State of Wisconsin
Department of Administration
Division of Energy

Environmental Research Program

Final Report
October 2005

Lichen Bioaccumulation and Bioindicator Study Near Alliant Energy – WPL Columbia Energy Center

Prepared by:
Susan Will-Wolf, principal investigator for UW-Madison, Department of Botany
Martha M. Makholm, principal investigator for WI-DNR, Bureau of Air Management
John A. Roth, collaborator, WI-DNR, Bureau of Air Management
Matthew P. Nelsen, **Anne H. Reis***, and **Marie T. Trest**,
UW-Madison, Department of Botany

This report in whole is the property of the State of Wisconsin, Department of Administration, Division of Energy, and was funded through the FOCUS ON ENERGY program.

focus on energy[sm]
The power is within you.

*current address WI-DNR

Table of Contents

Acknowledgements

We thank 22 Wisconsin private landowners who gave permission for this study to be conducted; only 5 are the same families as for the 1974 and 1978 studies. We thank the Wisconsin DNR, Columbia County, and Oxford Federal Prison for permission to survey sites on public lands. Susan Will-Wolf, Martha Makholm, and John Roth developed the project proposal; report authors are listed as chapter authors indicating contribution to the project. We thank David Rogers for discussions on data analysis. Corey Hart and John Wolf made helpful comments on drafts of the report. This study was funded by a grant from the Focus on Energy Environmental Research Program of the Wisconsin Department of Natural Resources.

Chapter 1
Introduction

Lichens are regarded as excellent organisms for biomonitoring of air pollution (Ferry et al. 1973; Nash & Wirth 1988; Nimis & Purvis 2002); they are widely used as both bioaccumulators (Martin & Coughtrey 1982; Bargagli & Mikhailova 2002) and bioindicators of impacts on communities (Ferretti & Erhardt, 2002; Will-Wolf & Scheidegger 2002; Will-Wolf et al. 2002). As lichens primarily lack specialized structures for water and gas exchange and depend largely on airborne sources for water and nutrients (Nash 1996), they have been found to be more sensitive to SO_2 and NO_x than vascular plants and to be reliable bioaccumulators of inorganic contaminants. Given these characteristics and the prior community surveys around the Alliant Energy – WPL Columbia Energy Center in 1974 and 1978 (Will-Wolf 1980a), a re-survey of the lichens incorporating current models and tissue analyses was ideal for evaluating modeling, bioaccumulation, lichen biomonitoring with a community subset, and long-term changes in communities.

Wisconsin Power & Light (the predecessor to Alliant Energy) constructed the Columbia Energy Center coal-fired power generating facility south of Portage during the mid to late 1970’s. One boiler became fully operational in 1976, and a second boiler came on-line in 1979. Researchers from the University of Wisconsin conducted baseline studies of various forms of vegetation, including lichens, in 1974 before the units began operating and restudied sites again in 1978. In 2003, we had the opportunity to resample lichens and forest vegetation at the original sites (see Fig 1-1). We also model pollutant concentrations for sites surrounding Columbia at two points during the history of operation as well as for the present time. This project provides the opportunity to "ground-truth" those mathematical model results using actual lichen tissue samples to assess whether dispersion models are good predictors of bioaccumulation in lichens.

This report is the culmination of studies around the point source Alliant Energy – WPL Columbia Energy Center. Mathematical modeling of deposition was combined with a bioaccumulation study of mercury, sulfur, and heavy metals in lichens as well as a lichen bioindicator. Mathematical modeling and bioaccumulation techniques address the levels and patterns of pollution distribution around the facility. This approach enabled us to evaluate modeling results with actual field data. The bioindicator surveys used the methods and sites of surveys in 1974 and 1978, providing the opportunity to (1) assess long-term biological responses in the area surrounding the facility, (2) assess long-term regional changes at the "background" sites farther away from the facility, and (3) evaluate the biological responses in light of the pollution levels indicated by the modeling and bioaccumulation data. Results of this study will be applicable to other areas in Wisconsin.

The following chapters detail the methods and discuss results of three facets of the study. Chapter 2 covers the Columbia dispersion model. Chapter 3 concerns lichen tissue analysis. Chapter 4 includes environmental data and tree community data as they relate to the lichen community data detailed in Chapter 5. Two peer-reviewed journal publications are in preparation: one with the results of models and tissue analysis by Makholm, Roth, and Will-Wolf, and a second with results of the lichen community studies by Will-Wolf, Makholm, Nelsen, and Trest. Results from this project are to be included in a chapter by Will-Wolf and

Nelsen for a book on ecological change in Wisconsin edited by Don Waller and Thomas Rooney, UW Department of Botany, with a tentative publication date of Winter 2006. Other publications may result from this project later. Following the publication of the data in peer-reviewed journals the tissues data (Chapter 3), tree data (Chapter 4), and lichen data (Chapter 5) will be provided to the DNR database for the Aquatic and Terrestrial Resource Inventory (ATRI).

All provisions of Article 6, Ownership of Intellectual Property of the original Research Grant Agreement, will be followed for publication of results of this study. Copies of all published papers will be supplied to the Focus on Energy Environmental Research Program and the Wisconsin Department of Natural Resources.

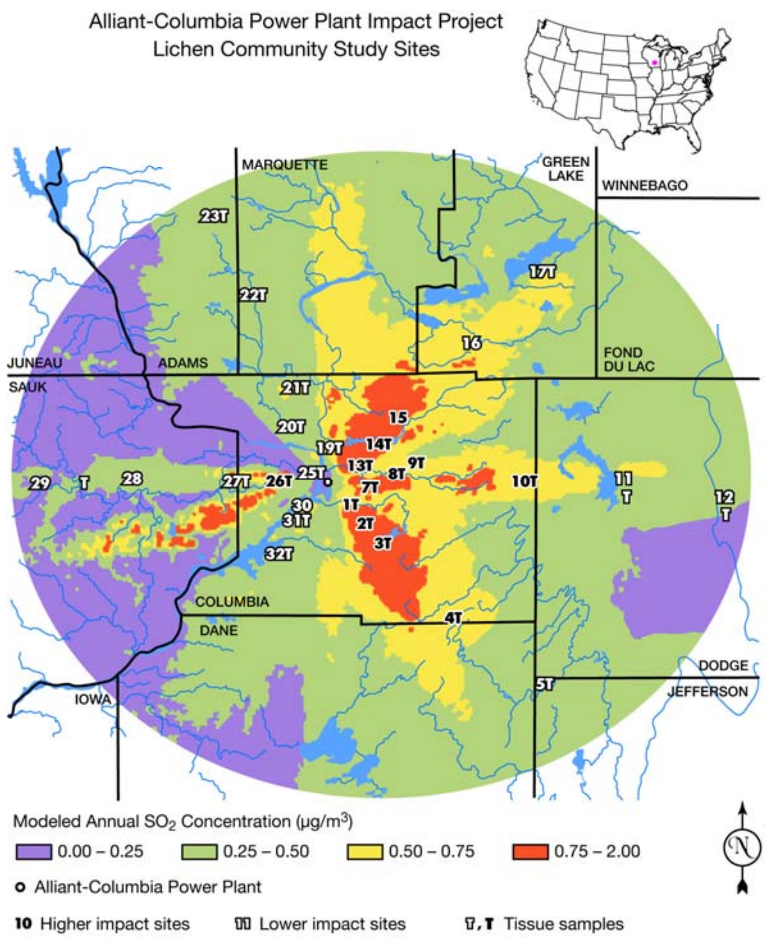

Figure 1-1. Map showing locations of lichen tissue sample sites ("T") and the 30 lichen communities sites (numbered) with modern (Model SO_2 -2003) modeled SO_2 concentration zones in color. Boundaries of concentration zones are extrapolated E to Site 12, which is beyond the range of the dispersion model grid (See Chapter 2). A "T" without a number marks a tissue sample site located several miles from the nearest lichen community sample site. Sites are classed as "higher impact" or "lower impact" based on Model SO_2 -2003 values (See Chapter 4). Tissue samples to represent background levels (control) were taken from Necedah Wildlife Refuge (Site 45), ~140 km NNW of the Alliant Energy – WPL Columbia Energy Center (off the map area to upper left). The oval of higher estimated SO_2 concentrations to the west of the power plant outlines the Baraboo Hills, where higher elevation ridges intersect the stack plume from the power plant.

Table 1-1. Community study site and tissue sample locations. Latitude and longitude are given to two decimal places and distance to km to protect landowner privacy. Sites with a T are tissue sample locations at or near a numbered community site (site number in parentheses if >3 km from tissue sample location). At tissue sample sites, f = *Flavoparmelia caperata*, p = *Punctelia rudecta*. Site 45 is a control tissue sample location far from any community study site. All analyses were based on exact plot locations, not the approximate locations given in this table.

Site #	Year	Elev. (m)	Lat. (°N)	Long. (°W)	Distance to Columbia (km)	County	Ownership
Community Site Locations							
1-Tf	1974-2003	247	43.45	89.38	4	Columbia	Rocky Run Creek SFA - WI DNR
2	1974-2003	311	43.43	89.35	8	Columbia	private
3	1974-2003	303	43.40	89.32	14	Columbia	Mud Lake SWI - WI DNR
4-Tf	1974-2003	290	43.29	89.18	29	Columbia	private
5	1974-2003	283	43.20	88.99	47	Jefferson	private
7	1974	242	43.50	89.34	6	Columbia	Columbia County Farm
7-Tf	2003	287	43.48	89.34	6	Columbia	private
8-Tf	1974-2003	244	43.50	89.29	11	Columbia	Wyona County Park
9	1974	249	43.51	89.25	14	Columbia	private
9-Tf	2003	250	43.51	89.25	14	Columbia	private
10	1974-2003	280	43.49	89.03	31	Columbia	private
11	1974-2003	287	43.49	88.83	48	Dodge	private
12	1974-2003	268	43.47	88.62	64	Dodge	Horicon Marsh SWI - WI DNR
13-Tf	1974-2003	247	43.51	89.36	5	Columbia	private
14-Tf	1974-2003	244	43.54	89.33	9	Columbia	private
15	1974-2003	296	43.58	89.28	15	Columbia	private
16	1974-2003	247	43.68	89.13	32	Green Lake	private
17	1974	249	43.80	89.00	49	Green Lake	private
17-Tfp	2003	271	43.79	89.00	48	Green Lake	private corporation
19	1974	238	43.53	89.42	6	Columbia	Swan Lake SWI - WI DNR
19-Tf	2003	238	43.54	89.43	6	Columbia	Swan Lake SWI - WI DNR
20	1974	250	43.57	89.50	11	Columbia	private
20-Tf	2003	245	43.57	89.50	11	Columbia	private
21	1974-2003	253	43.62	89.50	17	Columbia	private
22-Tf	1974-2003	279	43.76	89.58	33	Marquette	private
23-Tf	1974-2003	322	43.87	89.66	47	Adams	Oxford Federal Prison
25-Tf	1974-2003	252	43.50	89.47	4	Columbia	private
26-Tf	1974-2003	347	43.49	89.52	9	Columbia	private
27-Tf	1974-2003	314	43.49	89.61	16	Sauk	private
28	1974-2003	271	43.49	89.82	32	Sauk	private
29	1974-2003	302	43.49	90.00	47	Sauk	private
30	1974-2003	274	43.45	89.48	6	Columbia	private
31-Tf	1974-2003	248	43.43	89.49	8	Columbia	WI DNR
32	1974-2003	277	43.38	89.53	15	Columbia	private

Table 1-1 continued.

Site #	Year	Elev. (m)	Lat. (°N)	Long. (°W)	Distance to Columbia (km)	County	Ownership
Additional Sites for Tissue Samples							
3-Tf	2003	297	43.43	89.27	13	Columbia	private
(5)-Tfp	2004	243	43.20	88.96	49	Dodge	Waterloo SWA - WI DNR
(10)-Tfp	2004	282	43.47	89.05	30	Columbia	Paradise Marsh SWA - WI DNR
(11)-Tfp	2004	274	43.41	88.82	49	Dodge	Shaw Marsh SWA - WI DNR
(12)-Tp	2004	271	43.36	88.64	65	Dodge	private
21a-Tf	2003	253	43.62	89.50	16	Columbia	private
21b-Tf	2003	256	43.62	89.49	16	Columbia	private
(26)-Tf	2003	374	43.48	89.51	8	Columbia	private
(28-29)-Tf	2003	305	43.47	89.93	41	Sauk	private
45-Tf	2003	281	44.03	90.16	85	Juneau	Meadow Valley SWA - WI DNR
Alliant Energy – WPL Columbia Energy Center Location							
Columbia		246	43.4856	89.4196	0	Columbia	Alliant Energy

Chapter 2
Columbia Dispersion Model
John A. Roth, Martha M. Makholm, Susan Will-Wolf

Introduction

For this study a modern atmospheric dispersion model was used to estimate ambient concentrations of SO_2 around the Alliant Energy – WPL Columbia Energy Center. The United States Environmental Protection Agency (USEPA) has developed several models that are currently used for regulatory dispersion modeling applications (Turner 1994). As of Spring 2004, the Industrial Source Complex Short Term model version 3 (ISCST3) was the recommended model. This is a steady-state Gaussian plume model used for estimating the concentration of non-reactive pollutants. The modeling analysis was performed in 2003-2004 to assess the impact of current emissions on ambient air quality. Using the tools and models currently available, an analysis of past dispersion estimates at two points during the history of operation was also performed.

A model is a mathematical simulation, designed to predict what can or will happen in real-world scenarios. Atmospheric dispersion modeling is useful in predicting the impact a particular facility will have with respect to a given pollutant. The major benefit of dispersion modeling is that it is a relatively inexpensive way to determine the potential impact of a source. This information is vital in assessing a facility's compliance with respect to the National Ambient Air Quality Standards (NAAQS) as well as the various Hazardous Air Pollutant (HAP) standards, both federal and state mandated.

The Wisconsin DNR modelers currently use the ISCST3 (02035) model in order to calculate pollutant concentrations. In general, the model uses information about the facility in question, such as stack parameters, facility layout information such as orientation, and emission rates, along with hourly meteorological data in order to predict concentrations of pollutants surrounding the facility. The point of highest impact is determined through the use of a receptor grid that is set up by the modeler. The pollutant concentration at the point of highest impact added to a pre-determined background is compared to the corresponding ambient air quality standard.

Three different emission scenarios were modeled in this analysis. The first scenario (labeled Model SO_2-2003) used physical stack information from the 2001 emissions inventory and emission rates were the average of the years 1998-2001 inclusive. It estimates modern impact of Columbia on the surrounding area. A second scenario (labeled Model SO_2-1978) used information from the 1976 emissions inventory to estimate the impact of Boiler B21 with a 152.4m (500 ft) stack (S11) that became fully operational in 1976. This scenario estimates impact of the facility during the years 1976-1978, during the first biomonitoring study (Will-Wolf 1980a). A third scenario (labeled Model SO_2-1980) used information from the 1979 emissions inventory and so considered both boiler B21 and boiler B22 with a 198.1m (750 ft) stack (S12) that came on-line early in 1979. This third scenario estimates impact of the facility during the years 1979-1980 (-1987).

