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Urbanization and Atmospheric Deposition: Use of Bioindicators in Determining Patterns of Land-Use Change in West Georgia

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Abstract Changes in land use disrupt ecosystem patterns and processes and serve as precursors to other biotic and abiotic stressors. Forest ecosystems in the urban core typically differ structurally and functionally from those in rural areas. The overall objective of the study was to determine concentrations of selected air-borne contaminants (N, S, and heavy metals) over space and time and relate these to land-use changes. Elemental concentrations in lichens, soils, and tree cores were examined from 36 plots distributed along an urban-to-rural gradient surrounding Columbus, GA, USA. In situ lichen tissue exhibited the most significant differences among land-use types, with Cu, N, Pb, S, and Zn concentrations all significantly greater at urban sites. Lichen transplants revealed differences in concentrations between species, but not between land-use types. No discernable trends were observed regarding concentrations in soil and tree core data. Lichens appear to be a sensitive indicator of land-use change in this particular case study.

Keywords Atmospheric deposition · Bioindicator · Ecosystem · Forest health · Land-use change · Lichens · Metals · Urban-to-rural gradient

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1 Introduction

Forest ecosystems in urban and urbanizing (developing areas) locals are exposed to many and complex sources of localized air pollution due to increased human presence and may be impacted more than those in more rural/pristine areas. Atmospheric deposition of pollutants can affect various processes within a forest community, from individual plant to ecosystem levels, and exposure responses are often complex and not well understood (Smith and Siccama 1981; Muir and McCune 1988; McDonnell et al. 1997; Chappelka and Samuelson 1998). Forest health depends on many ecosystem processes that balance tree growth, mortality, and regeneration and maintain homeostasis (Hyland 1994). For example, air pollutants such as O_3, NO_2, NH_3, SO_2, and toxic metals (Pb, Cd, etc.) can affect plants at the leaf level, decreasing photosynthetic rates (Lefohn et al. 1997; McDonnell et al. 1997; Lovett et al. 2000; Gregg et al. 2003). Impaired photosynthetic processes can then disrupt overall plant growth and root dynamics, which can indirectly alter stand-level processes such as net primary productivity, decomposition rates, and soil nutrient cycling (Pouyat et al. 1995; Chappelka and Samuelson 1998; Lovett et al. 2000; Gregg et al. 2003). When these processes become impaired and the system is stressed, observations of pests, disease, and exotic plant species may increase (Percy and Ferretti 2004), while the incidence and abundance of sensitive lichen communities decrease (McCune et al. 1997). Therefore, analysis of bioindicators of these and other processes serve as useful tools in forest ecosystem health assessments.

A bioindicator is an organism that can be used to indicate an alteration in the environment, and these changes can be physical or chemical, as well as positive or negative (Manning 1993; Conti and Cecchetti 2001). Whether introduced or native to the area, these organisms can provide unique information regarding ambient air quality in a particular local or region (Manning 1993, 2003).

Typically, lichens are a good bioindicator because their anatomy and/or physiology (e.g., high surface area, lack of roots, rapid water absorption) are such that they are sensitive to small changes in the environment (Geiser and Reynolds 2002). Lichens are nonvascular composite organisms that lack a cuticle; as such, they are readily affected by pollutants that are deposited onto their bodies or absorbed into their tissue with the water and nutrients they largely obtain from the air for survival (Bergamaschi et al. 2007; Fenn et al. 2007). The anatomical structure and physiological processes by which they live and grow are such that pollutant deposition, accumulation, and uptake can be monitored (McCune 2000; Conti and Cecchetti 2001; Bergamaschi et al. 2007; Fenn et al. 2007; Geiser and Neitlich 2007). Use of lichens as bioindicators is especially valuable in ecological field research because the methods are repeatable, the data are more economical than instrumented monitoring, they allow rapid assessment, and changes can be tracked both spatially and temporally (McCune 2000; Geiser and Reynolds 2002).

Forest characterizations provide information about structure and composition, but bioindicators give us a good idea about the current state of ecosystem function. McLaughlin and Percy (1999) defined ecosystem health as the capacity to maintain adequate quantities of water, nutrients, and energy to be productive while being resistant or resilient to environmental stressors. Healthy forests display a balance among growth, mortality, and regeneration, are diverse, and have the ability to withstand or recover from exposure to stressors such as insect or disease outbreaks, adverse weather and climate conditions, and air pollutant deposition (Percy and Ferretti 2004). Standards for assessing forest health have been established and usually include a combination of several bioindicators of ecosystem health (McDonnell et al. 1993; McDonnell et al. 1997). These have proven useful in previous studies and are generally accepted as standard protocols for forest health assessments. Bioindicators best suited for air pollution analyses as well as overall forest health include foliar injury assessments (ozone, sulfur dioxide, fluoride, etc.), soil and tree nutrient status, and lichen incidence, abundance, species richness, and elemental concentrations in tissue (Muir and McCune 1988; Manning 2005; Geiser and Reynolds 2002). Previous research has shown that lichens provide a much clearer biological response to air pollution exposure than tree growth rate or visible foliar symptoms (Muir and McCune 1988).

Lichens are extremely sensitive to NO_2, NH_3, and SO_2 pollution (Nash and Gries 1986; McCune 2000; Gombert et al. 2004). It has been widely reported that lichen species diversity varies greatly with exposure to SO_2 and NO_2 (McCune et al. 1997; van Dobben et al. 2001; Frati et al. 2006; Fenn et al. 2007; Geiser and Neitlich 2007). The longevity of lichens and the processes by which they absorb air pollutants also make them useful as biomonitors of heavy metal deposition that has occurred within a forest in the recent past (Nash and Gries 1986; McCune 2000).

Heavy metal concentrations (e.g., Pb, Ni, Zn) in soils are a good indicator of forest health because these elements tend to accumulate and can alter biogeochemical cycles (Pouyat and McDonnell 1991; Smith and Siccama 1981). Prior studies have reported significant relationships between Pb concentrations in soil and proximity to major urban centers (Johnson et al. 1982; Pouyat and McDonnell 1991).

Whereas soil analyses can provide data over a broad spatial scale, tree core investigations offer an abundance of easily obtainable historical data. Positive relationships have been observed between soil and tree core Pb concentrations (Baes and McLaughlin 1984; Alberici et al. 1989; Jordan et al. 1990; McClenahen and Vimmerstedt 1993; Anderson et al. 2000). Mechanisms of Pb entry into tree xylem tissues probably include some combination of translocation from foliage (following deposition onto the tree) and root uptake from soil (Friedland 1990; Jordan et al. 1990; Anderson et al. 2000). Combining each of these methodologies enables researchers to gain a broader more complete view of the forest ecosystem such that inferences about landscape processes may be possible (Smith and Siccama 1981; Manning 2005).

