniken, D., and R.E. Showman. 1990. The macrolichens of Ohio: a revised checklist. Ohio Journal of Science 90:130-132.

Garcia, A.Z., T.H. Nash III, and M.A. Herrera-Campos. 2000. Lichen decline in Desierto de los Leones (Mexico City). Bryologist 103:428-441.

Godinho, R.M., H.T. Wolterbeek, T. Verburg, and M.C. Freitas. 2008. Bioaccumulation behaviour of transplants of the lichen *Flavoparmelia caperata* in relation to total deposition at a polluted location in Portugal. Environmental Pollution 151:318-325.

Gombert, S., J. Asta, and M.R.D. Seaward. 2003. Correlation between the nitrogen concentration of two epiphytic lichens and the traffic density in an urban area. Environmental Pollution 123:281-290.

Gustafsson, L. and I. Eriksson. 1995. Factors of importance for the epiphytic vegetation of aspen

Populus tremula with special emphasis on bark chemistry and soil chemistry. Journal of Applied Ecology 32:412-424.

Harris, R.C., and J.C. Lendemer. 2005. Contributions to the lichen flora of Pennsylvania: a checklist of lichens collected during the first Howard Crum bryological workshop, Delaware Water Gap National Recreational Area. Opuscula Philolichenum 2:1-10.

Hawksworth, D.L., and D.L. Hill. 1984. The lichen-forming fungi. Blackie, London.

Hill, R.J. 2000. Checklist for the lichen-forming, lichenicolous and allied fungi of Pennsylvania. Pennsylvania Department of Conservation and Natural Resources, Harrisburg, Pennsylvania.

Hinds, J.W. and P.L. Hinds. 2007. The macrolichens of New England. NY Botanical Garden Press, Bronx, New York.

Johansson, P., and J. Ehrlen. 2003. Influence of habitat quantity, quality and isolation on the distribution and abundance of two epiphytic lichens. Journal of Ecology 91:213-221.

Jovan, S. and B. McCune. 2005. Air-quality bioindication in the Greater Central Valley of California, with epiphytic macrolichen communities. Ecological Applications 5:1712-1726.

Kelly, M., and M. Besselman. 2009. Allegheny County Point Source Emission Inventory Report 2007. Allegheny County Health Department, Air Quality Program, Pittsburgh, Pennsylvania.

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. 2010. Lichen biomonitoring in National Capital Region: lichens as bioindicators of air pollution. George Mason University, Virginia. Available online: http://mason.gmu.edu/~jlawrey/CUE/sensitivity.

Lendemer, J.C. 2005. Contributions to the lichen flora of Pennsylvania: the lichen flora of the diabase region of Northern Bucks and Montgomery Counties. Opuscula Philolichenum 2:21-16.

Lendemer, J.C., and J.A. Macklin. 2006a. Contributions to the lichen flora of Pennsylvania: a preliminary checklist of the lichens of Nescopeck State Park. Opuscula Philolichenum 3:41-48.

Lendemer, J.C., and J.A. Macklin. 2006b. Contributions to the lichen flora of Pennsylvania: a preliminary checklist of the lichens of Worlds End State Park. Opuscula Philolichenum 3:53-64.

Lerond, M. 1978. Courbes d'isopollution de la region de Rouen obtenues par l'observation des lichen epiphytiques. Bulletin de la Societe Linne Normandie 106:73-84.

Loppi, S., L. Frati, L. Paoli, V. Bigagli, C. Rossetti, C. Bruscoli, and A. Corsini. 2004. Biodiversity of epiphytic lichens and heavy metal contents of *Flavoparmelia caperata* thalli as indicators of temporal variations of air pollution in the town of Montecatini Terme (central Italy). Science of the Total Environment 326:113-122.

Marmor, L., and T. Randlane. 2007. Effects of road traffic on bark pH and epiphytic lichens in Tallinn. Folia Cryptogamica Estonica, Fasc. 43:23-37.

McClenahen, J.R., D.D. Davis, and R.J. Hutnik. 2007. Macrolichens as biomonitors of air-quality change in western Pennsylvania. Northeastern Naturalist 14:15-26.