Methods

Ground level concentrations of sulfur dioxide (SO_2) from Columbia were produced using the ISCST3 model from facility emissions data and five years (1975-1979) of meteorological data. Surface data was collected in Madison, and the upper air meteorological data originated in Green Bay. The model used rural dispersion coefficients with the regulatory default options which allow for calm wind correction, buoyancy induced dispersion, and building downwash. The emissions of SO_2 were assumed to be an aerosol and gravitational effects are negligible.

To determine the current emissions from the facility, the Wisconsin Air Emissions Inventory was used. Electronic data exist from 1995, and at the time this study was performed, the most up to date information was from 2001. Sulfur dioxide emissions at Columbia come from the combustion of coal and fuel oil. The data reported by Alliant showed an unusually high value for B21 in 1997, so for the Model SO_2-2003 analysis the average of the 1998-2001 yearly emissions was calculated and modeled (see Table 2-1 for source details). To determine the emissions immediately after the first boiler (B21) became operational, the 1976 emissions report from the power plant was used (Model SO_2-1978). For the period immediately after the second boiler (B22) came on-line, the 1979 emission report was used (Model SO_2-1980). To be consistent with current emission calculations, the yearly total coal usage from each year was multiplied by the current USEPA emission factor as modified by the reported sulfur content of the coal, in formula form:

$$\text{Yearly Emissions} = 38.0\ \#\ SO_2/\text{ton} * \text{Tons Coal/year} * \%\ \text{sulfur}$$

Stack height and diameter are known quantities, but volumetric flow and gas temperature can vary. For the present analysis, the 2001 inventory report lists the average flow rate and temperature as derived from continuous emissions monitors located within the exhaust stacks. The 1976 and 1979 reports contained average information for each year as determined by stack tests.

Locations of the two main stacks were taken from digital orthophotos available within the DNR GIS system. The coordinate system used in Table 2-1 is a meter based system called Wisconsin Transverse Mercator (WTM); the standard Universal Transverse Mercator (UTM) system is not continuous across Wisconsin, consequently WTM was created by the state so that all data within the GIS system can be mapped. A grid of points with a 250 meter spacing was created extending 50 kilometers from the facility. The elevations for the 160,801 receptor points were derived from the 30 meter digitized elevation model of Wisconsin within the DNR GIS system.

All data were entered into the model, and the air concentrations were calculated for every hour of the five year period (43,824 hours). For each year of modeled data the annual average SO_2 concentration was calculated. Since the long-term impact is desired, the five yearly values at each receptor were averaged to produce a mean annual average for each receptor point. The highest value from any individual year and the highest averaged value are presented in Table 2-2. The averaged data were also imported into the GIS system for contouring and further analysis. The dispersion model was also run to obtain concentrations at specific locations for lichen community study sites, lichen tissue sample sites, and historic instrument ambient air monitor stations. As with the grids, the model results at the community sites were averaged over the five years with the results listed in Table 2-3. All air concentration data were collected into a geographic information system (GIS) for dissemination.

Table 2-1. Stack parameters used in dispersion model analyses. The elevation for the plant site is 245.9 m.

Alliant Energy – WPL Columbia Energy Center Stack Parameters						
ID	LOCATION (WTM: E, N)	HEIGHT (M)	DIA. (M)	VELOCITY (M/S)	TEMP (K)	SO_2 RATE (#/HR)
Model SO_2-2003						
S11	566844, 334913	152.4	6.40	28.18	357.4	3,542
S12	566844, 335042	198.1	6.40	29.90	405.8	3,337
Model SO_2-1980						
S11	566844, 334913	152.4	6.40	26.05	418.6	5,633
S12	566844, 335042	198.1	6.40	22.75	394.1	2,884
Model SO_2-1978						
S11	566844, 334913	152.4	6.40	26.05	418.6	5,633
S12	Not Constructed	-	-	-	-	-

Results and Discussion

Modeling analysis results (Tables 2-2 and 2-3) document that impact of SO_2 from Columbia probably peaked in the 1980's and has declined since. For present-day conditions (Model SO_2-2003) we generated a map of estimated SO_2 concentration zones in the study area (Figure 1-1) as well as values for specific locations (Table 2-3). Concentration of SO_2 from the power plant for site 45, a control site located ~140 km NNW of the power plant and well outside the modeling grid, was set at 0.001.

Table 2-2. Maximum estimated SO_2 for the three modeling scenarios.

Maximum Modeling Analysis Results (All Concentrations in μg/m^3)			
	Model SO_2-2003	Model SO_2-1980	Model SO_2-1978
Maximum Individual Year	2.39	3.10	3.08
Maximum 5-Year Average	1.89	2.30	1.83

Table 2-3. Site specific modeling analysis results for three dispersion model scenarios. Locations and elevations for each site are listed in Table 1-1. For tissue sample sites listed in Table 1-1 but not here, model values for nearby lichen community sites were used in analysis. Impact group for community sites is 1 for higher, 2 for lower impact, based on average for three scenarios.

Site Specific Modeling Analysis Results (All Concentrations in μg/m^3)

Site Number	Model SO_2-2003	Model SO_2-1980	Model SO_2-1978	Average	Impact Group
1	0.72	0.864	0.673	0.752	1
2	1.21	1.53	1.21	1.317	1
3	0.969	1.21	0.909	1.029	1
4	0.601	0.746	0.525	0.624	1
5	0.393	0.484	0.332	0.403	2
7	0.918	1.16	0.928	1.002	1
7 adjusted	1.201	1.554	1.271	1.342	1
8	0.592	0.729	0.535	0.619	1
8 adjusted	0.775	0.891	0.675	0.780	1
9	0.529	0.649	0.472	0.550	1
9 adjusted	0.702	0.882	0.659	0.748	1
10	0.653	0.811	0.577	0.680	1
11	0.518	0.643	0.451	0.537	2
12	0.344	0.427	0.296	0.356	2
13	1.05	1.25	0.898	1.066	1
14	0.932	1.14	0.824	0.965	1
15	1.06	1.3	0.933	1.098	1
16	0.544	0.668	0.459	0.557	2
17	0.453	0.558	0.38	0.464	2
19	0.604	0.678	0.451	0.578	2
20	0.394	0.465	0.332	0.397	2
21	0.353	0.407	0.286	0.349	2
22	0.321	0.375	0.259	0.318	2
23	0.325	0.388	0.271	0.328	2
25	0.251	0.289	0.222	0.254	2
26	0.774	0.931	0.72	0.808	1
27	0.46	0.55	0.398	0.469	2
28	0.261	0.316	0.217	0.265	2
29	0.246	0.303	0.208	0.252	2
30	0.393	0.461	0.342	0.399	2
31	0.337	0.409	0.293	0.346	2
32	0.418	0.503	0.357	0.426	2
Additional Lichen Tissue Sample Sites					
3-T	0.706	0.867	0.644	0.739	
21a-T	0.353	0.407	0.286	0.349	
21b-T	0.361	0.419	0.294	0.358	
(26)-T	1.161	1.385	1.087	1.211	
(28-29)-T	0.276	0.338	0.234	0.283	
45-T	0.001	0.001	0.001	0.001	

The ISCST3 dispersion model used for this study is a greatly improved version of the dispersion model (Ragland et al. 1980) used to estimate power plant impact for the original study (Will-Wolf 1980a), that had the same basic Gaussian plume assumptions but no option to account for terrain. In addition the modern version incorporates many more kinds of meteorological conditions and receptor locations. The relative impact of the power plant on lichen community sites should thus be better estimated with this modern model.

The current model has some limitations. The measured wind directions from the National Weather Service are provided in ten degree increments and although there is some randomizing that occurs during preprocessing, the model still produces 'fingers' of higher estimated concentrations aligned with major compass directions (Figure 1-1) that model smoothing does not eliminate. In between the major compass directions, the model underestimates SO_2 concentrations. For the most part this limitation has not been a problem for the study, since lichen community sites are also located along major compass directions from the power plant. Sites 7, 8, and 9 are exceptions; they were located off the major E compass direction from the power plant because of scarcity of suitable sites, and they are also located in an area where estimated SO_2 concentrations differ substantially across small distances. For these sites, additional model estimates were generated using the original longitude or easting of the study site but the latitude or northing of the power plant, thus locating them on the E compass direction (Table 2-3: 7, 8, 9 adjusted). These adjusted values are more accurate estimates of SO_2 concentrations at sites 7, 8, and 9, given model limitations. Elevation of true locations was used in all cases. For the same reason, model estimates for community sites 5, 10, 11, and 12 were used in analyses of impact of Columbia rather than model estimates for tissue sample locations (5)T, (10)T, (11)T, and (12)T which had similar distances to Columbia (Table 1-1) but were >3 km off the major compass direction from Columbia and thus in model underestimation zones.

The ISCST3 model results (Table 2-3) provide an excellent tool to represent relative impact of Columbia for comparison with tissue concentrations of sulfur (Chapter 3) and with data from the lichen community study (Chapter 5). We have achieved our goals for the models with respect to assessing relative impact of the power plant across the study area. ISCST3 model results should not be interpreted as absolute concentrations for comparing relative impact of Columbia with other potential sources of air pollution and with other studies on effects of SO_2 concentrations on lichens.

We currently cannot assess accuracy of model estimates for absolute concentrations of SO_2 from the power plant. Estimates from the 1980 model are higher than estimates from the ISCST3 model for the same general areas, and historic instrument SO_2 monitor data (available from US Environmental Protection Agency Aerometric Information Retrieval System: EPA-AIRS) from seven stations for 1977-1983 report concentrations 8-15 times greater than the ISCST3 model estimates for the instrument locations (Table 2-4). Several factors including other sources of SO_2 could affect monitor data. Further, monitor locations may be in model underestimation zones and/or the ICSTS3 model might underestimate SO_2 concentrations even in geographic zones of low model underestimation. Time to research historic monitor data, account for model limitations, and develop appropriate calibration with ISCST3 model values was cut from Makholm’s contribution to our project as a consequence of the 2003-2004 DNR budget crisis.

Table 2-4. ISCST3 model estimates and instrument monitor data from EPA AIRS for historic instrument ambient air monitor sites.

Historic Instrument Ambient Air Monitor Sites (All Concentrations in µg/m^3)

Monitoring Site	Elevation, Meters	Location Easting (UTM zone 16, Datum NAD83)	Location Northing (UTM zone 16, Datum NAD83)	ISCST3 model estimates: Model SO_2-1980	ISCST3 model estimates: Model SO_2-1978	EPA-AIRS Instrument Monitor Data (Averages, years with data for >90% of hours): 1979-1983	EPA-AIRS Instrument Monitor Data (Averages, years with data for >90% of hours): 1977-1978
Caledonia	311.0	297000	4819000	0.462	0.357	13.1	11.67
Dekorra	259.0	301000	4814500	0.457	0.331	11.27	9.84
Ft Winnebago	250.0	305100	4825500	0.872	0.59	9.05	11.25
Marcellon	274.0	313600	4829560	1.06	0.761	8	13.38
Portage	335.0	297150	4817450	0.873	0.683	8.15	no data
Springvale	265.0	318500	4815151	0.638	0.471	11.15	10.82
Wyocena	290.0	307700	4812500	1.38	1.12	9.13	10.76

Conclusions

We achieved the project goal of developing model estimates of relative impact of pollution from Columbia on lichen community and lichen tissue sample sites. We did not achieve the goal of using model results to estimate absolute SO_2 concentrations at sample sites.

Future Goals

It would be very useful to establish the accuracy of model estimates for absolute concentrations of SO_2 from the power plant. Two avenues of inquiry are available to pursue this. First, when the next generation of USEPA dispersion model is released (AERMOD), the three Columbia emission scenarios should be rerun with the new model to see how the modeled impacts from Columbia will change. This task is expected to take up to one week of work by a person with expertise equivalent to that of Roth.

Second, appropriate methods for comparing historic instrument SO_2 monitor data from seven stations for 1977-1983 from EPA AIRS with estimated SO_2 concentrations for scenarios "Model SO_2-1978" and "Model SO_2-1980" from both ISCST3 and the upcoming AERMOD dispersion models should be investigated. To our knowledge no modern instrument monitoring data are available for similar comparisons with "Model SO_2-2003" scenarios. This may allow estimation of accuracy and bias of concentration values from dispersion models, and generate conversion factors between model estimates and expected absolute concentrations. Tasks involve researching specifications and performance of the historical monitors, effect of monitor location on model estimation, and possible input from local lower-level SO_2 sources as well as high stacks from Columbia, before developing calibration protocols. This is estimated to require several weeks of work by a person with expertise equivalent to that of Makholm.

Chapter 3
Lichen Tissue Analysis

Introduction

In situations where direct measurement data are unavailable a useful technique for assessing deposition patterns of elements is to determine element concentrations in lichen tissue collected from the area of interest. Any geographic area can be approached this way as long as sufficient quantities of tissue (of an appropriate species) are available. Mapping of pollutant concentrations using lichen tissue analysis is a well-established technique for biomonitoring of pollutants (Jackson et al. 1993; Martin & Coughtrey 1982; Puckett 1988). Comparisons of lichen concentration data with direct measurement data have demonstrated that this approach provides an accurate depiction of deposition patterns; physiological studies have demonstrated that many elements, especially heavy metals, accumulate in lichen tissue through passive mechanisms of bioaccumulation below toxic levels (Bargagli & Mikhailova 2002).

For this study we have the opportunity to compare bioaccumulation results with model estimates of relative deposition from one source (Chapter 2), as well as with patterns of lichen diversity and community composition. We collected samples of naturally-growing lichen tissue for analysis of element content to address several project goals: 1) to investigate correlations among elements in lichen tissue samples to identify suites of elements with similar patterns of concentration, 2) to compare concentrations of elements in lichens with an estimator (Chapter 2) of relative impact of the Alliant Energy Columbia coal-fired power generating facility and with other variables relevant to identifying potential environmental sources of suites of elements, and 3) to develop a tissue element variable to relate to other site variables and to lichen community composition for the 29 lichen community sites in the 2003 samples.

Lichen species useful for tissue analysis need to be large and easily separated from their substrate, and need to be relatively common across the entire area to be studied. *Flavoparmelia caperata* and *Punctelia rudecta* are the only two lichen species large enough and easy enough to remove from bark that were known from earlier studies (Will-Wolf 1980a) to be abundant at many study sites. Both species have been successfully used for tissue element analyses in previous studies (Olmez et al. 1985; Rodrigo et al. 1999).

Methods

Field methods: We found early in field collection that *F. caperata* was both more abundant than *P. rudecta* and was also easier to collect, so the former species became our primary target, with the latter species serving as a secondary collection target. Replicate *P. rudecta* samples were collected at several sites for calibration between species, and were collected at several other sites where *F. caperata* samples were sparse or absent.