The overall goal of this project was to examine spatial and temporal trends of elemental concentrations (N, S, metals) in forests of West Georgia using bioindicators along an urban-to-rural gradient. The overall hypothesis was forest ecosystems in urban and rapidly developing areas are exposed to greater amounts of localized air pollutant deposition as a consequence of increased human presence, smaller area, more forest edges, and fragmented nature and thus can be subject to higher elemental concentrations in soils and plant tissues than those located in rural environments. Specific objectives were (1) to determine current background elemental concentrations at each site through the collection of in situ lichen tissue samples from plots across the gradient, (2) to detect short temporal deposition trends (1 year) across the region through the use of two common lichen species collected from a "reference" area and transplanted into plots along the gradient, (3) to establish broad spatial deposition trends in West Georgia by obtaining soil samples from each plot, and (4) to ascertain long temporal (50 years) trends in the region by acquiring tree cores from stand dominants (*Pinus taeda* L.) within each forest stand. Each of these datasets was analyzed for N, S, and metals (Cd, Cr, Cu, Ni, Pb, Zn) to provide information about deposition sufficient to establish trends in West Georgia over space and time. By assessing a variety of different media for elemental concentrations, commonalities in response could be evaluated to determine consistency in overall deposition trends.

2 Methods and Materials

2.1 Study Area

The study area (hereafter referred to as "West Georgia"; Fig. 1) includes Muscogee (location of the Columbus metropolitan area), Harris, and Meriwether counties in the west-central Georgia Piedmont and represents an urban-to-rural gradient in terms of land development (urban, developing, and rural, respectively). Urban growth around Columbus, GA, USA is constrained by Fort Benning (a large US military base) to the south and by the Chattahoochee River to the west, such that development is occurring to the north and east of Columbus.

Development in "West Georgia" has resulted in different demographic measures in these counties over the past 15 years. Muscogee County, which contains the city of Columbus, has had low population growth (3% from 1990–2005) but high density (333 people/km^2 in 2000). Harris County, adjacent to the northeast of Muscogee County, had extremely high population growth (56% from 1990–2005), far above the national average (19% from 1990–2005), but still maintains low (20 people/km^2 in 2000) density. Meriwether County, adjacent to the northeast of Harris County, has experienced a reduced population growth (2%) and low (17 people/km^2 in 2000) density from 1990–2005 (U.S. Census Bureau 2006).

2.2 Physiography and Climate

The study area lies within the Piedmont Physiographic Province of west-central Georgia, and plot elevations range from approximately 100 to 260 m above mean sea level. This province is characterized by gently rolling hills, deeply weathered bedrock, and isolated occurrences of granitic plutons (University of Georgia 2007). Soils in West Georgia are typical of the Piedmont Province and study sites are generally located on upland sandy loams (Table 1). The climate of the west Georgia Piedmont is moist and temperate. Precipitation is evenly distributed across the region and is mainly in the form of rainfall, as snowfall in the area is rare (Southeast Regional Climate Center 2007). The 50-year average of mean annual precipitation in the area is 1,245 mm, while annual temperatures range from a mean monthly minimum of 12°C in January to a mean monthly maximum of 24°C in July (Southeast Regional Climate Center 2007). Prevailing winds during the growing season are from the south–southwest, flowing up from the Gulf of Mexico, while in winter large frontal systems typically force air down the eastern side of the Appalachian Mountains resulting in strong northwesterly winds across Georgia (Styers 2005).