McCune, B. 1988. Lichen communities along O_3 and SO_2 gradients in Indianapolis. The Bryologist 91:223-228.

McCune, B., C.C. Derr, P.S. Muir, A. Shirazi, S.C. Sillett, and W.J. Daly. 1996. Lichen pendants

for transplant and growth experiments. Lichenologist 28:161-169.
McCune, B., J. Dey, J. Peck, K. Heiman, and S. Will-Wolf. 1997. Regional gradients in lichen communities of the southeast United States. The Bryologist 100:145-158.
McCune, B. 2000. Lichen communities as indicators of forest health. The Bryologist 103:353-356.
Mozingo, H.N. 1948. Western Pennsylvania lichens. The Bryologist 51:38-56.
NALH. 2010. Consortium of North American Lichen Herbaria. Available online: http://symbiota.org/nalichens/index.php.
Nash III, T.H. 1976. Sensitivity of lichens to nitrogen dioxide fumigations. The Bryologist 79:103-106.
NCDC. 2004. Climatography of the United States No. 20, 1971-2000. Station: Pittsburgh International Airport, PA. National Climatic Data Center, Asheville, North Carolina.
Nimis, P.L., A. Ciccarelli, G. Lazzarin, R. Barbagli, A. Benedet, M. Castello, D. Gasparo, D. Lausi, S. Olivieri, and M. Tretiach. 1989. I licheni come bioindicatori di inquinamento atmosferico nell'area di Schio-Thiene-Breganze (VI). In Bolletino del Museo Civico di Storia Naturale di Verona, 16. CO.GE.V. s.r.l., Verona, Ecothema s.r.l., Trieste.
Nylander, W. 1866. Les lichens du Jardin du Luxembourg. Bulletin de la Societe Botanique de France 13:364-372.
Orange, A., P.W. James, and F.J. White. 2001. Microchemical methods for the identification of lichens. British Lichen Society, London.
PADEP. 2006. Ambient Air Quality Monitoring Report. Pennsylvania Department of Environmental Protection, Bureau of Air Quality, Harrisburg, Pennsylvania.
PennDOT. 2009. Annual average daily traffic maps, 2008. Pennsylvania Department of Transportation. Available online: http://www.dot.state.pa.us/Internet/Bureaus/pdPlanRes.nsf/infoBPRTrafficInfoTrafficVolumeMap.
Perlmutter, G.B. 2010. Bioassessing air pollution effects with epiphytic lichens in Raleigh, North Carolina, U.S.A. The Bryologist 113:39-50.
Richardson, D.H.S. 1988. Understanding the pollution sensitivity of lichens. Botanical Journal of the Linnean Society 96:31-43.
Santamaria, J.M., and A. Martin. 1997. Tree bark as a bioindicator of air pollution in Navarra, Spain. Water, Air, and Soil Pollution 98:381-387.
Scheidegger, C., and B. Schroeter. 1995. Effects of ozone fumigation on epiphytic macrolichens: ultrastructure, CO_2 gas exchange and chlorophyll fluorescence. Environmental Pollution 88:345-354.
Showman, R.E. 1985. Gavin area air quality biomonitoring studies. American Electric Power Service Corp., Environmental Engineering Division. Columbus, Ohio.
Showman, R.E. 1997. Continuing lichen recolonization in the Upper Ohio River Valley. The Bryologist 100:478-481.
Sochting, U. 2004. Flavoparmelia caperata-a probably indicator of increased temperatures in Denmark. Graphis Scripta 15:53-56.
Tang, W., T. Raymond, B. Wittig, C. Davidson, S. Pandis, A. Robinson, and K. Crist. 2004. Spatial variations of $PM_{2.5}$ during the Pittsburgh air quality study. Aerosol Science and Technology 38:80-90.
UCS. 2008. Climate change in Pennsylvania: impacts and solutions for the Keystone State. Union of Concerned Citizens Publications, Massachussetts.
USCB. 2002. Census 2000 population finder. U.S. Census Bureau, Washington, D.C. Available