Wherever possible two samples of *F. caperata* of 2 to 4 g each were collected. To obtain representative data each sample included material from five to ten trees. Tissue was collected from 0.25 to 2.5 m above ground level from trees (of various species) that were well exposed to air movement and did not appear to be influenced by any observable local pollution sources. The tissue was pulled or scraped from tree bark with a sterile scalpel blade (new blade for each sample) directly into glass jars cleaned to required EPA standards for trace element studies. Careful collection techniques in the field reduced the need to clean samples in the lab, reducing potential contamination from handling. Sample collection took 15-45 minutes per sample in the field.

Samples were collected at the lichen community sample site when the above collection conditions were met there; in some cases it was necessary to collect at a nearby location. At a few localities there was only enough material for one sample of *F. caperata*, and at some sites no material of either species could be collected in the vicinity. Samples were collected from or near most of the 29 lichen community sites, and from an additional site near Necedah, Wisconsin (NW of the study area) to provide background level data (Figure 1-1 and Table 1-1). Most samples were collected in summer and fall of 2003; additional samples were collected in spring 2004 and spring 2005 following the identical sample protocol.

Laboratory analysis methods: Samples were thoroughly air-dried in air-conditioned conditions immediately after collection. Those kept for more than t`hree weeks before lab analysis were stored frozen. 2004 and 2005 samples were cleaned of bark particles in the lab as necessary after drying; samples were handled in clean lab space in an air-conditioned lab on a fresh 100% cotton bond herbarium sheet using a fresh sterile scalpel blade and alcohol-wiped forceps by a person wearing alcohol-wiped disposable lab gloves. Cleaning took 30-60 minutes per sample.

2003 and 2004 samples were delivered to the Wisconsin State Lab of Hygiene in spring 2004 for initial preparation (air dried and ground to pass through a 2 mm screen in a Wiley ED-5 laboratory mill) and for mercury (Hg) analysis. Analysis used a research-grade atomic fluorescence spectrometer with a Brooks-Rand detector gold amalgam trap that measures Hg at levels down to 0.1 ng/g or ppb Hg in a state-of-the-art Trace Metal Clean Laboratory (TMCL) designed and certified to permit the analysis of ultra-trace metals in environmental samples at part per trillion levels (additional information may be found at <http://www.slh.wisc.edu/ehd/biomonitoring/index.php>). Spring 2005 tissue samples were not analyzed for Hg.

Element analyses other than Hg (Al, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, Li, Mg, Mn, Mo, Ni, N, P, Kg, Na, S, and Zn) were completed at the University of Wisconsin - Extension Soil and Plant Analysis Lab (UW Lab) using three options: heavy metals, total minerals, and total N. 2005 samples were dried and ground at the UW Lab before analysis using protocol identical to that above; 2003 and 2004 samples were prepared for Hg analysis (above) before analysis by the UW Lab. Samples were then wet digested (samples for heavy metals analysis were ashed before digestion) and analyzed using an inductively coupled plasma emission (ICP) spectrophotometer. All results are reported on a dry weight basis. UW Lab quality assurance and control protocols QA/QC include verifying results primarily based on instrument performance, duplicate analysis and estimation of elemental recovery based on reference materials. For total N the Lab QA/QC protocols include digested blanks, digested reference materials and duplicate analysis (additional information at <http://uwlab.soils.wisc.edu>).

All data validation procedures were conducted on the set of 2003 and 2004 *F. caperata* samples

(N = 43 study samples, 26 sites -16 sites with duplicate samples) plus 4 control samples of an international standard. A number of elements were dropped from the data analysis because data quality was poor. Co, Mo, and Ni had a high percentage of results (56%, 80%, and 87% of samples, respectively) below detection levels, interfering with our ability to investigate variation among sites. Cd, Mo, and Pb had high levels of variability in analysis of control samples (standard error > 1.4 x mean) and Cd, Co, Mn, Pb, Ca, and Mn had high levels of variability between duplicate samples collected at the same site (most duplicates with standard error >0.5 x mean). The remaining 14 elements - Fe, Al, Cr, Cu, B, Li, Na, S, Hg, N, Zn, Mg, P, and K – had data quality adequate to warrant further analysis.

To establish accuracy of UW Lab results we compared four control samples of an international lichen tissue standard tested at the UW Lab with certified values of the standard having known accuracy (Heller-Zeisler et al. 1999) for eight elements - Al, Cr, Cu, Fe, K, Na, P, and Zn.

Data analysis methods: In several cases we perform many statistical tests for each analysis and "experiment-wide" error is an important factor, but many of our variables are strongly correlated with each other, and it is not clear to us how to accurately count number of independent variables for formal adjustment of probability levels. We therefore report categories of probability values rather than relying on particular levels of significance, and assume that some of our test results with probabilities suggesting rejection of our null hypothesis occur by chance. Different data sets and analysis methods were used to address our questions; they are detailed below.

Data analysis methods 1) Correlations between tissue elements: Pearson correlation of concentrations of 14 tissue elements (averages for replicate samples) in 2003 - 2004 *F. caperata* samples is used to examine relationships between the different elements. Use of original values in ppm allows presence of extreme values and differences in ranges of values to affect the correlation coefficient, and reduces the impact of small differences in order of values between elements. We felt this was an appropriate choice since general patterns of correlation were of more interest than specific correlation coefficients (Sokal & Rohlf 1995).

Data Analysis Methods 2) Correlation of tissue elements with external variables: Spearman correlation of data is used to examine relationships of tissue element concentrations (averages for duplicate samples) for 14 elements in 2003 - 2004 *F. caperata* samples to three geographic (based on Table 1-1) and two Columbia impact variables at the same sites. Distance to Columbia (Table 1-1) assumes equal impact of Columbia in all directions, while Model SO_2-2003 (Table 2-3) estimates impact using a sophisticated dispersion model reflecting winds and site elevation as well as other parameters. Using the non-parametric Spearman correlation has the effect of ranking each variable is independently; for instance, sites are ranked based on tissue S concentrations, and sites are also ranked based on Distance to Columbia. Correlation then compares rank order of sites for the two variables rather than their original values. This approach reduces impacts of non-normal data distributions, reduces the effect of extreme values, and eliminates effects of differences in ranges of values. We chose this more conservative data treatment for these analyses because we wished to interpret relationship to potential causal variables from the tests (Sokal & Rohlf 1995).

More intensive analysis of relationships of tissue S concentrations with geographic and

Columbia impact variables used ranked data (for the same reasons as above) and included 2003 - 2004 tissue samples for both lichen species (averages for replicate samples). We used two-way analysis of variance (ANOVA) of three sites (3, 22, 28-29, Fig 1-1, Table 1-1) by two species by two replicates using 2005 data to test whether element concentration levels are different for the two species. We used two-way ANOVA of the same three sites by two years by two replicates using 2003 and 2005 *Flavoparmelia caperata* data to test whether element concentrations for that species are different for samples collected two years apart. After determining that data for *F. caperata* and *Punctelia rudecta* could be combined without applying a correction factor, we averaged tissue S data for both species and included all sites with tissue data for either species in analyses.

For combined tissue S data we performed simple Pearson and Spearman rank correlation analyses with original values to identify the most useful version of a group of related external variables. Then we performed partial correlation analysis on ranked data to identify which subsets of useful variables gave the strongest correlation with tissue S at sites. We also investigated the impact on correlations of site 22, which we noticed has anomalously high tissue element concentrations for its location relative to other nearby sites and to environmental gradients represented by external variables.

For most external variables, values were for the exact site where tissue samples were taken. Model SO_2-2003 values for community sites 7, 8, and 9 adjusted and for community sites 5, 10, 11, and 12 were substituted for values at the related tissue sample sites to overcome a model limitation: underestimation of SO_2 away from cardinal compass directions (Chapter 2, Table 2-3).

Data analysis methods 3) Estimating tissue concentrations at lichen community sites without samples: Five community sites, Sites 15, 16, 28, 29, and 30 had no lichen tissue element data associated with them. A sixth site, 22, had anomalously high measured lichen tissue element values. We developed estimates of tissue Sulfur values for these six sites using two independent methods:

1) We estimated tissue S at Sites without measured values from tissue S values at nearby sites to create the variable Tissue Sulfur. For Sites 15, 16, 22, 28, and 29 we performed simple linear interpolation or extrapolation of tissue S from tissue S values at other sites on the same compass direction from the power plant using distances between sites. Sites 28 and 29 were estimated using tissue site 28-29 in addition to other lichen community sites, so one degree of freedom should be subtracted for estimation at Sites 28 and 29. We assigned Site 30 the tissue S value of Site 31 to three digits, since there were only three sites on that compass direction from Columbia and sites closest to the power plant on other compass directions did not consistently have tissue S higher or lower than the next site out on the same compass direction. Degrees of freedom for statistical tests for this variable at 29 sites are 23 rather than 28, to account for estimation.

2) Linear regression models were developed in SPSS 12.0 for lichen tissue S values explained by external site variables, with variables and sites chosen based on single and partial correlation analyses above. We tested full models only, comparing models by including or excluding variables manually. The model with the combination of sites and independent variables

producing the strongest explanatory power, the smallest standard deviation, and the lowest p value was chosen for estimation.

Results and Discussion

Accuracy of lab tissue analyses: Mercury (Hg) data from the Wisconsin State Laboratory of Hygiene met or exceeded their stringent quality control standards. Comparison of an international standard lichen tissue sample (Heller-Zeisler et al. 1999) with UW Lab analyses shows that for seven of eight elements with useful data, averages of UW Lab values are outside (below) the expanded confidence interval for the standard and thus show important bias (Table 3-1, Cu is the exception). The average UW Lab value is 80% of the standard value (range 57%-88%) for these eight elements; we extrapolate that lichen tissue values for other elements tested at the UW Lab likely average about 80% of total concentrations. Based on this finding, for comparison with tissue concentration values from other studies with standardized values we recommend multiplying our values by 120%.

Table 3-1. Comparison of UW Lab analyses of IAEA-336 *Evernia prunastri* control samples with standard values established for IAEA-336.

	HEAVY METALS[1]				MINERALS[1]			
Sample ID	Cr ppm	Cu ppm	Fe ppm	Zn ppm	P ppm	K ppm	Al ppm	Na ppm
IAEA-336 sample 2004-1	0.76	2.85	367	26.1	469.586	1477.056	391	259
IAEA-336 sample 2004-2	0.92	2.98	367	26.9	466.518	1473.375	392	266
IAEA-336 sample 2004-3	0.75	4.17	374	26.8	480.880	1503.058	386	261
IAEA-336 sample 2004-4	0.78	2.53	369	27.5	475.850	1488.655	394	268
Average	0.803[2]	3.133[2]	369.250	26.825[2]	473.208	1485.536	390.701	263.432
[3]IAEA-336 Standards (mg/kg)	Cr†	Cu*	Fe*	Zn*	P†	K*	Al†	Na*
Average	1.06	3.600	430.000	30.400	610.000	1840.000	680	320.000
[4]Expanded Confidence Interval	0.89-1.23	3.1-4.1	380-480	27.0-33.8	490-730	1640-2040	570-790	280-360

[1]Results reported on a 'dry weight' basis. Units: ppm = mg/kg. P and K originally reported as %: 1% = 10,000 ppm.
[2]One of the 4 replicates fell within the expanded confidence interval for IAEA-336 standard values
[3]IAEA-336 standard values are from Heller-Zeisler et al. 1999.
[4]Expanded confidence interval is 95% CI with 5% added to account for any variation due to sample inhomogeneity.
*Recommended values meet the strictest criteria; †Information values meet less strict criteria.

Tissue analysis: We had three goals for tissue analysis: 1) to investigate correlations between tissue element concentrations to identify elements with common sources, 2) to investigate relation of tissue concentration patterns for all elements to external variables, and 3) to develop a tissue sulfur concentration variable for correlation with lichen community data. We used the more abundant *Flavoparmelia caperata* data to address goals 1) and 2). Forty-one *F. caperata* tissue samples were collected from 25 sites (Table 1-1); at nine sites we collected only one sample. Tables 3-2, 3-3, and 3-4 present results based on these data. We used all available data from both *F. caperata* and *P. rudecta* to address goal 2) in greater detail for sulfur and to address goal 3). Forty-nine tissue samples from *F. caperata* and *P. rudecta* combined were collected from 26 sites (Table 1-1); at some sites we had three replicates, while six sites have only one sample of either species.

Results and discussion 1) Correlations between tissue elements: Correlations between eight of the elements - Fe, Al, Cr, Cu, B, Li, S, and Hg - are notably high; most of the pairwise correlations are ≥ 0.5, with most p ≤ 0.01 (Table 3-2). Fe, Al, Cr, and Cu have particularly strong intercorrelations, with the most likely explanation that they are arriving at proportional rates at the different study sites and are attached to the same particles which are arriving from common sources. B, Li, S, and Hg have moderately strong correlations with each other and with the four elements above, suggesting they are probably attached to many of the same particles and mostly arrive from the same common sources. Two additional elements - Na and Mg – are correlated with several of the above eight elements, suggesting they have at least some arrival mode and sources in common with the above eight elements. To the degree that they are more weakly correlated with other elements, data for Na and Mg suggest the potential for at least some independence in arrival mode or sources from the eight more strongly intercorrelated elements. Two other elements, N and Zn, have few strong correlations with the group of eight elements. The study area is heavily agricultural and it is possible that dust from agricultural fields is an important source of the N in lichen tissue. Zn has weak associations with other elements, suggesting it may not arrive via the same particles and/or have sources different from the rest.

Table 3-2. Pearson correlations between elements (concentration values in ppm). Elements with acronyms in **bold** have relatively strong correlations with one another. P and K are distinct from all other elements. N = 43 samples from 26 sites; p ≤ 0.10, indicated by **bold**; p ≤ 0.05, indicated by shading; p ≤ 0.01, indicated by **shading and bold**.

	Fe	**Al**	**Cr**	**Cu**	**B**	**Li**	**S**	**Hg**	Na	Mg	N	Zn	P
Fe	1												
Al	**0.951**	1											
Cr	**0.894**	**0.860**	1										
Cu	**0.785**	**0.812**	**0.701**	1									
B	**0.693**	**0.687**	**0.586**	**0.606**	1								
Li	**0.682**	**0.722**	**0.526**	**0.605**	**0.636**	1							
S	**0.599**	**0.657**	**0.513**	**0.744**	**0.505**	0.314	1						
Hg	**0.437**	**0.474**	**0.552**	0.318	0.358	**0.299**	**0.437**	1					
Na	0.351	0.305	0.308	0.333	0.146	**0.267**	0.001	-0.070	1				
Mg	0.313	0.156	0.121	0.166	0.325	0.323	-0.15	-0.371	0.107	1			
N	0.171	0.184	0.151	**0.282**	0.117	-0.035	**0.491**	0.161	0.006	-0.123	1		
Zn	0.164	0.116	**0.293**	0.071	0.211	0.127	-0.105	0.052	0.115	0.223	-0.175	1	
P	-0.080	-0.032	-0.082	-0.009	-0.028	**-0.403**	0.186	-0.211	-0.074	0.087	0.120	-0.077	1
K	-0.163	-0.091	-0.189	-0.066	-0.064	**-0.425**	0.163	-0.243	**-0.263**	0.071	0.119	-0.171	**0.909**

Interpreting patterns of intercorrelation as evidence of arrival via attachment to the same particles and deposition from common sources is supported by many studies (review by Bargagli & Mikhailova 2002). Olmez et al (1985) examined trace elements in *F. caperata* and *P. rudecta* near a coal-fired power plant in Maryland, USA, and concluded that the similarity of concentration in airborne particles (from a source similar to Columbia) and lichens "strongly suggests a close relationship between lichens and suspended particles." Rodrigo et al. (1999) found in a comparison of *F. caperata* tissue concentrations with bulk deposition, throughfall and leaf-wash in two oak forests in Spain, that the order of abundance of trace metals in lichens is similar to that in bulk deposition measurements.