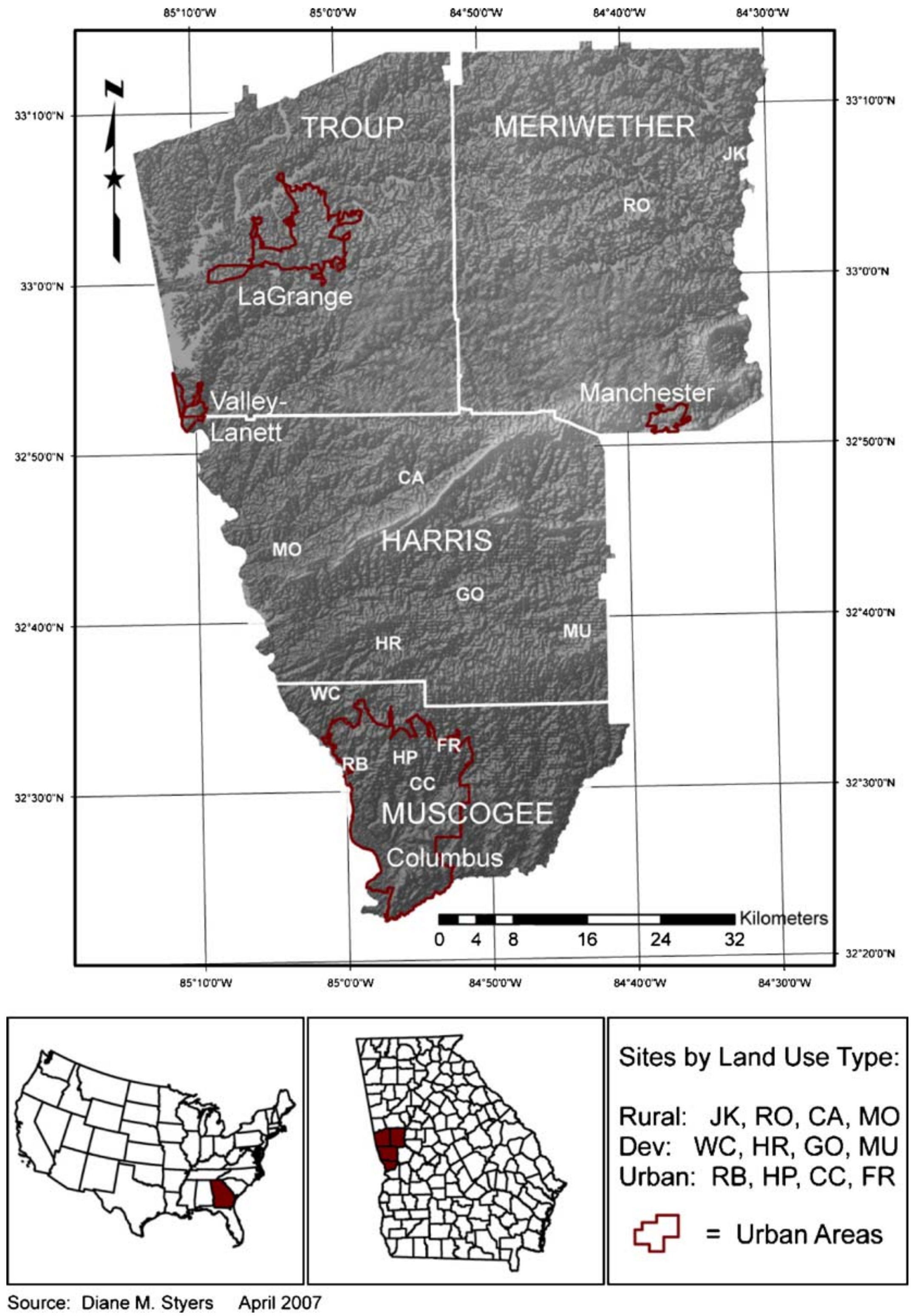

Fig. 1 Map of "West Georgia" study area. Urban Sites: *RB* Roaring Branch, *HP* Heath Park, *CC* Cooper Creek, *FR* Flat Rock; developing sites: *WC* Whiskey Creek, *HR* Hunter Road, *GO* Goolsby, *MU* Mulberry Creek, rural sites: *MO* Mountain Oak, *CA* Callaway, *RO* Red Oak, *JK* Joe Kurz WMA

Table 1 West Georgia site characteristics measured during the study (2005–2006)

Site[a]	Latitude	Longitude	Elevation (m)	Soil series	Hardwood (%)	Canopy (%)	BA (m^2/ha)	Stand age (year)
RB	32.5271	−84.9903	106	Wedowee sandy loam, 10–35% slopes	69	95	26.59	34
HP	32.5336	−84.9321	101	Dothan-Urban land complex, 2–5% slopes	74	74	39.15	45
CC	32.5076	−84.9125	100	Esto-Urban land complex, 8–25% slopes	70	90	37.08	53
FR	32.5448	−84.8814	208	Pacolet sandy clay loam, 10–15% slopes	54	70	41.53	51
WC	32.6016	−85.0226	126	Pacolet sandy loam, 15–25% slopes	83	94	32.89	40
HR	32.6456	−84.9489	188	Pacolet sandy loam, 6–10% slopes	94	94	39.30	47
GO	32.692	−84.8535	139	Pacolet sandy loam, 10–15% slopes	77	85	26.83	47
MU	32.6541	−84.7304	205	Chewacla sandy loam, 0–2% slopes	100	91	21.45	30
MO	32.7393	−85.0642	157	Pacolet sandy loam, 6–10% slopes	71	94	30.25	48
CA	32.8068	−84.9186	246	Cecil sandy loam, 6–10% slopes	57	94	31.11	60
RO	33.0687	−84.6508	254	Cecil sandy clay loam, 10–15% slopes	89	90	35.07	53
JK	33.1165	−84.5360	239	Cecil sandy clay loam, 6–10% slopes	97	95	35.02	39

Urban sites: *RB* Roaring Branch, *HP* Heath Park, *CC* Cooper Creek, *FR* Flat Rock; developing sites: *WC* Whiskey Creek, *HR* Hunter Road, *GO* Goolsby, *MU* Mulberry Creek; rural sites: *MO* Mountain Oak, *CA* Callaway, *RO* Red Oak, *JK* Joe Kurz WMA

2.3 Pollution Sources and Monitoring

The 21-county pollution source region (US EPA 2006) in the vicinity of Columbus, GA includes the study area and several surrounding counties in Georgia and Alabama. The US EPA AirData database (US EPA 2006) indicated there are currently 105 facilities in the 21-county area that are monitored for emissions of CO, NH_3, NO_x, VOCs, SO_2, PM10, and PM2.5. The majority of these are located in Russell and Lee counties, AL, and include industrial, manufacturing, textile, lumber, paper, and brick-assembly factories, as well as sources from excavation and paving activities. However, two Georgia Power coal-fired steam-electric power plants are located in Heard and Coweta counties, GA, just north of Troup and Meriwether counties, respectively. In addition, there are several highways in the area (I-85, I-185, US 280, US 431, US 27) that are major routes for automobiles and transfer truck traffic and therefore are mobile sources of pollutants (e.g., CO, NO_x, etc.).

Pollutants monitored by stations in the Columbus, GA/Phenix City, AL metropolitan area include O_3, SO_2, Pb, PM10, and PM2.5 (US EPA 2006). No continuous monitors are located in the surrounding counties included in our study area so comparisons are difficult to make. Ogawa Model 3300 O_3 and NO_x passive samplers (http://www.rpco.com/products/ambprod/amb3300/) were installed at each sampling site location on trees at approximately 1.5 m in height to supplement the continuous monitoring data. Pollutant concentrations were determined through laboratory analyses conducted at the University of Minnesota (Krupa, personal communication). The devices were located within seven passive sampling sites (two urban, two developing, three rural) along the urban–rural gradient. Samples were collected approximately biweekly from May 18–September 21, 2005. However, due to sample contamination, only NO_x samples collected from July 27–September 21 were included in data analyses.

2.4 Plot Selection

Thirty-six permanent 0.05-ha circular plots (three plots per site; four sites per land-use type—urban, developing, and rural) were established in the winter of 2004–2005 along an urban-to-rural gradient using criteria adapted from the USDA Forest Inventory and Analysis National Program (FIA) guidelines (USDA Forest Service 2006) to assess the spatial extent of elemental concentrations in West Georgia. Approximately one half of our sites were previously selected by other researchers conducting water quality experiments in the region (Lockaby et al. 2005). Additional sites selected were added to extend the gradient farther to the northeast, so that the gradient extends approximately 100 km from southwest to northeast and is approximately 75 km in width (Fig. 1). Since other studies were conducted within these same locations, there was a more deliberate selection of specific plots. Individual plot locations were selected based on specific criteria: upland, interior (at least 30 m from an edge), forested locations within pine-oak woodlands containing mature loblolly pine and oak (*Quercus* spp.) of a similar age class, elevation, slope, aspect, uniformity of soils, and site histories (Table 1; see Styers 2008 for more specific information on plot characterization).