online: http://factfinder.census.gov/servlet/SAFFPopulation?_submenuId=population_0&_sse=on.
USFS. 2010. Air pollution sensitivity ratings for Pacific Northwest macrolichens. U.S. Forest Service, National lichens and air quality database and clearinghouse, Washington, D.C. Available online: http://gis.nacse.org/lichenair/index.php?page=sensitivity.
van Herk, C.M. 1999. Mapping of ammonia pollution with epiphytic lichens in the Netherlands. Lichenologist 31:9-20.
van Herk, C.M., A. Aptroot, and H.F. van Dobben. 2002. Long-term monitoring in the Netherlands suggests that lichens respond to global warming. Lichenologist 34:141-154.
Washburn, S.J., and T.M. Culley. 2006. Epiphytic macrolichens of the greater Cincinnati metropolitan area-part II: distribution, diversity and urban ecology. The Bryologist 109:516-526.
Wetmore, C.M. 1989. Lichens and air quality in Cuyahoga Valley National Recreation Area, Ohio. The Bryologist 92:273-281.

APPENDIX A

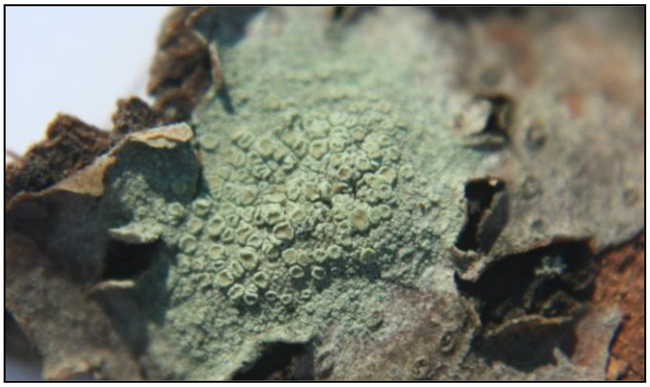
The following is a list of presentations made at scientific conferences to promote the study. The authors are also working on a peer-reviewed manuscript to be submitted to The Bryologist and a user-friendly lichen identification guide for southwestern Pennsylvania.

(1) Roy, J.D., M.R. Opdyke, and B. Dolney. 2010. Lichen distribution in southwestern Pennsylvania as an indicator of environmental conditions. Paper presented at Pennsylvania Academy of Science Meeting, Abstract 26.
(2) Dolney, B.E., M.R. Opdyke, and L. Frost. 2009. Lichen distribution across a landscape gradient as an indicator of air pollution and habitat alteration. Poster presented at Ecological Society of America Meeting, Abstract PS 95-189.
(3) Dolney, B.E., M.R. Opdyke, and L. Frost. 2009. Lichen distribution across an urban to rural landscape gradient as an indicator of air pollution and habitat alteration. Poster presented at Pennsylvania Academy of Science Meeting, Abstract 57.

APPENDIX B

The following photographs of common lichens found in southwestern Pennsylvania were taken by Matthew R. Opdyke.

CRUSTOSE LICHENS



Lecanora stobolina
Mealy rim-lichen

Lepraria lobificans
Fluffy dust lichen

unknown sterile crustose

FOLIOSE LICHENS

Candelaria concolor
Candleflame lichen

Flavoparmelia caperata
Common greenshield lichen

Hypogymnia physodes
Monk's-hood lichen

Parmelia sulcata
Hammered shield lichen

Parmotrema hypotropum
Powdered ruffle lichen

Phaeophyscia pusilloides
Pompon shadow lichen

Phaeophyscia rubropulchra
Orange-cored shadow lichen

Physcia millegrana
Mealy rosette lichen

Punctelia caseana
Powdered speckled shield lichen

Punctelia rudecta
Rough speckled shield lichen

SQUAMULOSE LICHENS

Cladonia caespiticia
Stubby-stalked cladonia

Cladonia ochrochlora
Smooth-footed powderhorn

Cladonia parasitica
Fence-rail cladonia

APPENDIX C

The enclosed CD includes digital copies of the final report and raw data generated from the study.