Concentrations of P and K in lichen tissue have strong positive correlation with each other, and are not or are negatively correlated with all other elements (Table 3-2). P and K are only weakly held at ionic attachment sites compared to many other elements and can be displaced by them. As heavy metal tissue concentrations increase, P and K concentrations decrease. Concentrations of P and K in lichen tissue are thus related to factors other than their own deposition rates. No conclusions regarding P and K deposition levels or sources can be made with the present data.

Results and discussion 2a) Correlation of all tissue elements with external variables: **C**orrelations between tissue element concentrations and external variables using only *Flavoparmelia caperata* data (Table 3-3) show a pattern of stronger correlation with Columbia impact than with general geographic variables for the eleven elements that show at least some evidence of common sources (Table 3-2); nine of the eleven showed evidence of lower tissue concentrations as Distance to Columbia increased. Only Hg of the eight strongly intercorrelated elements showed no correlation with Columbia variables; it showed correlations with Distance West and Distance NW. . Additional strong correlation of Li and weak correlations of Cr and B with Distance West and Distance Northwest hint at a response of the entire suite of correlated elements to a geographic gradient. The strength of correlations with a Columbia impact variable compared to the weaker relationships with geographic variables indicates that Columbia has an influence detectable above background levels, and separable from other regional trends.

Table 3-3. Spearman correlations between *F. caperata* tissue element concentrations and environmental variables (non-parametric correlation of unranked values). P and K are not included because their concentrations reflect internal tissue processes as much as external element sources (see text). N = 26 sites, from 43 samples (multiple *F. caperata* samples from one site are averaged); $p \leq 0.10$ indicated by **bold**; $p \leq 0.05$ indicated by shading; $p \leq 0.01$ indicated by **shading and bold**.

	Distance to Columbia	Model SO2-2003	Distance W	Distance NW	Distance N
Fe	**-0.631**	0.288	0.316	0.267	0.162
Al	**-0.556**	0.292	0.316	0.272	0.16
Cr	**-0.569**	0.285	**0.352**	**0.336**	0.215
Cu	**-0.702**	**0.385**	0.307	0.198	0.091
B	**-0.505**	0.099	**0.373**	**0.378**	0.279
Li	-0.399	0.03	**0.531**	**0.524**	0.405
S	-0.486	0.312	0.209	0.203	0.205
Hg	-0.187	0.027	0.404	0.411	0.158
Na	-0.461	0.431	0.168	**-0.338**	**-0.357**
Mg	-0.236	-0.019	0.132	0.123	0.197
N	**-0.581**	**0.573**	0.005	-0.123	-0.202
Zn	-0.057	0.003	0.202	0.287	**0.348**

Of particular interest are sulfur, because of its role in acidic precipitation, and mercury, an important toxin. S concentrations are strongly correlated with eight other elements in the group, indicating some common sources (Table 3-2), and show evidence in this data set of stronger linkage with Columbia impact than with geographic variables (Table 3-3).

Mercury tissue concentrations are not correlated with Columbia impact, rather Hg values increase with Distance West (r = 0.404; p = 0.041) and Distance Northwest (r=0.411; p=0.037) in the study area. This suggests that 1) ground-level Hg levels are more strongly determined by trends over a larger area and 2) Columbia emissions have little direct influence on local ground-level Hg levels. Mercury emissions from Columbia are probably going into long-range transport rather than directly impacting the local area.

Lower element concentrations (minimum values, Table 3-4) are found at sites farther from Columbia (site data not shown) for the eight strongly correlated elements (Table 3-2). These lower values are similar to those at the background site (Necedah; Table 3-4), suggesting the influence of Columbia on element concentrations in lichen tissue is undetectable at the outer sites, approximately 50-60 km away. Higher values of most elements were three to four times higher than the lower values, although N, Fe and Al show approximately a seven-fold range. Maxima for the ten correlated elements were uniformly much higher than the higher of the two background values, adding further support to indications of Columbia as an important source for elements in the study area.

Table 3-4. Element averages and ranges in ppm at *F. caperata* tissue sites (Table 1-1) in the main study area compared with two background samples (Site 45). N = 41 replicates at 25 sites in the main study area.

	Fe	**Al**	**Cr**	**Cu**	**B**	**Li**	**S**	**Hg**	Na	Mg	N	Zn
average	849	799.5	1.63	6.89	7.37	0.89	1893.5	0.140	33.40	752.80	15395	35.8
120% of avg	1019	959.4	1.96	8.26	8.85	1.07	2272.2	0.167	40.08	903.36	18474	43.0
std dev	290	249.0	0.57	1.56	1.72	0.27	314.9	0.030	10.17	197.48	3295	11.6
min. value	197	177.8	0.74	3.43	3.08	0.33	987.0	0.089	21.21	485.62	2900	22.0
max. value	1410	1252.0	3.04	9.60	10.17	1.49	2365.4	0.235	71.81	1605.74	20200	82.8
120% of max	1692	1502.4	3.65	11.52	12.21	1.79	2838.5	0.282	86.18	1926.89	24240	99.4
background values	515	532.6	1.01	6.28	6.04	0.80	1772.3	0.118	31.60	861.31	13500	31.6
	391	383.9	0.82	5.13	8.17	0.81	1458.2	0.092	32.68	934.59	10700	70.8

Since the choice of naturally-growing large, loosely attached lichen species was very limited in the study area, we used *Flavoparmelia caperata* and *Puctelia rudecta*, species not commonly used for element studies. Data that are directly comparable are not widely available. Olmez, et al. (1985) measured element levels in *F. caperata* at five sites from 1.6 to 20 km in four directions from the Dickerson Power Plant (about 60 km northeast of Washington D.C.). That study does not provide the systematic comparison of distance and direction possible in the present study. Five elements are included in both studies: Al, Cr, Fe, Na, and Zn. In every case 120% of the average value from the present study is well below that of Olmez et al. (1985), expected because we include several sites far from the power plant. 120% of four of our maximum values (near Columbia) are within one standard deviation (lower) of their averages. Our maxima were at sites closer on average to Columbia than their sample sites were to their power plant, suggesting impact in our study area is no more than, and is probably less than, in their area.

Results and discussion 2b) Correlation of tissue sulfur with external variables: We concentrated on sulfur (S) for a more in-depth analysis of tissue element concentrations because of the long history and huge body of literature linking airborne SO_2 with adverse impacts on lichens (Nash & Gries 2002; Nimis & Purvis 2002) and because we have model-based estimates of relative impact of SO_2 released from Columbia stacks. Lichen tissue S is also at least moderately correlated with seven of the nine other elements in the group linked to airborne particles and common origin. So S can represent the group in further investigation of relation of tissue element concentration to Columbia impact and geographic variables.

Two-way ANOVA of sulfur concentration in 2005 tissue samples for two replicates each of *F. caperata* and *P. rudecta* at three sites (3, 22, 28-29, Fig 1-1, Table 1-1) showed strong differences among sites ($p = 0.002$), but no difference between species ($p = 0.576$, interaction $p = 0.671$), showing that we are justified in combining data from the two species. Two-way ANOVA of sulfur concentration in two replicates each of *F. caperata* for 2003 and 2005 tissue samples from the same three sites showed strong differences among sites ($p = 0.007$), but no difference between years ($p = 0.247$, interaction $p = 0.920$), showing that 2005 data comparisons may be applied to 2003 and 2004 data. Based on these findings, we averaged tissue S data for both species and included all sites with tissue data for either species, adding seven replicates and one site. This modest increase in sample size is actually of great benefit; at four of the most eastern sites number of replicate samples increased from one to two or three, and the one site added was the most eastern site of all. This increases our ability to distinguish between relation to Columbia impact and to geographic gradients.

Simple Pearson and Spearman correlations of combined *F. caperata* and *P. rudecta* tissue S data with external variables indicated that Model SO_2-2003 and Distance to Columbia showed the strongest correlation with lichen tissue S. Distance West (lower p), Distance Northwest and Elevation were not correlated with $p \leq 0.1$, and no other variables provided helpful insights.

Partial correlations on ranked data of S with all combinations of these variables showed that Model SO_2-2003 always gave stronger partial correlations than did Distance to Columbia, with tissue S higher where Columbia impact is estimated to be higher, so the former was retained to represent impact of Columbia. Both Distance West and Distance Northwest had strong partial correlation with tissue S; tissue was S higher to the W and NW. Distance West always gave stronger partial correlations, so that variable was retained to represent the geographic gradient. Elevation was never significant, but inclusion of Elevation in partial correlation models always resulted in higher partial correlation with the target variable, so Elevation was retained. Partial correlation of tissue S rank with rank of Model SO_2-2003 accounting for ranks of Distance West and Elevation was stronger than partial correlation of rank of Distance West accounting for ranks of Model SO_2-2003 and Elevation, though both were very strong. These results support the conclusion that lichen tissue S has two external sources, with Columbia the primary source and a strong secondary pattern of higher S in the western part of the study area.

We noticed that Site 22 tissue S values are anomalously high with respect to nearby sites and with respect to the site's position on gradients with a geographic component. Site 22 tissue values for nine of the ten correlated elements are also higher than expected based on values for nearby sites. We repeated the above correlation and partial correlation analyses with Site 22 data excluded and in every case strength of correlation increased. We concluded that Site 22 may have been influenced by some relatively recent unknown pollution event or source, of which no trace remains at or near the site. With Site 22 excluded, partial correlation of tissue S rank with Model SO_2-2003 rank accounting for Distance West rank and Elevation rank was 0.648 ($p = 0.001$), and with Distance West rank accounting for Model SO_2-2003 rank and Elevation rank was 0.540 ($p = 0.008$).

Results and discussion 3) Estimating tissue concentrations at lichen community sites without samples: Estimating tissue S concentration by interpolation proved to be more satisfactory than estimation by linear regression.

Estimation by interpolation: Simple linear interpolation of distance between sites vs. tissue S concentrations for adjacent sites gave tissue S estimates for Sites 15, 16, 22, 28, and 29 (Table 3-4). For Site 30, nearest to Columbia on a compass direction with only three sites, we used the tissue S concentration of Site 31 as our estimate. By interpolating based only on a single compass direction from Columbia in each case, we accounted for both the estimated impact of the power plant and relation of other general geographic gradients to tissue S values at a site.

Linear regression models: Several linear regression models were constructed, with tissue S rank as the dependent variable, including Model SO_2-2003 rank and Distance West rank as independent variables in all models, Elevation rank in or out as an independent variable, and Site 22 in or out. Models with Site 22 out had consistently higher coefficients and lower standard errors, so Site 22 was removed. We found that including Model SO_2-2003, Distance West, and Elevation with 25 sites produced the best model. This model had an adjusted r-square of 0.432, standard error of 5.767 ranks, and F-value of 7.327 ($p=0.001$). Model SO_2-2003 rank ($p = 0.000$) had the most influence on the regression model, followed by Distance West rank ($p = 0.020$), with Elevation rank having the least influence ($p = 0.044$), based on unstandardized regression coefficients (below; Constant $p = 0.000$). This regression equation was used to estimate the S tissue rank at sites without measured tissue S values:

$$\text{Tissue S value rank} = 17.001 + (0.685 \times \text{Model } SO_2\text{-2003 rank}) - (0.353 \times \text{Elevation rank}) - (0.591 \times \text{Distance West rank})$$

We interpolated ranks of the three independent variables for sites without measured tissue S values; values were entered into the above formula to estimate tissue S concentration rank at that site (Table 3-5).

We chose to include the tissue S values estimated by interpolation for the six sites in a variable (Tissue Sulfur) to compare with other data for the twenty-nine 2003 lichen community sites Tissue Sulfur used measured tissue S for those sites with tissue samples and interpolated tissue S for those sites without samples. The linear regression model estimating rank of tissue S accounts for less than half the variation in rank of tissue S (regression r^2 above), and the standard error for the model gives a 95% confidence interval of +/- 11.3 ranks, so we felt the model was not adequate for estimation. All six tissue S values estimated from interpolation have ranks within the 95% confidence interval of tissue ranks estimated from the regression model; half are within three ranks of the model estimation and half are 5-7.5 ranks from the model estimation.

Table 3-5. Values and ranks of variables used in linear regression model for five sites where tissue data were not collected and for Site 22, where measured tissue S was excluded. Estimates from regression and interpolation are in the two right columns. Average measured tissue S concentration for Site 22 is included in parentheses after estimated tissue S value.

Site #	Model SO_2-2003 Value	Model SO_2-2003 Rank	Elevation, m Value	Elevation, m Rank	Distance West, m Value	Distance West, m Rank	Regression Model Estimate Tissue S Rank	Interpolation Estimate Tissue S Value
15	1.060	4	296	7.29	315604	7.78	12.519	0.180
16	0.544	13.7	247	20	328329	5.53	16.075	0.170
22	0.321	23.1	279	11.5	292242	22.42	15.421	0.159 (0.200)
28	0.261	24.6	271	15	271995	24.62	13.899	0.189
29	0.246	25	302	6.22	257154	25.35	16.217	0.179
30	0.393	19	274	13.13	299700	16.45	15.640	0.192

Conclusions

Element data derived from lichen tissue concentrations provide a clear signal of local increase in elements from Alliant Energy – WPL Columbia Energy Center emissions. The geographic extent of Columbia impact falls entirely within the area of study and tissue S concentrations are strongly correlated with a model of Columbia impact based on measured SO_2 emissions. There does not appear to be a local effect of Columbia on mercury levels, suggesting most mercury from Columbia may go into long-distance transport. Lichen tissue sulfur concentration pattern clearly also has a geographic component independent of Columbia, suggesting other sources of S are important in the area. Increased tissue S to the west of the study area is not correlated with any known pollution gradient or industrial source of S. A larger-scale regional gradient of higher pollution in southeastern Wisconsin to lower pollution in northwestern Wisconsin (NADP website) associated with adverse impacts to lichen communities toward the southeast (Makholm 2003; Makholm & Mladenoff 2005) runs counter to the local geographic trend seen in our study area. The increase of tissue S with distance to the west in the study area is currently unexplained, though it is correlated with some land use and forest composition variables (See Chapter 4).