2.5 Lichen Tissue Collections

Lichens are known to be very sensitive indicators of ecosystem health (Nash and Gries 1986; McCune 2000; Gombert et al. 2004), so in situ tissue samples were collected and analyzed for elemental concentrations. Since exposure time was unknown in the in situ lichen collection and to account for any seasonal variability encountered due to seasonal differences (time of year collected), three separate collections were conducted over the course of 9 months (December 2005; June and September 2006). A minimum of 20 g (dry weight) of lichen tissue (any fruticose and foliose species) was collected from trees and recently fallen branches for elemental analysis to determine background N, S, and metal concentrations for each site similar to the methodology used by Geiser (2004). Due to difficulty in removal, no crustose lichen species were collected. According to Geiser (2004), 20 g of tissue is sufficient to offset any variability due to differences in lichen age within a single sample. Dead or dying tissue was not collected, as these samples could introduce error into the dataset. After removal of soil and debris from the samples to prevent contamination, lichen tissue samples were immediately transported from the field to the Auburn University Soil and Plant Tissue Testing Lab for elemental analysis (see Section 2.7 below).

To control for unknown plot variability within the in situ lichen tissue collection, a transplant experiment was conducted. Healthy samples of *Usnea strigosa* and *Parmotrema perforatum* located on black cherry (*Prunus serotina*) bark were collected from a common reference area (The Preserve at Callaway, located in West Georgia) during August 2005. Pollution emission concentrations in this area are generally regarded as low (Maxwell-Meier and Chang 2005). *U. strigosa* and *P. perforatum* are both common and conspicuous lichen species in the Georgia Piedmont (McCune et al. 1997; Morin et al. 2006; Georgia Botanical Society 2007). Although pollution sensitivity has not previously been determined for *U. strigosa*, many eastern *Usnea* species are highly pollution sensitive (McCune et al. 1997; Morin et al. 2006; USDA Forest Service 2007). Similarly, pollution sensitivity for *P. perforatum* has not been previously reported, and other eastern *Parmotrema* species are ranked all along the pollution sensitivity range, from tolerant to intermediate to sensitive (McCune et al. 1997; Morin et al. 2006; USDA Forest Service 2007). Based on these references, it is suggested here that *U. strigosa* is relatively pollution sensitive, while the sensitivity of *P. perforatum* is unknown. A tentative pollution sensitivity ranking of “intermediate” is suggested for *P. perforatum* based on the results of Styers (2008).

An initial elemental analysis was conducted on a portion of the lichen tissue samples to determine the average nutrient concentrations of the entire collection prior to transplantation. For each transplant sample, approximately 6 g of tissue was mounted onto a 10× 10-cm wooden board using liquid nails (Pearson 1993) and then transplanted to nine sites (18 plots—three each urban, developing, and rural). Transplants were placed on the northeast side of *Liquidambar styraciflua* L. trees at heights ranging between 1.4 and 1.7 m to maintain consistency, mimic optimum habitat location, and avoid interference with a separate but simultaneous lichen cover analyses on water oak (*Quercus nigra*; Styers, unpublished). An elemental analysis was conducted on one randomly selected transplant sample every 3 months for *U. strigosa* and every 6 months for *P. perforatum* (due to less available tissue sample material) during a 12-month exposure period (September 2005 through September 2006). Lichen tissue transplant samples were immediately transported from the field to laboratory for elemental analysis. Transplanted lichen tissue samples were analyzed for N, S, and metals, but due to limited sample quantities, not all analyses could be conducted for each of the sampling sites. Transplants were placed into several sites within each land-use type and site-specific values were averaged to land-use type for statistical analysis.

2.6 Soil and Tree Core Samples

Soil sampling locations were selected at the four cardinal directions at the plot boundary within each of the study plots and were cored using standard

National Forest Health Monitoring Program methods (USDA Forest Service 2006). O horizon material was brushed aside until the top of the A horizon was reached and then soil was sampled to a depth of 10 cm using a standard 5-cm-diameter stainless steel hand auger. To prevent cross contamination, the hand auger was washed with Alconox and rinsed with deionized water between sample collections. Soils were sieved (5.6 mm mesh size) to remove roots and rocks in the field and then taken to the laboratory for drying and elemental analysis. Samples were collected during December 2005 concurrent with tree core sampling.

Tree core samples were collected in an attempt to establish a longer historical record of elemental concentrations in the West Georgia region. Up to six *P. taeda* individuals per plot were randomly selected from within each of the study plots and cored using standard dendrochronological methods (Swetnam et al. 1985). These trees were selected because they were present in all sites, dominant within the study area, and had the potential to provide the longest chronologies for elemental analyses. Samples were collected during December 2005 concurrent with soil sampling.

Trees were cored using 5.15 mm inside diameter increment borers. A minimum of two cores were extracted from each tree at breast height (1.55 m), parallel to the slope. To prevent cross contamination, increment borers were washed with Alconox and rinsed with deionized water between samples. Cores were temporarily stored in paper straws and allowed to air dry for 1 week at 25°C prior to preparation for analysis. One core from each tree was used to determine age. These cores were mounted into grooved wooden mounts then sanded with a series of three grits (120, 400, 600) of sandpaper to enhance ring visibility (Swetnam et al. 1985). Ring patterns were measured and cross-dated and chronologies were developed. The other core was used for elemental analyses. These selected cores were cut into 10-year increments (up to 50 years) using the aged cores as a guide. Woody tissue samples were then transported to the laboratory for elemental analysis.

Decadal tree core samples were first statistically analyzed to gain a better understanding of temporal trends in the region by examining changes in metal concentrations from 1956–2005. After these long-term trends had been established, changes between land-use types during the past decade (1996–2005) were examined in more detail. Due to the small tissue sample amounts, there was only enough tissue to analyze for metals, and as a result, N and S concentrations were not examined in tree core tissues.

2.7 Elemental Analyses

Preparation of soil and plant materials and elemental analyses were conducted by the Auburn University Soil and Plant Tissue Testing Lab (Kirsten 1979; Plank 1992; Odom and Kone 1997). To check for quality control, the appropriate National Institute of Standards and Technology standards were used in all sample analyses. All samples were dried in a forced air oven at 60°C to a constant weight. Soil and plant materials were then weighed (0.1 and 0.2 g, respectively) and placed into tinfoil cups. Nitrogen was analyzed by combustion at 950°C using a LECO TruSpec C/N and S by combustion at 1,450°C using a LECO SC-432 (Kirsten 1979). Soil minerals were determined by Melich I extraction (Odom and Kone 1997) and solutions were analyzed by inductively coupled plasma emission spectroscopy (ICP-ES) using a Varian Vista-MPX Radial Spectrometer.

Plant tissue minerals were determined by dry ashing (organic matter destruction). Approximately 0.5 g of dried plant material was weighed into ceramic crucibles and ashed for 8 h in a muffle furnace at 500°C. Samples were digested on a hot plate using 1 N nitric acid and 1 N hydrochloric acid and filtered into approximately 50 ml volumetrics brought to volume with deionized water. Solutions were then analyzed by ICP-ES using a Varian Vista-MPX Radial Spectrometer to obtain mineral concentrations in each of the lichen and tree core samples (Odom and Kone 1997).