We have derived reliable and informative analyses of tissue element data from two lichen species, *Flavoparmelia caperata* and *Punctelia rudecta,* not extensively used for such investigations before. While less convenient to use than more loosely attached lichen species commonly used for such studies, they have much potential for use in biomonitoring because both are common and widespread across eastern United States. We have demonstrated that the two species can be used together for analysis. We achieved adequate general analysis of tissue element data using only one species, while for a more focused analysis we achieved better interpretation of relationships using both species.

Future Goals

The exact nature of the east-west gradient in lichen tissue Sulfur concentration should be investigated further. Our gradient might possibly be related to soil nutrients or even to land use practices, but we cannot establish this with only current data. Explanation of this east-west gradient will improve interpretation of lichen tissue element concentrations used for biomonitoring of pollutants.

Chapter 4
Tree Communities and Environmental Variables

Introduction

The main focus of the lichen study was investigation of lichens on selected substrates as biomonitors of pollution; however, lichen communities are also affected by many aspects of their environment, including general forest structure, disturbance, and climate. The direct effect of substrate on lichens was standardized by sampling only on Red/Black oak group (*Quercus* section *Lobatae*, Flora of North America Editorial Committee 1997) tree species. Other environmental factors may also exert indirect influence on lichen communities of the target habitats.

To facilitate investigation of how environmental context relates to lichen communities of the target substrate trees we surveyed the overall tree species composition of each site and compiled information on site environmental characteristics.

Methods

Field methods – forest vegetation: Original site selection criteria (Will-Wolf 1980a) were that woods must be at least 2 ha, with a strong component (at least 30 trees) of Red/Black oak group trees present, and no evidence of recent internal disturbance (no recent stumps, evidence of widespread tree disease, etc.). 1974 target locations were selected at 5, 10, 16, 32, and 48 km (+54 km E) from Columbia along NE, E, SE, SW, W, and NW directions (Fig. 1-1). More locations were selected in the eastern part of the study area, to give about equal representation of locations expected to have moderate and higher impact vs. lower to no impact from Columbia based on predictions of a dispersion model (Will-Wolf 1980a). The closest suitable woods to each target location was selected as a sample site for the study. Twenty-nine sites were selected within 1.5 km of their target location. Twenty-four of the original sites were available for resampling in 2003 (Table 4-1); three sites were no longer oak forest and two sites were inaccessible. Five sites were selected to replace those sites; three were within 500m of the 1974 site and had the same ownership, while two (7 and 17) were up to 2 km from the 1974 site with different ownership.

Table 4-1. Lichen community study site tree vegetation. Woodland has lower tree density than forest. For sites where the two tree species with the highest Curtis (1959) Importance Value (and at least 10% IV) between years changed or were different, both are noted. Site Groups were developed during analysis. Group 1 has higher IV of disturbance increaser species and Group 2 has higher IV of later succession increaser species.

Site #	Site Location 1974-2003	Site Group	Two Most Important Trees and Habitat
1	same	1	*Prunus serotina/Quercus macrocarpa* forest both years
2	same	1	*Q. alba/Q. rubra* to *Q. alba/Q. macrocarpa* forest
3	same	1	*Q. alba/Q. rubra* forest both years
4	same	1	*Q. alba/Q. rubra* to *P. serotina/Q. alba* forest
5	same	2	*Robinia pseudoacacia/Q. rubra* forest – dominants reversed in 2003
7	different	2	*Q. velutina/Q. macrocarpa* woodland - *Q. alba/Q. velutina* young forest

Table 4-1 continued.

Site #	Site Location 1974-2003	Site Group	Two Most Important Trees and Habitat
8	same	1	*Q. alba/Q. velutina* to *P. serotina/Q. alba* forest
9	different	1	*Q. velutina/Q. alba* woodland - *Q. alba/P. serotina* forest
10	same	2	*Q. rubra/Q. alba* to *Q. rubra/Tilia americana* forest
11	same	1	*P. serotina/Q. alba* forest both years (*Q. velutina* declined)
12	same	2	*Juglans cinerea/T. americana* to *Q. rubra/T. americana* forest
13	same	2	*Q. alba/Q. ellipsoidalis* forest to *Q. ellipsoidalis* young forest
14	same	2	*Quercus ellipsoidalis/Q. alba* forest – dominants reversed in 2003
15	same	1	*Q. velutina/Q. alba* forest to *P. serotina/Q. alba* young forest
16	same	1	*Quercus velutina/Q. alba* to *Q. velutina/P. serotina* forest
17	different	1	Both *Q. rubra/Q. alba* forest
19	different	2	*Q. ellipsoidalis/Q. alba* woodland - *Q. ellipsoidalis* forest
20	different	1	*Q. velutina/Q. alba* forest - *P. serotina/Q. velutina* forest
21	same	1	*Q. velutina/Q. alba* forest both years
22	same	1	*Q. rubra/Q. alba* forest – dominants reversed in 2003
23	same	1	*Q. velutina/Q. alba* forest – dominants reversed in 2003
25	same	1	*Q. velutina* to *Q. velutina/Celtis occidentalis* forest
26	same	1	*Q. alba/Q. ellipsoidalis* to *Acer rubrum/Q. alba* forest
27	same	1	*Q. rubra/Q. alba* to *A. rubrum/Q. rubra* forest
28	same	1	*Q. velutina/Q. alba* to *A. rubrum/Q. velutina* forest
29	same	1	*Q. rubra/Carya ovata* to *A. rubrum/Q. rubra* forest
30	same	1	*Q. rubra/Q. alba* to *Q. rubra/Populus grandidentata* forest
31	same	1	*Q. ellipsoidalis* to *Pinus strobus/Q. ellipsoidalis* forest
32	same	2	*Q. velutina* to *Q. velutina/Q. alba* forest

At each site, up to twenty circular 0.01 ha plots were located at 15 m intervals in a rectangular grid pattern established from a random start point. Species and basal area were recorded for all trees >15 cm diameter in each plot. This original 1974 protocol was repeated for 2003 surveys at both original sites and replacement sites. In addition, 2003 field surveys included recording for each plot % cover of the shrub layer including seedlings, and notes on most common species of shrubs, seedlings above 1 m tall, and saplings.

1974 surveys were conducted in winter; tree species identification was based on bark and bud characteristics. 2003 surveys were conducted in summer. Based on identification for a few relocated lichen sample trees and on 2003 surveys, we concluded some 1974 species assignments within the Red/Black oak group were probably mistaken. After analysis of 2003 data including notes on possible hybridization (for which the Red/Black oak group is notorious), we modified 1974 data for this group to better match our 2003 data. This removed 1974-2003 differences due only to identification differences for this important but difficult group, and provided us with conservative data for comparing tree species composition between surveys.

Environmental data: Slope, aspect, topographic position, and other site characteristics were recorded in the field in 2003. Similar site characteristics for sites not resampled in 2003 were derived from site notes and detailed maps drawn in 1974 (Will-Wolf 1977). 2003 site location and elevation were recorded with a hand-held GPS (NAD83) in the field and all site locations and elevations were checked and corrected as needed by locating the study site on TopoZone (website) in the office. Climate data (1971-2000 averages) for each site location were extracted from Spatial Climate Analysis Service (website) using ArcGIS Desktop 9 (2004).

1974 sites had little evidence of recent disturbance (Will-Wolf field notes). Sites were assigned a disturbance intensity score for 2003 based on detailed field notes and information from landowners on the 1974-2003 history of the woods: score 0 = no significant disturbance; score 1 = some disturbance from logging, wind damage, or disease; score 2 = significant disturbance from logging, wind damage, or disease. Sites were assigned a Forest Fragmentation Index (Table 4-2) based on field notes about what was adjacent to the site in 1974 and 2003, plus indications of forest cover adjacent to the site from printed USGS topographic maps and TopoZone (website). 1974 and 2003 resurveyed sites could have received different fragmentation indexes in each year, but in all cases each site had the same fragmentation index in both years.

Table 4-2. Forest Fragmentation Index.

Index	Description
1	surrounded on all sides by woods, connected to larger woods
2	1-2 sides open to non-forest, but non-forest is natural environment
3	1 side open to agricultural field, other very disturbed, or 2-3 sides open but most non-forest is natural environment
4	2 sides open to agricultural field, other very disturbed
5	3 sides open to agricultural field, other very disturbed
6	4 sides open to agricultural field, other very disturbed, isolated, but +/- large
7	4 sides open to agricultural field, other very disturbed, isolated and small

Data Summary and Analysis: Tree species composition of a site was summarized by calculating a Relative Importance Value (IV) for each species following Curtis (1959); the average of relative frequency, relative density, and relative dominance (basal area). IV for a site is always 100%, so differences in total tree number or summed basal area between sites are ignored and differences in proportions of species are emphasized. Several summary variables for tree species composition and forest structure were derived to aid in explanation of variation among sites (Table 4-3); several of these are estimators of the amount of sun reaching the forest understory including tree trunks. When appropriate, we derived a change estimate for a variable by subtracting its 1974 value from its 2003 value. Such change estimates emphasize magnitude of change and are compared to each other and to similarity values for sites between the two years. Shrub layer index for sites in 1974 was estimated from field notes and maps. Light level in forest understory was not directly measured in 1974, so indirect estimators of this variable from data available in both years were used.

Table 4-3. Composite variables based on tree species composition at a site.

Variable Name	Explanation
Tree Species #	The total number of tree species at each site.
Tree CI	The Curtis (1959) Continuum Index placing an upland forest on a gradient from dry/early succession (minimum 100) to moist/late succession (maximum 1000). Mostly southern Wisconsin, and some northern Wisconsin Continuum Adaptation Values were used for tree species.
Shade: Tree BA	Basal Area of each tree species multiplied by a shade score (1 least to 5 most) and summed for a site; high value indicates more canopy shade.

Table 4-3 continued.

Variable Name	Explanation
Shade: Tree IV	IV of later successional and other "increaser" tree species (*Acer, Celtis, Fraxinus, Prunus, Tilia, Ulmus*) summed for a site; high value indicates more canopy shade.
Shrub Layer	Shrub layer score (5: sparse shrub layer to 1: high density of tall (>1.5m) shrub layer) estimates potential to shade tree trunks; includes tall seedlings and saplings.
Sun: Tree BA + Shrubs	Rank of "Shade: Tree BA" (rank 1 = highest value) plus "Shrub Layer;" high value indicates more sun. Shade: Tree BA (ranks 1-24) has about 5 x the influence on the index as Shrub Layer (scores 1-5).
Sun: Tree IV + Shrubs	Rank of "Shade: Tree IV" (rank 1 = highest value) plus "Shrub Layer;" high value indicates more sun. Shade: Tree IV (ranks 1-24) has about 5 x the influence on the index as Shrub Layer (scores 1-5).
"Disturbance" Tree IV	Summed IV of *Acer negundo*, *Prunus serotina*, and *Ostrya virginiana*, species found to increase in disturbed upland forests in southern Wisconsin.
Late Succession Tree IV	Summed IV of *Acer rubrum, Celtis* sp., *Fraxinus* sp., and *Tilia* sp., natural later succession tree species that have increased at Columbia sites.

Data for the 24 sites (Table 4-1) surveyed in both 1974 and 2003 were used to investigate changes over time. To investigate patterns of variation just in 1974 or 2003 we used data from all 29 sites for that year.

Tree communities of sites were described and compared using local nonmetric multidimensional scaling (NMS) ordination (Legendre & Legendre 1998; McCune & Grace 2002) with Sørenson distance (= 1- Sørenson Similarity, below) to extract major gradients of variation (PC-ORD v. 4.3 software, McCune & Mefford 1999) from the data set of tree species IVs for sites. NMS, a widely used and reliable unconstrained ordination technique, is one of the most robust and effective methods to extract important differences in composition. NMS gives multiple possible solutions; in each case the selected best solution (significantly non-random, lowest final stress and instability of 40 to >100 solutions tested) displayed three axes of variation and displayed 80-90% of variation among sites in the data set. Environmental variables and summary vegetation variables (Table 4-2) are secondarily related to the tree composition gradients as aids to interpreting them, so correlations between variables do not interfere with analysis.

The similarity of a site between 1974 and 2003 is an index of how much it changed over that time; a higher similarity means less change has occurred. Average similarity between sites in a year is an estimate of the variability of all sites in that year. A higher average value is evidence that tree communities are more similar across the study region. We used the Sørenson similarity index (also known as coefficient of community, McCune & Grace 2002) as a measure of similarity between sites:

$$\text{Sørenson Similarity} = \quad 3 \min | IV_{ah}, IV_{bh} |, \ h = 1 \text{ to } s \text{ (all species at a site)}$$

where a and b are any two sites (or the same site in 2 years), and IV_{ah} and IV_{bh} are the relative IV importance values of species h at sites a and b. The index ranges from 0 for no species in common to 1.0 for all species having the same importance values; it can be multiplied by 100 and interpreted as % similarity. We compared 24 sites surveyed in both 1974 and 2003 with a paired samples T-test and the non-parametric Wilcoxon Signed Ranks test, using Sorenson similarity index values for each site with all other sites of that year (N = 276 each year).

Results and Discussion

We found two main factors in the tree communities – variation among sites and change over time.

Variation among sites: The most important gradient of variation in forest vegetation in both years and for all sites is Curtis (1959) tree CI, which is lower at drier sites often with mostly early successional trees and higher for more moist sites often with some later successional trees. This gradient is Axis 1 (most important) in ordinations for all 1974 and 2003 sites and for the 24 sites surveyed in both years (Figure 4-1A: Axis 1 displays about 41% of variation). For the sites chosen for this study, sites with lower CI have more Black oak group species – *Q. ellipsoidalis* and/or *Q. velutina* while sites with higher CI have more Red oaks – *Q. rubra*. Other forest composition variables are correlated with this gradient: tree species number and shade indexes are higher at higher CI sites while sun indexes are higher at lower CI sites. None of the other environmental variables are correlated with this gradient.

Two other gradients of variation in forest vegetation are important. Lower CI sites are differentiated by the proportion of Hill's oak vs. Black oak at the site. This is the second most important gradient for the 24 sites of 1974-2003 (Figure 4-1A, Axis 2 displays about 27% of variation) and for the 29 sites of 1974. It is only the third most important gradient for the 29 sites of 2003. No other forest composition or environmental variables are strongly correlated with this gradient.

Some sites have a higher proportion of "disturbance" increaser tree species while others have a higher proportion of "later succession" increaser tree species; this is the third most important gradient for the 24 sites of 1974-2003 (Figure 4-1B, Axis 3 displays about 18% of variation) and the second most important gradient for the 29 sites sampled in 2003. This is not an important gradient for the 1974 sites. Black cherry (*P. serotina*) is by far the most common of the disturbance increaser species, while Red maple is the most common of the later succession increaser species. This gradient is correlated with geographic variables; more sites in the west and northwest of the study area have lower proportions of disturbance increaser species.

Change over time: There has been notable change in forest composition from 1974 to 2003 at the 24 sites surveyed in both years. Average similarity between years for the same site is 0.5676, or about 57% similar (high about 79% for Sites 11 and 21, low about 28-30% for Sites 26 and 27; the same site surveyed twice using random sampling is usually about 85-90% similar). Average number of tree species at a site has increased, with other species establishing and increasing at sites originally chosen because they were primarily oak forests. Tree communities at the study sites have become significantly less similar over time ($n=276$: paired t-test $t=3.5417$, $p<0.000$; Wilcoxon signed rank test, $z=3.1129$, $p=0.002$); average similarity between sites in 1974 was about 34%, while it was only 30% in 2003. Oaks have decreased in importance and other species have increased (Fig 4-2). Evidence of recent disturbance was minimal in 1974 (only two sites with evidence of some recent disturbance) as one of the site selection criteria; disturbance was much more prevalent by 2003 (21 of 29 sites with some disturbance 1974-2003, mostly logging, with five sites heavily disturbed and three sites no longer oak forest).

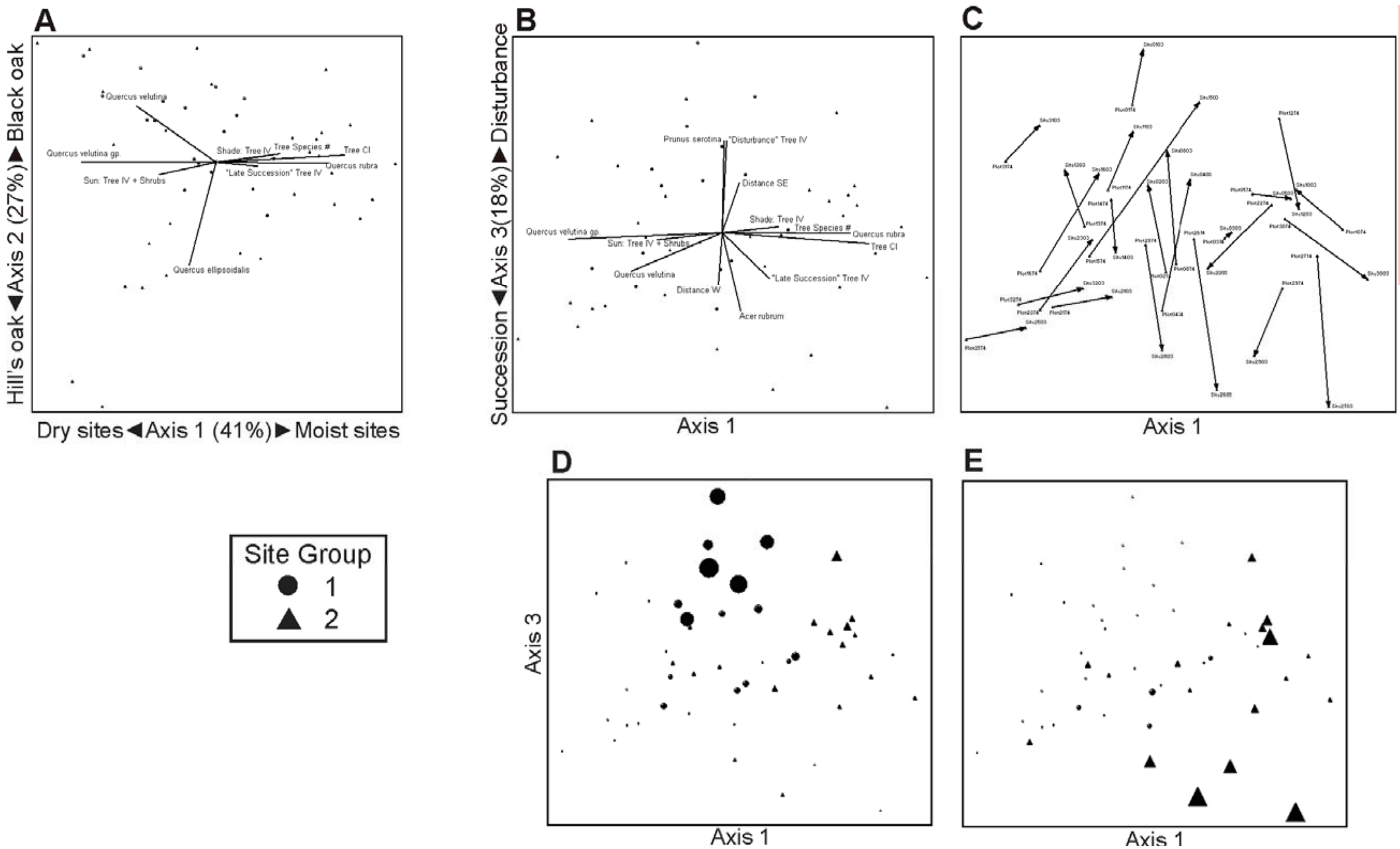

Figure 4-1. NMS ordination of 1974 and 2003 sites based on tree species composition. Dots (each site each year) close together indicate similar site composition. Axis labels include interpretation of the gradient represented, and proportion of total variation on that axis. Lengths of lines from center on diagrams A and B represent strength of variables correlated with axes; direction from center points to sites with higher values of that variable. “Disturbance” tree species increased more at Group 1 sites (circles), while in “Late successional” tree species increased more at Group 2 sites (triangles). **A.** The two most important gradients are drier vs more moist sites (Axis 1) and Hill’s oak vs Black oak sites (Axis 2). **B.** Axis 1 again and the third most important gradient: “Late successional” tree increase vs “Disturbance” tree increase (Axis 3). **C.** Axis 1 and Axis 3 showing arrows from the site in 1974 to the same site in 2003. **D.** Axis 1 and Axis 3: symbol size increases with IV of “Disturbance” tree species. **E.** Axis 1 and Axis 3: symbol size increases with IV of “Late successional” tree species.

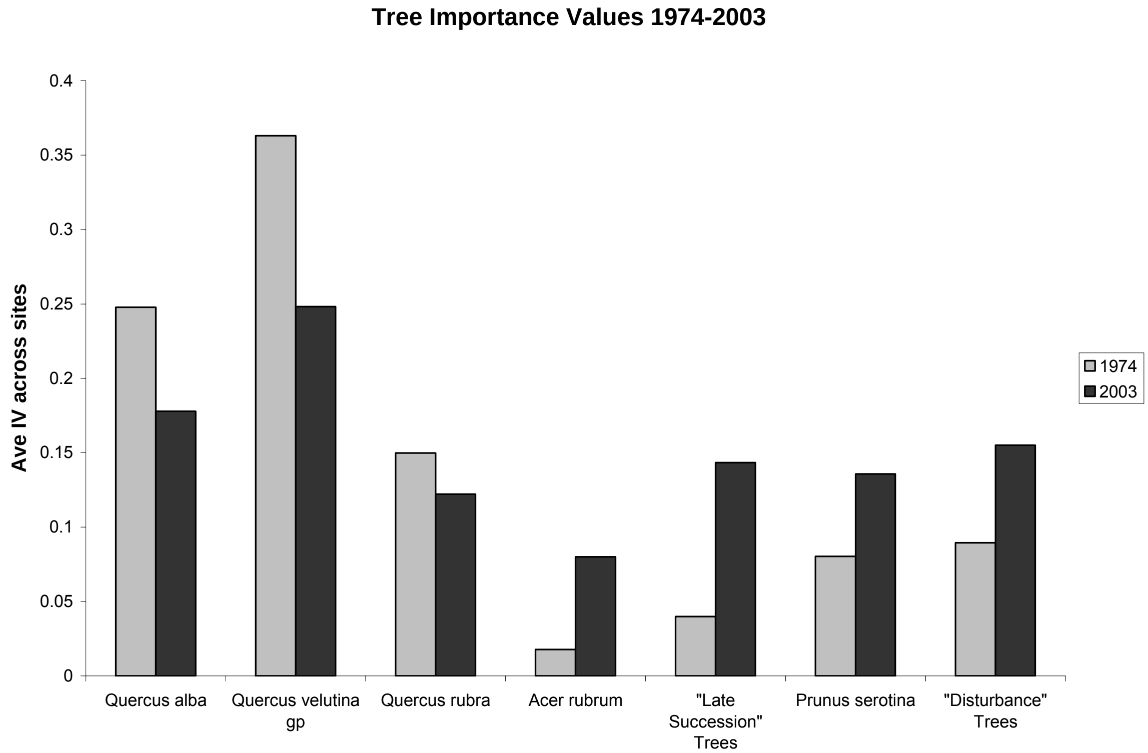

Figure 4-2. Change in tree species 1974-2003. *Acer rubrum* is the most common of the late succession increasers and *Prunus serotina* is the most important of the disturbance increasers. For all but *Quercus rubra*, the change between years is significant (95% or 99% confident) with a Wilcoxon signed ranks test.

The 1974-2003 ordination diagrams indicate that rather than a unified major gradient of change over time in tree species composition, there have been two distinct pathways for change at sites. Direction of arrows in Fig. 4-1C from a site in 1974 to the same site in 2003 indicate the pathway of change; length of an arrow estimates magnitude of change. Up arrows indicate increase in "disturbance" tree species as noted on Axis 3 legend and vectors in Fig 4-1B; down arrows and those mostly pointing right indicate increase in "later succession" tree species. Note that in Fig 4-1C, arrows in the upper left are mostly up, and arrows in the lower half and to the right are mostly down or to the right.

Based on the ordination pattern we examined the original tree species data. Sites that have more 1974-2003 increase in "disturbance" tree species are assigned to Site Group 1, and those with either more 1974-2003 increase in "later succession" tree species or little change in either species group are assigned to Site Group 2. Most of the larger symbols in Fig 4-1D for higher IV of "disturbance" tree species are circles marking Site Group 1, and most of the larger symbols in Fig 4-1E for higher IV of "later succession" tree species are triangles marking Site Group 2. For the 1974-2003 data set, nine sites are assigned to Site Group 1 – disturbance, and 15 sites to Site Group 2 – succession. The five additional 2003 sites were assigned to a site group based on IV of the two species groups, resulting in 11 in Site Group 1 and 18 in Site Group 2 (Table 4-1).

Importance values of the species groups were strongly correlated with their site group; Model SO_2-2003 was positively correlated with Site Group 1. Forest Fragmentation Index (an estimator of landscape disturbance) had a weak positive correlation with Site Group 1, while disturbance intensity (an estimator of within-site disturbance) and tree CI were not correlated with Site Group designation. Overall Forest Fragmentation Index is negatively correlated with Distance West and for 2003, with Tissue S (Chapter 3, partial correlation after accounting for model SO_2-2003 from Chapter 2).

Within each site group 1974-2003 site similarity was strongly correlated with changes in the characteristic species group; this indicates changes in these two species groups accounted for most of the 1974-2003 changes at sites. Within Site Group 1 (disturbance), IV of disturbance increasers is negatively correlated with Distance West, and positively correlated with Forest Fragmentation Index, but not correlated with within-site disturbance intensity. Forest Fragmentation Index is negatively correlated with Distance West and positively correlated with Distance Southeast. Within Site Group 2, IV of late succession increasers is positively correlated with site disturbance intensity, but not correlated with either Forest Fragmentation Index or Distance West

The strongest gradient in tree species composition is related to general site habitat, ranging from Hill's oak or Black oak at sites with drier conditions to Red oak at sites with more moist conditions. This was found in all three data sets, 1974, 2003, and 1974-2003, and is expected from general knowledge of southern Wisconsin forests (Curtis 1959). A second gradient, variation in proportion of Hill's oak and Black oak at drier sites, was also found in all three data sets. Relation of a site to these two gradients has the potential to explain variation in lichen community composition, so these gradients are used as variables in analysis of lichen species composition (Chapter 5).

A third variation in tree species composition is of particular interest in understanding change at study sites over time. This gradient, from sites with disturbance increasers more important to sites with later succession increasers more important, appears from ordination analysis increasingly important through time; not at all important for 1974 data, the third most important gradient for 1974-2003 data, and second for the 2003 data set. Analyses show clearly that the two sets of increaser species help define two different pathways of 1974-2003 change at different sets of sites, and that different environmental variables correlate with degree of change over time within the different site groups. Variation within Site Group 1, sites mostly in landscapes with more fragmented forest cover, is linked to landscape context and land use external to the site. In contrast, variation within Site Group 2, sites mostly in landscapes with less fragmented forest cover, is linked more to within-site disturbance history.

Conclusions

This strong difference between the two site groups in how tree species composition changed could potentially affect variation in lichen communities and change from 1974 to 2003, so it must be accounted for in analysis of lichen community data. Since the differences in how tree species change developed over the same time period when lichens were subject to potential impact from Columbia, the possibility must be considered that these two potential drivers of change interact to affect lichen communities.

While it appears from our simple index that forest fragmentation around our sites has not changed in three decades, it does seem that the effect of forest fragmentation on forest vegetation dynamics has changed, as evidenced by our two site groups important in 2003 but not 1974. Patterns of change in Site Group 2 are generally consistent with natural succession patterns studied by plant ecologists in southern Wisconsin (Peet & Loucks 1977), but patterns of change in Site Group 1 and the existence of two distinct pathways of change must be viewed from a much broader perspective. Since degree of forest fragmentation is correlated with site group (higher in Site Group 1) and is also negatively correlated with Tissue S (Tissue S from Ch 3, ranked data, partial correlation –0.392, $p=0.04$ accounting for Model SO_2-2003 from Ch 2) (Chapter 3), and all three variables correlate with Distance West, the possibility exists there is an underlying cause linked to all three variables related to variation in intensity of human land use across the study area.

Future Goals

Evidence for two distinct pathways of change in southern Wisconsin oak forests should be investigated for a broader range of forested sites. The possibility of an underlying cause linking forest fragmentation, pathway of forest change, and lichen tissue Sulfur needs to be explored. Such an underlying driver of variation in forest vegetation has the potential to be important for understanding vegetation change across southern Wisconsin, so further investigation is strongly recommended.

Chapter 5
Lichen Communities

Introduction

Monitoring of lichen community composition around a point source of pollution provides both useful estimates of impact of pollution on lichens and identifies bioindicators for pollution impact on health of the larger ecosystem (Smith et al. 1993; Van Haluwyn & Van Herk 2002). Our current lichen community survey repeats a study conducted in 1974 and 1978 (Will-Wolf 1980a), allowing us to compare short-term (4 years) and long-term (29 years) biological impacts in areas close to the Alliant Energy – WPL Columbia Energy Center with estimation from a dispersion model (Chapter 2) of potential impact. We are able to re-evaluate the original estimates of impacts on community composition from this source (Will-Wolf 1980a), and in addition, we now compare impacts of this source with other potential sources of impacts to lichen communities and evaluate their relative importance. We can also evaluate our ability to predict long-term effects from short-term studies. Through linking this local study with widespread pollution monitoring of the state by the DNR, we can also evaluate potential long-term regional "background" pollution impacts relevant to a wider area.