Of the elements determined by the aforementioned laboratory methods, only the following were statistically analyzed as part of this study: Cd, Cr, Cu, Ni, N, Pb, S, and Zn. Due to a calibration error in the LECO TruSpec C/N analyzer, N data for the June 2006 lichen transplant collection were omitted from the study.

2.8 Experimental Design and Statistical Analysis

Distributions and descriptive statistics for each variable were examined for the 12 sites (averaged from the 36 plots—3 per each site) using JMP IN® 5.1.2 (SAS Institute Inc. 2003), which was the statistical software package used in each of the analyses described hereafter. Since the data values were an average of three plots, they each displayed approximately normal distributions and were thus kept in their original format for analysis. Following preliminary explorations of the data, standard one-way analysis of variance (ANOVA) was performed to test for differences among the means between each land-use type for soil samples, since they were collected at one point in time. Differences in group means were then compared using Tukey–Kramer honestly significant difference, which tests for all differences among the means and adjusts for multiple comparisons made by controlling for the overall error rate. Tukey–Kramer comparisons were used to evaluate significance ($p<0.05$) since a broad range in elemental values for developing land uses may have obscured significant differences between urban and rural land-use types in the standard ANOVA analysis. In situ lichen samples, lichen transplants, and tree core samples were evaluated using repeated measures multivariate analysis of variance to account for both spatial and temporal differences. Lichen transplant data were analyzed in two distinct ways: one to compare differences in elemental concentrations between the two lichen species (*U. strigosa* vs. *P. perforatum*) and another to examine alterations in *U. strigosa* over time (seasonal changes) as well as across the land-use gradient.

3 Results and Discussion

3.1 Ambient Pollutant Concentrations

As shown from the data in Fig. 2, ozone concentrations were low in 2006 and were similar among sites for both the active and passive monitors. In an attempt to correlate ozone concentrations with injury to ozone-sensitive bioindicator plants including blackberry (*Rubus* spp.), yellow poplar (*Liriodendron tulipifera*), sweetgum, and black cherry were sampled at all sites using protocols developed from USDA FIA guidelines (USDA Forest Service 2006). No visible ozone symptoms were found at any of the sites sampled for any species observed. These results are related to the low ozone concentrations and micro-meteorological factors such as low soil moisture in 2006 (data not shown). Based on these data, determination of visible foliar symptoms may not consistently be a good indicator of ecosystem health as related to urbanization in the Columbus, GA area. Cumulative–seasonal NO_x concentrations were the greatest in the urban locations (Fig. 2) indicating a potential greater pollution load. However, as previously mentioned, no continuous NO_x monitors are located in the Columbus area to provide verification of the passive sampling data.

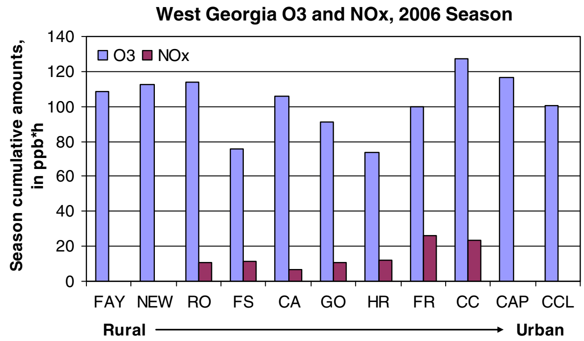

Fig. 2 Seasonal O_3 and NO_x concentrations collected from continuous and passive samplers located in the vicinity of Columbus, GA during the 2006 growing season (May 18–September 21). Passive monitors—urban sites: *CC* Cooper Creek, *FR* Flat Rock; developing sites: *WC* Whiskey Creek, *HR* Hunter Road, *GO* Goolsby; rural sites: *CA* Callaway, *RO* Red Oak. Continuous monitors—*FAY* Fayetteville, GA; *NEW* Newnan, GA (rural); *CAP* Columbus airport; *CCL* Columbus crime lab (urban), O3 monitoring only, provided by US EPA, AirData, 2006 (US EPA 2006). NO_x monitored only July 27–Sept 21

3.2 Elemental Concentrations in In Situ Lichen Tissue

Elemental concentration means are reported as the overall collective mean from the three bulk in situ lichen collections. *F* test comparisons indicate that urban environments consistently had significantly ($p\leq0.01$) higher concentrations of Cu, N, Pb, S, and Zn than either rural or developing land-use types (Table 2). Overall Cu concentrations in urban areas were 1.5-fold greater in lichens from developing and rural forests. Pb in the USA has dramatically decreased since the 1970s (Mielke 1999); small yet significant differences were found in urban vs. rural and developing areas. Zn concentrations in West Georgia lichens varied greatly between land-use types, with concentrations in urban areas 2.18- and 1.75-fold greater in rural and developing areas, respectively. *F* test comparisons also suggest significant ($p\leq0.01$) differences in concentrations of N and S between urban vs. rural

Table 2 Elemental concentration means (±SE) for in situ lichen tissue samples collected over 9 months (December 2005–September 2006) in West Georgia

Overall means (mg kg^{-1}) ± SE				Prob > *F*		
Element	Urban	Developing	Rural	Land use	Time	Time × LU
Cd	<0.1±0.02	<0.1±0.01	<0.1±0.01	–	–	–
Cr	3.73±1.17	3.45±0.91	2.97±0.89	0.86	<0.01**	0.96
Cu	26.75±0.99 (a)	18.27±0.84 (b)	17.47±1.86 (b)	<0.01**	<0.01**	0.48
N	14,340±995 (a)	10,329±280 (b)	9,768±627 (b)	<0.01**	0.06*	0.13
Ni	13.36±6.11	9.06±2.82	10.73±3.48	0.79	0.07*	0.69
Pb	3.39±0.47 (a)	1.79±0.16 (b)	1.73±0.09 (b)	<0.01**	0.06*	0.82
S	2,064±108 (a)	1,454±41 (b)	1,444±84 (b)	<0.01**	<0.01**	0.06*
Zn	41.61±2.25 (a)	23.77±1.75 (b)	19.06±1.06 (b)	<0.01**	<0.01**	0.10*

Number of study sites sampled for each land use type $n=4$ (total $n=12$); elemental concentrations reported in mg kg^{-1}; significant differences in land use and time assessed by *F* test and in interaction term (land use × time) by Wilks' lambda; overall land use means in a row with different letters (variables in bold) are significantly different ($p<0.05$) based on overall means from three collections. Cd was below detectable limits and therefore not analyzed (–)

SE standard error of the mean, *LU* land use

*$p\leq0.10$; **$p\leq0.01$

and developing areas. Lichens collected in urban land-use types had higher N and S, which were 1.4- to 1.5-fold greater than those in developing and rural areas, respectively. Of the nine elements analyzed, only Cd, Cr, and Ni concentrations were not significantly different between land-use types.