The 1974 study design (Will-Wolf 1980a) targeted lichen species on three black oak group species, Black oak (*Quercus velutina*), Red oak (*Q. rubra*), and Hill's oak (*Q. ellipsoidalis*) to limit variability in lichen communities due to differences in substrate pH and physical characteristics (Brodo 1973). Hale (1955) showed that these three tree species have similar bark pH and harbor similar lichen communities in southern Wisconsin. Many study sites were selected to balance variation in forest habitat across the estimated pollution gradient. We repeated the original sampling procedures so that new results can be validly compared with older results. The study design and sampling protocol used by Will-Wolf (1980a) are similar to study approaches currently recommended to detect subtle alterations in community structure (Smith et al. 1993; Van Haluwyn & Van Herk 2002; Will-Wolf 1988).

We investigated relationship of lichen community composition to the forest vegetation and environmental variables developed in Chapters 2, 3, and 4 to generate hypotheses about causation.

Methods

Data collection methods: The goal of the field sample protocol was repeatable sampling of a community subset suitable for assessing subtle impacts of pollution. In the 2003 resurvey, 24 of the 29 1974 sites (Fig. 1-1) were sampled, and five replacement sites were selected near unavailable sites using the same criteria as for 1974 sites (Chapter 4), for a total of 29 sites (see Table 1-1) surveyed in 2003.

Ten black oak group trees (size range 8-50 dm^2) per study site were chosen in 1974 for sampling, one tree near every other forest vegetation sample point (Chapter 4). Lichen species seen, with some bark substrate, were collected from two 25 x 25 cm square vertical trunk plots per tree (1.3m height, NE aspect + direction facing Columbia). For 1978 and 2003 surveys, sample trees were selected at each site to match size and location of 1974 sample trees and the 1974 sample protocol was repeated. The 1974 and 1978 field surveys were conducted in winter, and no species identifications were made in the field. The 2003 surveys were conducted in summer, and some macrolichen specimens unambiguously identifiable to species in the field were recorded on data sheets, with samples not collected. Presence of crust lichen taxa and other macrolichen taxa was noted on the field data sheets, but samples were always collected with substrate for identification in the lab.

Specimen identification and data summary methods: Field specimens were identified to species as possible and presence in each individual bark plot was recorded. Bark pieces were examined thoroughly for species not noted in the field. Nomenclature follows Esslinger (website). Voucher specimens for each species encountered are deposited in the Wisconsin State Herbarium (UW-Madison); original data will be archived in ATRI after publication. Laboratory scrutiny of bark samples and identifications of lichens for 1974 and 1978 surveys were conducted under time constraints; reports were submitted within 6 months to 1 year of field collection. 2003 bark samples were allotted at least three to four times the lab time for scrutiny and identification of lichens as the 1974 and 1978 surveys. Additionally, lichen identification expertise was greater for the 2003 project than for 1974 and 1978 surveys.

For comparison of 2003 surveys with 1974 and 1978, only data from the 24 sites surveyed in all years were included. All older nomenclature was updated and corrected as necessary, consulting original voucher specimens. Several 2003 species were combined for the comparison data set as needed to accommodate broader species concepts accepted for Will-Wolf (1980a). Three modern crustose lichen taxa probably not recognized by Will-Wolf as lichen species for 1974 and 1978 studies were removed from the comparison data set. For analysis of 2003 data alone, all modern species were entered as separate taxa, and all 29 sites were used for most analyses. The complete lichen species list (Table 5-1) includes notes on taxonomic nomenclature and which species are included in each data set. All lichen abundance data were rechecked from original data sheets (including 1974 and 1978) and were modified as needed before entry into electronic data sets.

Frequency data from the 20 plots on ten trees at each site were combined to represent the lichen community at the site. Recording frequency of lichen species in multiple bark quadrats is a widely recommended semi-quantitative measure of lichen abundance (Smith et al. 1993; Van Haluwyn & Van Herk 2002; Will-Wolf 1988). Abundance for a species thus varied from 0 to 20 (present in all bark plots at a site).

Data analysis methods: We analyzed data in several ways to address study goals. Overall patterns of variation in lichen communities and relation to environmental variables were explored with multivariate community analysis. General changes over time were evaluated with statistical tests based on similarity between sites. When numerically appropriate, we derived a change estimate for a variable by subtracting its 1974 value from its 2003 value. Such change estimates emphasize magnitude of change and are compared to similarity values for sites between the two years. Evaluations of responses of particular lichen guilds and species and relationships with environmental variables including impact of Columbia were conducted with statistical tests. Will-Wolf's (1980a) statistical evaluations of lichen community response to impact of Columbia were repeated both to reevaluate the original analysis in light of improved estimation of relative impact and to compare short-term and long-term evaluation of relative impact. All standard statistical analyses were performed using SPSS 12.0 (2003).

Analyses comparing 1974 and 1978 use data from all 29 original sites. Analyses comparing 1974 and 2003 with or without 1978 use data from the 24 sites surveyed in all three years. Most analyses of 2003 alone use data from 24 resurveyed sites plus 5 replacement sites. See Table 1 for species included in each data set. Because we perform many statistical analyses we must consider experiment-wide error. Because there is much intercorrelation among our many species and environmental variables we do not formally adjust p values for assessing significance of multiple tests. Instead we report exact p values and assess relative importance. We expect that about 5% of our p = about 0.05 occur by chance when the null hypothesis should not be rejected. Tests with $0.01 < p < 0.05$ are considered by us to be weakly or marginally significant.

Data analysis methods 1) Lichen communities: Exploration of variation in lichen communities was conducted with local non-metric multidimensional scaling ordination (NMS) with Sørenson distance, an appropriate multivariate analysis technique to identify major correlates of variation in plant communities at different sites without *a priori* assumptions about relationships (see Chapter 4). Analyses were performed using PCORD software (McCune & Mefford 1999). Species found at fewer than three sites in the data set were removed and species abundance was relativized to site totals, setting summed site abundance to 1.0. Differences in total abundance between sites are thus ignored and differences in proportions of species are emphasized in analysis. NMS gives multiple possible solutions; in each case the selected best solution (significantly non-random, lowest final stress and instability of 40 to >100 solutions tested, both final stress and instability acceptably low; McCune & Grace 2002) displayed three axes of variation and displayed 85-90% of variation among sites in the data set. Environmental and forest vegetation variables (Chapters 2, 3, 4) were correlated with ordination axes to aid in interpreting relationships of variation in lichen communities to those variables.

The similarity of a site between years is an index of how much it changed over that time; a higher similarity means less change has occurred. Average similarity between sites in a year is an estimate of the variability of all sites in that year. A higher average value is evidence that lichen communities are more similar across the study region. Changes in lichen communities at individual sites from 1974 to 1978 and from 1974 and 2003 (n = 24 for each) were measured with the Sørenson index of similarity based on relativized data. Average similarity between sites within year was compared for 1974-1978 and for 1974-2003 (n=276 for each), using the paired sample t-test and the Wilcoxon Signed Ranks test.

1974-2003 site similarity values for lichen species composition were compared with other variables and change in other variables through correlation and partial correlation on ranked data. Significance of changes in lichen communities were also tested with the χ^2 test of association on raw abundance of species at a site, repeating an analysis of Will-Wolf (1980a). Species with low abundance at sites were combined into classes such that no more than 20% percent of classes had an expected abundance >1 but < 5.

1974-2003 site similarity values for lichen communities were also compared for two sets of site groups to investigate links of lichen community change with other variables: Site Groups 1 and 2 based on tree species change (Ch. 4), and site groups for higher and lower Columbia impact. Will-Wolf's (1980a) division of sites into Columbia impact groups was revised based on new dispersion model estimates (Ch. 2): sites with average modeled SO_2 concentration of >0.575 mcg/m3 (Table 2-3) are assigned to a higher impact group, and those with lower values are assigned to a lower impact group.

Data analysis methods 2) Lichen guilds: Species were grouped into two sets of guilds, one based on morphological characteristics and one based on genus of algae found as the photosynthetic partner in the lichen (see Table 5-1). Eight morphological guilds (variable name Morpho-guilds) were designated: large foliose, medium foliose, small foliose, fruticose, cladoniiform, corticate crustose, granular/ecorticate crustose and embedded crustose (includes species without thallus apparent on the surface of the substrate). These Morpho-guilds are hypothesized to be related to ecophysiological processes linked to thallus surface area, such as water retention and nutrient absorption.

We drew from the taxonomic literature to assign species to guilds based on genus of the algal partner (variable name Algal-guild). These Algal-guilds (Table 5-1) are hypothesized to be related to differences in physiological characteristics of the algae, such as photosynthetic efficiency and shade tolerance. Three guilds were created based on the algal genus associated with a lichen species: *Trebouxia*, *Asterochloris* and *Trentepohlia*. Not all lichen taxa could be assigned to Algal-guilds, due to uncertainty about the identity of the algal partner for a lichen species. The *Trebouxia* guild was divided into three subguilds based on thallus morphology: crustose, small foliose and large foliose. The small foliose algal subguild is identical to the small foliose morpho-guild, and the large foliose algal subguild is the sum of the medium and large foliose morpho-guilds, so relatively little additional analysis was done on the macrolichen Algal-guilds. The *Asterochloris* guild was divided into two subguilds: cladoniiform (macrolichens with a *Cladonia*-like growth form) and leprose (granular/ecorticate crustose species).

Guild abundance at each site in 2003 was compared with Columbia impact (Model SO2-2003 from Ch. 2), Tissue S, and with numerous shade and geographic variables (see Ch. 4) using Spearman, Pearson's, and Kendall's tau correlation tests (p values for two-tailed tests of significance). Rank of 2003 minus 1974 abundance for a Morpho-guild at sites was also compared to most of the same variables, or to their change over time. Due to small sample sizes, we did not include the cladoniiform, fruticose and embedded crust Morpho-guilds in analyses. We used Paired t-tests, Wilcoxon Signed rank tests, and Chi-square tests to test whether guild abundance changed over time within and across sites. For some variables, separate tests were conducted on sites within two Columbia impact groups (higher and lower, see Table 2-3) to investigate effect of pollution on these guilds.

Table 5-1. Lichen species list for 1974, 1978, and 2003 with presence at comparable sites between years. For guild designations, Cr = crustose; Fo = foliose. A '-' indicates that the guild or presence was not used due to lack of data or synonomy issues of species between years.

Species	Morpho-Guild	Algal-Guild	Presence: 29 sites 1974	Presence: 29 sites 1978	Presence: 24 sites 1974	Presence: 24 sites 2003	Presence: 2003 all
Acrocordia megalospora (Fink) R. C. Harris	Cr-Thallus Embedded/ Absent	Trentepohlioid	-	-	-	-	3
Amandinea polyspora (Willey) E. Lay & P. May	Cr-Corticate	Trebouxioid	0	0	0	7	10
Amandinea punctata (Hoffm.) Coppins & Scheid.	Cr-Corticate	Trebouxioid	7	16	5	7	10
Amandinea sp.	Cr-Corticate	Trebouxioid	0	0	0	1	1
Anisomeridium polypori (Ellis & Everh.) M.E. Barr	Cr-Thallus Embedded/ Absent	Trentepohlioid	-	-	-	-	22
Arthonia caesia (Flotow) Körber	Cr-Granular/ Ecorticate	-	28	29	23	24	29
Arthonia radiata (Pers.) Ach.	Cr-Thallus Embedded/ Absent	Trentepohlioid	0	0	0	1	1
Arthonia sp. Harris	Cr-Thallus Embedded/ Absent	Trentepohlioid	0	0	0	3	3
Buellia schaereri De Not.	Cr-Corticate	Trebouxioid	3	8	3	1	1
Caloplaca discolor (Willey) Fink	Cr-Corticate	Trebouxioid	1	2	0	0	0
Candelaria concolor (Dickson) Stein	Fo-Small	Trebouxioid	12	19	10	24	29
Candelariella efflorescens R.C. Harris & W.R. Buck	Cr-Granular/ Ecorticate	Trebouxioid	25	29	20	23	28
Candelariella xanthostigma (Ach.) Lettau	Cr-Granular/ Ecorticate	Trebouxioid	9	11	7	12	15
*Catinaria atropurpurea (*Schaerer) Vezda & Poelt	Cr-Granular/ Ecorticate	-	0	0	0	3	3
Cladonia cf. *cryptochlorophaea* Asah.	Cladoniiform	Asterochloris	0	0	0	1	1
Cladonia coniocraea (Flörke) Sprengel	Cladoniiform	Asterochloris	0	0	0	3	3
Cladonia grayi G. Merr. ex Sandst.	Cladoniiform	Asterochloris	0	0	0	4	4
Cladonia ochrochlora Flörke	Cladoniiform	Asterochloris	0	0	0	2	2
Cladonia rei Schaerer	Cladoniiform	Asterochloris	0	1	0	4	4
Coenogonium pineti (Ach.) Lücking & Lumbsch	Cr-Thallus Embedded/ Absent	Trentepohlioid	0	0	0	7	9
Evernia mesomorpha Nyl.	Fruticose	Trebouxioid	3	1	2	0	0
Flavoparmelia caperata (L.) Hale	Fo-Large	Trebouxioid	22	24	17	11	14
Flavopunctelia flaventior (Stirton) Hale	Fo-Large	Trebouxioid	5	17	4	2	3
Flavopunctelia soredica (Nyl.) Hale	Fo-Large	Trebouxioid	12	6	9	2	4
Graphis scripta (L.) Ach.	Cr-Corticate	Trentepohlioid	17	17	16	18	21
Heterodermia speciosa (Wulfen) Trevisan	Fo-Small	Trebouxioid	0	1	0	0	0
Hyperphyscia adglutinata (Flörke) H. Mayrh. & Poelt	Fo-Small	Trebouxioid	0	10	0	23	28
Hyperphyscia syncolla (Tuck. ex Nyl.) Kalb	Fo-Small	Trebouxioid	4	2	4	0	0
Lecanora hybocarpa (Tuck.) Brodo	Cr-Corticate	Trebouxioid	23	24	21	14	16
Lecanora hypoptoides (Nyl.) Nyl.	Cr-Corticate	Trebouxioid	8	18	6	0	0
Lecanora impudens Degel.	Cr-Corticate	Trebouxioid	0	0	0	12	15
Lecanora strobilina (Sprengel) Kieffer	Cr-Granular/ Ecorticate	Trebouxioid	17	14	15	10	11
Lecanora symmicta (Ach.) Ach.	Cr-Granular/ Ecorticate	Trebouxioid	6	8	5	2	4
Lecanora thysanophora Harris	Cr-Granular/ Ecorticate	Trebouxioid	19	12	17	22	26
Lecidea albohyalina (Nyl.) Th. Fr.	Cr-Granular/ Ecorticate	-	0	0	0	2	3
Lepraria caesiella R.C. Harris	Cr-Granular/ Ecorticate	Asterochloris	-	-	-	-	4
Lepraria lobificans Nyl.	Cr-Granular/ Ecorticate	Asterochloris	7	3	7	8	9
Melanelia subaurifera (Nyl.)	Fo-Medium	Trebouxioid	2	5	2	1	1
Micarea peliocarpa (Anzi) Coppins & R. Sant.	Cr-Thallus Embedded/ Absent	-	-	-	-	-	2
Micarea sp. 1	Cr-Thallus Embedded/ Absent	-	-	-	-	-	1
Mycobilimbia epixanthoides (Nyl.) Vitik., Ahti, Kuusinen, Lommi & T. Ulvinen	Cr-Corticate	-	21	22	18	22	26

Table 5-1 continued.