Natural background elemental concentration ranges for lichen species in the Southeast have not been previously reported. A literature search was conducted to locate any reported concentrations for these elements in any lichens of the same genera used in this study. Geiser and Neitlich (2007) proposed that lichens located in western Washington and Oregon in the northwestern USA having values of N>5,900 mg kg^{-1}, S>730 mg kg^{-1}, and Pb>15 mg kg^{-1} be considered "enhanced". Based on these criteria, percent background N and S in lichen tissue across West Georgia may be considered enhanced, but individual and interspecific variation in lichen tissue chemical composition between locals needs to be considered before any definitive conclusions can be made (Table 3). A relative comparison of elemental concentrations between these sites is still noteworthy, since there are no reported species-specific data across regions with which to compare. The concentrations reported in the West Georgia study are slightly higher than those reported by Fenn et al. (2007) for lichens in urban areas near the Columbia River Gorge, located along the Washington/Oregon border. However, N concentrations in Naples, Italy reported by Vingiani et al. (2004) were eightfold greater and S concentrations were15-fold greater than those reported for West Georgia (Table 3). Naples is a major seaport in a highly industrialized area of Italy, which could explain the extremely high N and S concentrations observed there. With the exception of Cu and Ni, metal concentrations were all substantially higher in Naples and Pavia, Italy (Adamo et al. 2003; Bergamaschi et al. 2007) than in West Georgia.

Anthropogenic sources of atmospheric N and S can be emitted and deposited both locally from vehicles and roadways (mobile sources) and regionally from power plants and other industrial point sources (Lovett et al. 2000; Gregg et al. 2003; Pitcairn et al. 2006; Fenn et al. 2007; Geiser and Neitlich 2007). Several stationary pollution emission sources are present in the West Georgia region (US EPA 2006). However, without the analysis of pollutant-specific transport models, the exact locations of pollutant deposition from these sources cannot be determined.

The largest anthropogenic sources of metals, however, originate locally as dry-deposited particulate matter from roadways, and deposition typically decreases with distance from roads (Zechmeister et al. 2005). Road density values within census tracts in urban areas of Columbus are four- and fivefold greater than those in developing and rural census tracts, respectively (Styers, unpublished). Sources of

Table 3 Comparisons of concentrations (mg kg^{-1}) of selected elements in lichen tissue in several studies

Author(s)	Location	Lichen species	Cd	Cr	Cu	N	Ni	Pb	S	Zn	Exposure
Geiser and Neitlich 2007	Western WA/OR, USA	Locally abundant target species (bulk collection)	–	–	–	>5900	–	>15	730	–	If values > "enhanced"
Fenn et al. 2007	Columbia River, WA/OR, USA	Locally abundant target species (bulk collection)	–	–	–	13,600	–	–	1,220	–	Urban mean
Vingiani et al. 2004	Naples, Italy	*Pseudevernia furfuracea* (L.) Zopf (fruticose transplants)	–	–	–	11,9900	–	–	31,600	–	Urban mean (after 17 weeks exposure)
Adamo et al. 2003	Naples, Italy	*Pseudevernia furfuracea* (L.) Zopf (fruticose transplants)	0.64	4.09	42.78	–	9.07	59.95	–	171.59	Urban mean (after 17 weeks exposure)
Bergamaschi et al. 2007	Pavia, N. Italy	*Pseudevernia furfuracea* (L.) Zopf (fruticose transplants)	0.48	8.4	23	–	4.6	36	–	162	Urban mean (after 5 months exposure)
Bergamaschi et al. 2007	Pavia, N. Italy	*Usnea* gr. *hirta* (fruticose transplants)	0.23	4.3	17	–	2.3	16	–	106	Urban mean (after 5 months exposure)
Present study	Columbus, GA, USA	Locally abundant fruticose and foliose species (bulk collection)	<0.1	3.73	26.75	14,300	13.36	3.39	2,060	41.61	Urban mean
Present study	Columbus, GA, USA	*Usnea strigosa* (Ach.) Eaton (fruticose transplants)	<0.1	1.2	23.01	6,800	<0.1	1.18	1,360	0.11	Urban mean (after 6 months exposure)
Present study	Columbus, GA, USA	*Parmotrema perforatum* (Jacq.) A. Massal (foliose transplants)	<0.1	1.11	14.5	8,300	<0.1	1.01	1,560	1.14	Urban mean (after 6 months exposure)

roadway particulate matter responsible for metal deposition include tire wear (Cd, Pb, Zn), brake wear (Cr, Cu, Pb, Zn), vehicle body degradation (Ni), engine fluid spills (Cr, Ni, Zn), and exhaust emissions (Cr, Cu, Pb, Zn), and deposition is typically greater where braking and/or accelerating are greatest (Tam et al. 1987; Sutherland and Tolosa 2000; Charlesworth et al. 2003; Adachi and Tainosho 2004; Zanders 2005; Zechmeister et al. 2005; Sabin et al. 2006). It is possible that the greater concentrations of Cu and Ni in West Georgia compared to Naples and Pavia may be attributed to deposition from these sources. However, cause and effect relationships cannot be proven in the current study. Further, natural elemental sources (e.g., biological decay, lightning strikes, forest fires, oceans, bedrock weathering, windblown dust) and other anthropogenic sources (e.g., present and historical applications of fertilizers and pesticides to lawns, golf courses, and agricultural fields) cannot be entirely ruled out. It is also possible that observed differences between land-use types are due to natural variation in soil and/or lichen tissue chemical compositions. However, based on the consistently higher elemental concentrations observed in urban vs. rural and developing areas, it is entirely possible that air pollution is a contributing factor to the greater elemental concentrations in the metropolitan Columbus, GA area.

3.3 Elemental Concentrations in Transplanted Lichen Tissue

3.3.1 Elemental Concentration Trends in U. strigosa vs. P. perforatum

Significant species-specific differences were observed between *U. strigosa* and *P. perforatum* regarding concentrations of N and S (Table 4). In each case, *P. perforatum* had significantly ($p \leq 0.01$) greater concentrations of N and S based on least squares means across the entire 1-year exposure period. This not only could be due to natural variation in lichen tissue chemical composition but may also indicate that *P. perforatum* has a greater tolerance to these elements than *U. strigosa*. Further, *P. perforatum* exhibited greater elemental concentrations for all of the metals analyzed except Pb, although these differences were not statistically significant. In other studies, some eastern lichen species in the genus *Parmotrema* have

Table 4 Elemental concentration means for *U. strigosa* and *P. perforatum* lichen transplant tissues collected over 1 year (September 2005–September 2006) at seven different sites along the urban–rural gradient in West Georgia

Species LS means (mg kg^{-1})					Prob > *F*				
Element	*P. perforatum*	*U. strigosa*	Species	Land Use	Spp × LU	Time	Time × Spp	Time × LU	Time × Spp × LU
Cd	<0.1	<0.1	–	–	–	–	–	–	–
Cr	3.64	1.71	0.14	0.63	0.59	0.01***	0.52	0.22	0.95
Cu	21.17	17.27	0.68	0.68	0.79	0.46	0.52	0.59	0.79
N	7,970.39 (a)	6,506.78 (b)	0.01***	0.48	0.48	0.06*	0.82	0.29	0.36
Ni	0.51	0.45	0.81	0.32	0.59	0.04**	0.81	0.32	0.59
Pb	0.95	1.02	0.54	0.01	0.54	0.72	0.21	0.03**	0.97
S	1,490.42 (a)	1,055.92 (b)	<0.01***	0.80	<0.01***	<0.01***	0.74	0.68	0.31
Zn	15.23	13.24	0.50	0.33	0.87	<0.01***	0.61	0.47	0.92

Elemental concentrations reported in mg kg^{-1}; significant differences in species, land use, and time assessed by *F* test and in interaction terms by Wilks' lambda; mean values in a row with different letters (variables in bold) are significantly different ($p<0.05$) based on least squares means; Cd was below detectable limits and therefore not analyzed (–)

Spp species, *LU* land use

*$p\leq0.10$; **$p\leq0.05$; ***$p\leq0.01$

been identified as pollution tolerant (McCune et al. 1997; Will-Wolf, personal communication). Based on our results, we suggest a tentative pollution sensitivity ranking for *P. perforatum* of "intermediate" given that the concentrations of N and S found in *P. perforatum* are greater than the maximum N and S concentrations exhibited by other sensitive species (USDA Forest Service 2007). These data support the findings from the background lichen study (see above) where urban land-use types had the greatest N and S concentrations. Further, although N and S concentrations were greater in in situ lichen tissues, concentrations in both *U. strigosa* and *P. perforatum* transplanted tissue were higher after only 6 months of exposure (Table 3) than the threshold concentrations suggested by Geiser and Neitlich (2007).

Pb exhibited distinct differences across land-use types, while a unique species/land-use interaction was observed with S (Table 4). Across elemental concentrations, several significant differences were observed over time, but there were no time/species interactions observed. Time/land-use interactions were observed only in Pb while no time/species/land-use interaction was observed (Table 4).

3.3.2 Elemental Concentration Trends in U. strigosa over Time

Using repeated measures analysis, the lichen transplant study also revealed significant temporal (seasonal) differences in elemental concentrations in *U. strigosa* tissue samples. Pollution sensitivity has not previously been determined for *U. strigosa*, but many other eastern *Usnea* species have been documented as pollution sensitive (McCune et al. 1997; Morin et al. 2006; Will-Wolf, personal communication; USDA Forest Service 2007). Since a greater amount of *U. strigosa* reference sample material was available for collection, these transplants were collected every 3 months resulting in elemental concentration data for each season over the course of 1 year. Least squares means indicate only N was significantly different ($p\leq0.10$) between land-use type, in which urban (mean=6,782 mg kg^{-1}) and developing (mean= 6,681 mg kg^{-1}) land-use types had higher concentrations of N than rural (mean=6,332 mg kg^{-1}) areas (Table 5). This enabled analysis of seasonal fluctuations in elemental concentrations over time for a single species (*U. strigosa*). Significant differences over time were observed for Cr ($p=0.10$), Ni, S, and Zn ($p<0.01$) as shown in Table 5. However, these data were not further examined since an in-depth analysis of seasonal nutrient cycling dynamics for *U. strigosa* was beyond the scope of this project (Hovenden 2000).

3.4 Elemental Concentrations in Soils

Soil samples were obtained to gain additional information about spatial differences in background elemental concentrations between sites. However,

Table 5 Repeated measures elemental concentration means for *U. strigosa* lichen transplant tissues collected over 1 year (Sept 2005–Sept 2006) in West Georgia

Land use LS means (mg kg^{-1})				Prob > *F*		
Element	Urban	Developing	Rural	Land use	Time	Time × LU
Cd	<0.1	<0.1	<0.1	–	–	–
Cr	3.90	4.05	5.74	0.46	0.10*	0.51
Cu	23.87	15.41	18.49	0.16	0.26	0.20
N	6,782.82	6,681.73	6,332.71	0.09*	0.27	0.34
Ni	0.98	1.95	2.54	0.28	0.01**	0.21
Pb	1.13	1.04	0.97	0.69	0.41	0.27
S	1,069.50	1,056.69	990.25	0.41	<0.01**	0.20
Zn	13.60	13.29	13.37	0.87	<0.01**	0.81

Number of study sites sampled; Elemental concentrations reported in mg kg^{-1}; significant differences in land use and time assessed by *F* test and in interaction term (land use × time) by Wilks' lambda. Cd was below detectable limits and therefore not analyzed (–)

LU land use

*$p \leq 0.10$; **$p \leq 0.01$

the majority of the elements analyzed were either below detectable limits (<0.1 mg kg^{-1}; Cd, Cr, and Ni), or not significantly different between land-use types (data not shown). The only exception is N, where Tukey–Kramer comparisons indicated urban environments had significantly ($p<0.05$) higher concentrations of N (mean=833 mg kg^{-1}) than either rural (mean=92 mg kg^{-1}) or developing (mean=150 mg kg^{-1}) land-use types. These results may be attributed to natural variation in soil chemical composition, natural elemental sources, or pollution sources such as emissions from fossil fuel combustion, particle dust from roadways, demolition, and construction activities, or a combination of these factors (Lovett et al. 2000; Gregg et al. 2003; Pitcairn et al. 2006; Fenn et al. 2007; Geiser and Neitlich 2007).

3.5 Elemental Concentrations in Tree Core Tissues

Tree core samples were collected to ascertain a long-term historical record (50 years) of elemental concentrations in West Georgia forests. For all elements except Zn, metal concentrations in almost all decades for all samples were below the detectable limit (<0.1 mg kg^{-1}). Zn is considered a micronutrient necessary for plant growth and vitality, and all concentrations observed fell within the sufficiency range (28–53 mg kg^{-1}) for *P. taeda* (Mills and Jones 1996) indicating the concentrations were not high enough to be considered toxic (i.e., a pollutant). The exceptions were several plots (all at developing sites) which at different times during the 50-year period had higher concentrations of one or more of the metals analyzed. However, there were no significant differences between site, decade, or metals (data not shown).

3.6 Comparison of Results from Different Assessments

Four different assessments were conducted allowing an examination of commonality and consistency within forest stand elemental concentration trends. Since tree growth and nutrient uptake can be influenced by soil nutrients and lichen growth can be related to the physical traits of a particular tree (e.g., bark roughness, bark pH, canopy cover), it is possible that each medium is potentially exposed to similar elemental concentrations and that those could be transferred from one to another (Muir and McCune 1988). However, our results suggest that correlations between each of the different media used in this study are inconclusive, or simply may not exist. Much of the bulk lichen tissue collected was from recently fallen crown branches (majority of material on the ground), which are potentially exposed to greater amounts of atmospheric deposition since exposure is typically greater (and duration is longer) in tree canopies due to their elevated locations (Weathers et al. 2001). Results indicate land-use differences in the bulk lichen samples but not in the lichen transplants, which were located at breast height and implies an urban association across time and irrespective of species. Had only lichen transplant samples been collected, one might have reported that there are no differences in elemental concentrations between land-use types. Additional studies are needed to examine atmospheric deposition effects using transplants located at different tree heights to test this hypothesis. Further, had the lichen transplant study been conducted for longer than 1 year, the results might have shown different temporal trends altogether.

Soil scientists regularly test soils for elemental concentrations, and had that been the only assessment conducted, one might have also concluded that there were essentially no differences in elemental concentrations in urban vs. rural areas, with the exception of N. Dendroecologists, on the other hand, would have a very difficult time interpreting elemental concentrations using tree rings in West Georgia, although this method has worked in many other locations (Baes and McLaughlin 1984; Alberici et al. 1989; Jordan et al. 1990; McClenahen and Vimmerstedt 1993; Anderson et al. 2000).

Jordan et al. (1990) conducted a similar study within 50 km of the present research study area (in Auburn, AL) and also failed to correlate metal concentrations in decadal tree core samples with those obtained from soils. Translocations of metals are dependent on many factors, such as soil texture, size, and chemical structure of the element measured and soil pH. We tried to ensure homogeneity among sites; however, in field studies, this is not always feasible, for example, soil pHs ranged from 4.2–6.0 among plots, a factor which may potentially affect translocation from soil to plant. Since it is known that some tree species translocate various elements to their leaves (Jordan et al. 1990; Anderson et al. 2000), the inclusion of analysis of leaf tissue elemental concentrations may have been more appropriate for comparison to lichen tissues. However, since the original goal was to obtain long-term historical data regarding elemental concentrations in West Georgia forests, tree cores were selected instead.

While the bulk lichen data overall suggest greater elemental concentrations in urban areas, these trends were not observed in soils and lichen tissue transplants. One exception is that of N, in which differences were also observed between land-use types in soils and in transplanted *U. strigosa* tissues. Passive NO_x monitoring data for the West Georgia region from 2006 indicate that atmospheric concentrations in urban areas were 2.4- and 2.1-fold greater than in rural and developing areas, respectively. Overall, however, these concentrations are relatively low, and the sources are unknown. It is recommended that continuous NO_x samplers be located in the region to determine within and among season variability.

Results from a separate study (Styers 2008) examining the incidence, abundance, and species richness of lichens located on trees within the same sample plots imply similar land-use-related trends. Values for each of these lichen community attributes were significantly greater in rural and least in urban forests of West Georgia. The differences observed could possibly be linked to greater pollution emissions and increased forest fragmentation, both resulting from rapid increases in urbanization in the Columbus area.

3.7 Conclusions

Results reported in this study suggest the necessity of testing several different indicators of forest elemental concentration assessments due to the likelihood that each will result in different conclusions. Each media has its own strengths and weaknesses but can used in combination to gain a better understanding of elemental concentrations at various locations within the ecosystem and to discern spatial and temporal differences. Tree cores may provide long-term historical records, while soil data can provide broad spatial documentations of elemental concentrations. Results from this study suggest that lichens appear to be useful bioindicators of potential pollution exposure to Southern forests. Elemental concentrations in West Georgia are lower compared to larger metropolitan areas in the world (Adamo et al. 2003; Vingiani et al. 2004; Frati et al. 2006; Bergamaschi et al. 2007); however, discernible trends are still evident.

Overall, land-use type is related to Cu, N, Pb, S, and Zn concentrations in urban environments, where values are significantly greater than in rural locations. Although natural sources cannot be eliminated, these values may be attributed to greater emissions in urban areas, as a greater number of stationary and mobile sources are usually found in cities (Lovett et al. 2000; Gregg et al. 2003; Pitcairn et al. 2006; Fenn et al. 2007; Geiser and Neitlich 2007). These findings illustrate the utility of the urban-to-rural gradient approach, since analyses suggest that elemental concentrations observed in urban areas (and not in rural or developing areas) may be related to pollution exposure.

Differences in elemental concentrations between lichen species were also observed. *P. perforatum* had overall higher concentrations of each of the metals tested except Pb, and significant differences were observed in N and S values. Again, this not only could be due to natural variation in lichen tissue chemical composition but could also possibly be related to the ability of *P. perforatum* to tolerate or even thrive under "N- and S-enhanced" atmospheric conditions. Based on these findings and published literature and compared to results for *U. strigosa*, it is suggested that *P. perforatum* has an "intermediate" pollution sensitivity ranking while *U. strigosa* is relatively pollution sensitive.

This study was correlative in nature and cause and effect relationships cannot directly be proven. Further, natural elemental sources (e.g., biological decay, lightning strikes, forest fires, oceans, bedrock weathering, windblown dusts) and other anthropogenic sources (e.g., present and historical applications of fertilizers and pesticides to lawns, golf courses, and agricultural fields) may have contributed to the deposition total. However, based on the consistently higher elemental concentrations observed in urban vs. rural and developing areas, air pollution cannot be ruled out as a contributing factor to the greater elemental concentrations observed in the metropolitan Columbus, GA area. Further studies are needed to verify these results, such as those using radioisotopic markers of pollutants from selected emission sources, or lichens in controlled-environment studies.

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