Species	Morpho-Guild	Algal-Guild	Presence: 29 sites 1974	Presence: 29 sites 1978	Presence: 24 sites 1974	Presence: 24 sites 2003	Presence: 2003 all
Myelochroa aurulenta (Tuck.) Elix & Hale	Fo-Medium	Trebouxioid	23	24	19	21	25
Myelochroa galbina (Ach.) Elix & Hale	Fo-Medium	Trebouxioid	12	12	9	3	3
Opegrapha varia Pers.	Cr-Thallus Embedded/ Absent	Trentepohlioid	0	0	0	1	1
Parmelia squarrosa Hale	Fo-Medium	Trebouxioid	6	3	4	1	1
Parmelia sulcata Taylor	Fo-Medium	Trebouxioid	16	19	11	4	6
Parmotrema hypotropum (Nyl.) Hale	Fo-Large	Trebouxioid	0	0	0	1	1
Pertusaria pustulata (Ach.) Duby	Cr-Corticate	-	20	13	16	12	13
Phaeophyscia adiastola (Essl.) Essl.	Fo-Small	Trebouxioid	-	-	-	-	7
Phaeophyscia ciliata (Hoffm.) Moberg	Fo-Small	Trebouxioid	0	0	0	2	2
Phaeophyscia hirsuta (Mereschk.) Essl.	Fo-Small	Trebouxioid	0	2	0	4	5
Phaeophyscia pusilloides (Zahlbr.) Essl.	Fo-Small	Trebouxioid	24	25	20	21	23
Phaeophyscia rubropulchra (Degel.) Essl.	Fo-Small	Trebouxioid	23	18	19	24	29
Physcia aipolia (Ehrh. ex Humb.) Fürnr. var. *aipolia*	Fo-Small	Trebouxioid	2	2	2	1	1
Physcia millegrana Degel.	Fo-Small	Trebouxioid	29	29	24	24	29
Physcia stellaris (L.) Nyl.	Fo-Small	Trebouxioid	24	24	20	3	4
Physciella chloantha (Ach.) Essl.	Fo-Small	Trebouxioid	-	-	-	-	22
Physconia leucoleiptes (Tuck.) Essl.	Fo-Small	Trebouxioid	0	1	0	1	2
Punctelia bolliana (Müll. Arg.) Krog	Fo-Large	Trebouxioid	20	21	16	5	7
Punctelia missouriensis G. Wilh. & Ladd	Fo-Large	Trebouxioid	0	0	0	4	4
Punctelia perreticulata (Räsänen) G. Wilh. & Ladd	Fo-Large	Trebouxioid	0	0	0	1	1
Punctelia rudecta (Ach.) Krog	Fo-Large	Trebouxioid	23	28	20	20	25
Pyrenula pseudobufonia (Rehm) R. C. Harris	Cr-Corticate	Trentepohlioid	17	18	15	9	10
Pyxine sorediata (Ach.) Mont.	Fo-Small	Trebouxioid	0	2	0	4	6
Ramalina americana Hale	Fruticose	Trebouxioid	3	6	3	0	0
Ramalina sinensis Jatta	Fruticose	Trebouxioid	2	1	2	0	0
Rinodina cf. *degeliana* Coppins	Cr-Corticate	Trebouxioid	0	1	0	6	8
Rinodina pachysperma H. Magn.	Cr-Corticate	Trebouxioid	0	0	0	2	4
Rinodina papillata H. Magn.	Cr-Corticate	Trebouxioid	25	24	21	23	28
Scoliciosporum chlorococcum (Stenh.) Vezda	Cr-Granular/ Ecorticate	-	17	19	16	23	27
Sterile Crust 1	Cr-Granular/ Ecorticate	-	0	0	0	18	21
Sterile Crust 2	Cr-Corticate	Trebouxioid	0	0	0	11	14
Sterile Crust 3	Cr-Granular/ Ecorticate	-	0	0	0	11	12
Usnea hirta (L.) F. H. Wigg.	Fruticose	Trebouxioid	1	0	0	0	0
Xanthomendoza fallax (Hepp) Søchting, Kärnefelt & S. Kondr.	Fo-Small	Trebouxioid	1	7	1	12	13

Data analysis methods 3) Lichen species: Our estimate of lichen abundance is the frequency of lichen species occurrence in 20 quadrats on a site. Lichen species abundance was ranked for each of the sites in 2003 for species present in at least 20 quadrats across all sites. For species present in at least 20 quadrats across the resampled 1974-2003 sites, 2003 abundance minus 1974 abundance was our measure of change; a positive value indicates an increase in abundance in 2003. Correlations of rank of 2003 abundance, rank of 1974 and 2003 abundance, and change in abundance (2003 minus 1974) with a variety of environmental variables (see Chapters 2, 3, 4, and Lichen guilds, above) were calculated. Pearson correlation is reported for ranked data, and Spearman's rho correlation coefficient is reported for unranked data (two-tailed tests for both).

Results and Discussion

A total of 75 species were found in all years of the study (Table 5-1), with the most diversity recorded for 2003. Some of the 2003 species were truly new finds; others are species segregated from groups recorded in 1974 and 1978 as single species. Tufted or hanging fruticose species were very scarce in earlier studies and were not recovered in bark plots in 2003, though their presence outside sampled plots at 2003 sites was confirmed (off-plot vouchers).

We found both variation among sites in lichen community composition in all years as well as changes in lichen community composition within sites between 1974 and 2003. Environmental factors relating to these include 1) variation in forest habitat, 2) changes in forest structure and composition at least partly associated with increased shade, 3) an E and SE vs W and NW geographic gradient, and 4) modeled impact of Columbia. Apparent increase in lichen species richness at sites is likely due at least in part to more thorough scrutiny of bark samples in 2003.

Results and Discussion 1) Lichen communities: Ordination of lichen communities indicates there are two major factors relating to variation in lichen community composition in all years: 1) forest structure and composition associated with shade and moisture gradients, and 2) an E and SE vs W and NW geographic gradient. In all single years 1974, 1978, 2003, and in 1974-78 (none shown), these are the two most important gradients in lichen community composition. The relative importance of these factors and the variables related to them changed over time. For 1974, 1978, and 1974-78, forest composition was the more important, while for the 2003 ordination (not shown), importance of lichen composition gradients was reversed, with the geographic gradient the more important.

The strong correlation of tree community composition variables with composition of lichen communities only from Black oak group tree trunks demonstrates that even for this more in-depth investigation within the standardized substrate community (Hale 1955), species distribution is notably affected by general site environment. Correlation of lichen community composition with shade and sun variables as well as tree composition, and lack of correlation with physical site variables such as elevation, suggests that the underlying cause is likely forest structure affecting shade at trunks.

The importance of variation of lichen communities along an E and SE vs W and NW geographic gradient mirrors somewhat the geographically-linked variation in both tree species composition (Ch. 4- 1974 and 2003) and Tissue S (Ch. 3 - 2003). Sites to the W and NW of the study area in general have higher lichen species abundance, more typical successional patterns of change in tree species, less fragmented forests, and higher Tissue S (after accounting for Columbia). Lichen species richness is also higher at W and NW sites, mostly in those sites with more sun. The greater importance of geographic variation in 2003 suggests there might have been a switch between 1974 and 2003, from within-site variables (Tree CI and shade) to environmental context (forest fragmentation) as the most important general constraints on lichen communities.

The two gradients most important in single years were displaced for the 1974-2003 dataset to second and third place. The second most important gradient in lichen community composition for this dataset (Fig. 5-1A, Axis 2) correlates with the gradient in forest composition, from shady moist sites positively correlated with Tree CI (plus a shade variable and tree species richness), to sunny drier sites, positively correlated with IV of Black oak group trees (plus a sun variable and also lichen species richness). The third most important gradient in lichen community composition (Fig 5-2A, Axis 3) for 1974-2003 is the E and SE vs W and NW geographic gradient, correlated here primarily with lichen abundance (higher to the W and NW). Two species that changed little in relative importance over the time of the study illustrate the geographic gradient. *Myelochroa aurulenta* (Fig. 5-2B), a medium foliose lichen (Table 5-1), is relatively more important at sites to the W and NW that have higher lichen abundance, while *Rinodina papillata* (Fig. 5-2B), a corticate crust lichen, is relatively more important at sites to the E and SE that have lower lichen abundance. Geographic variation in lichen communities is only partially linked to geographic variation in tree communities. Tree variables linked to the two pathways of change in forest vegetation at sites (Fig. 4-1) are weakly correlated with lichen gradients in ordinations, and not always with the geographic gradient, for instance see Later Succession Trees (Fig. 5-2A).

For the 1974-2003 dataset, change over time was by far the most important gradient for lichen communities, accounting for more than half the variation (Fig. 5-1A, Axis 1). In contrast to tree communities, lichen communities all changed similarly between 1974 and 2003; arrows for sites in both years all point to the left on ordination Axis 1 (Fig 5-1B), and converge, suggesting lichen communities became more similar over time (Axis 2, Fig 5-1A & B), at the same time tree species communities diverged. Change in lichen communities is strongly correlated with the onset of pollution from Columbia and increase of within-site disturbance and also is correlated with increases in shade and in species richness of both lichens and trees. We believe much of the apparent increase in lichen species richness at sites is due to increased effort with sample scrutiny and expertise with identification, so we do not discuss this as an important change in lichen communities over time.

Patterns worth investigating are illustrated by four common lichen species. *Flavoparmelia caperata* (Fig. 5-1C), a large foliose lichen (Table 5-1) and our primary tissue sample species (Ch. 3), is much more important at drier, sunnier sites; it decreased in relative importance over the study period. *Physcia millegrana* (Fig 5-1E), a small foliose lichen found across the sun-shade gradient (no correlation with Axis 2); also decreased in importance over time. In contrast, *Phaeophyscia pusilloides* (Fig. 5-1D), a small foliose lichen more important at shadier and more moist sites, showed relatively little change over the study period while *Candelaria concolor* (Fig. 5-1F), also a small foliose lichen more important at shadier sites, increased over time.

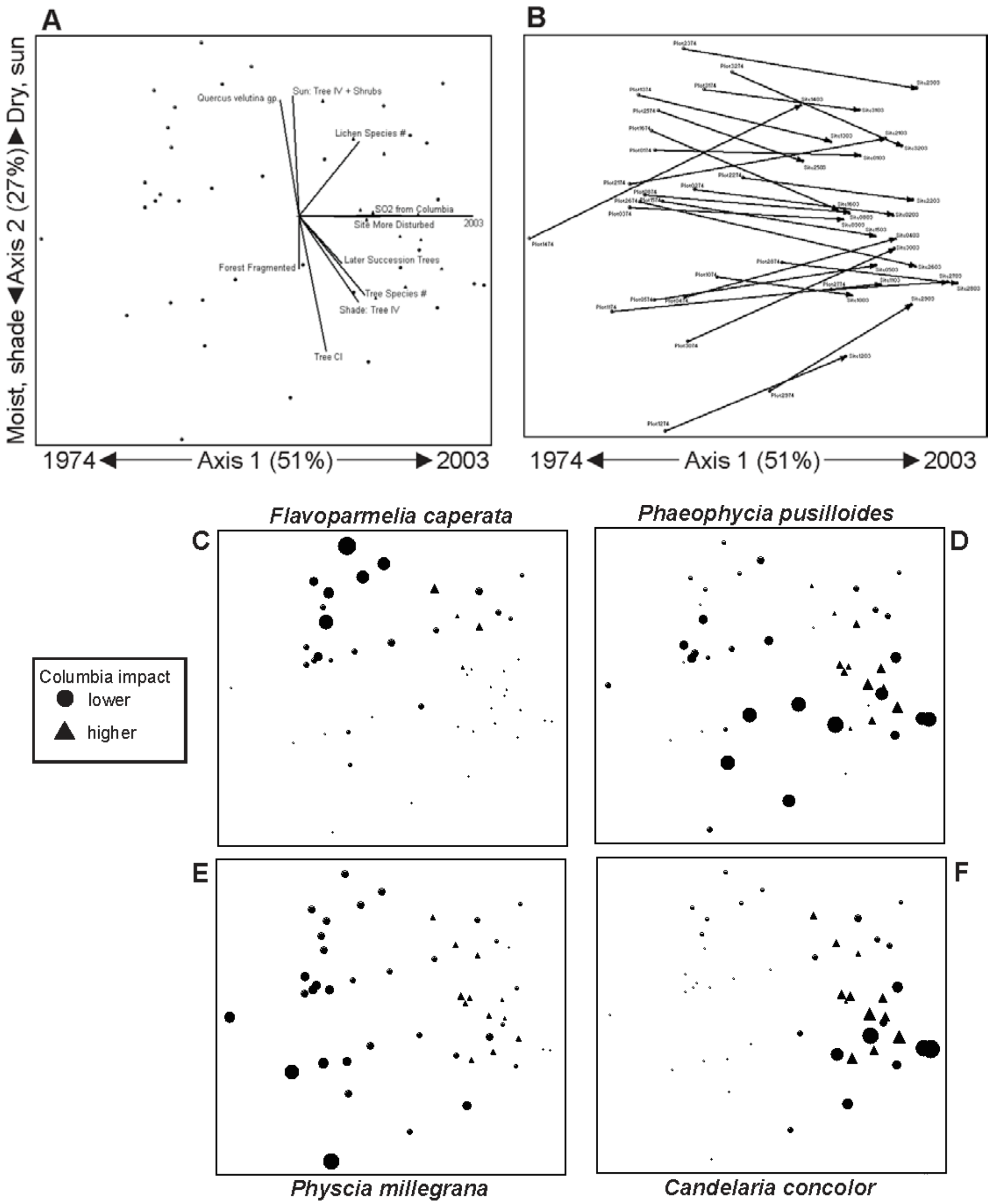

Figure 5-1. Two important gradients (NMS ordination) of variation in lichen communities at 24 sites in 1974 and 2003. All graphs have the same two axes. **A**.1974-2003 change is the most important gradient (Axis 1, 51% of variation): increases in lichen species richness, Columbia impact, and site disturbance. Variation from drier, sunnier sites to more moist, shadier sites with higher Tree CI is the second most important (Axis 2, 27% of variation). **B**. Arrows connecting a site 1974-2003 converge to the right; lichen communities were more similar to each other in 2003 than in 1974. Response is displayed for four common species.(larger symbol = more abundant): **C.** prefers sun, decreases over time; **D**. prefers shade, shows little change over time; **E.** is broadly distributed across the sun-shade and Tree CI gradient and decreases over time; **F.** prefers shade and increases over time.

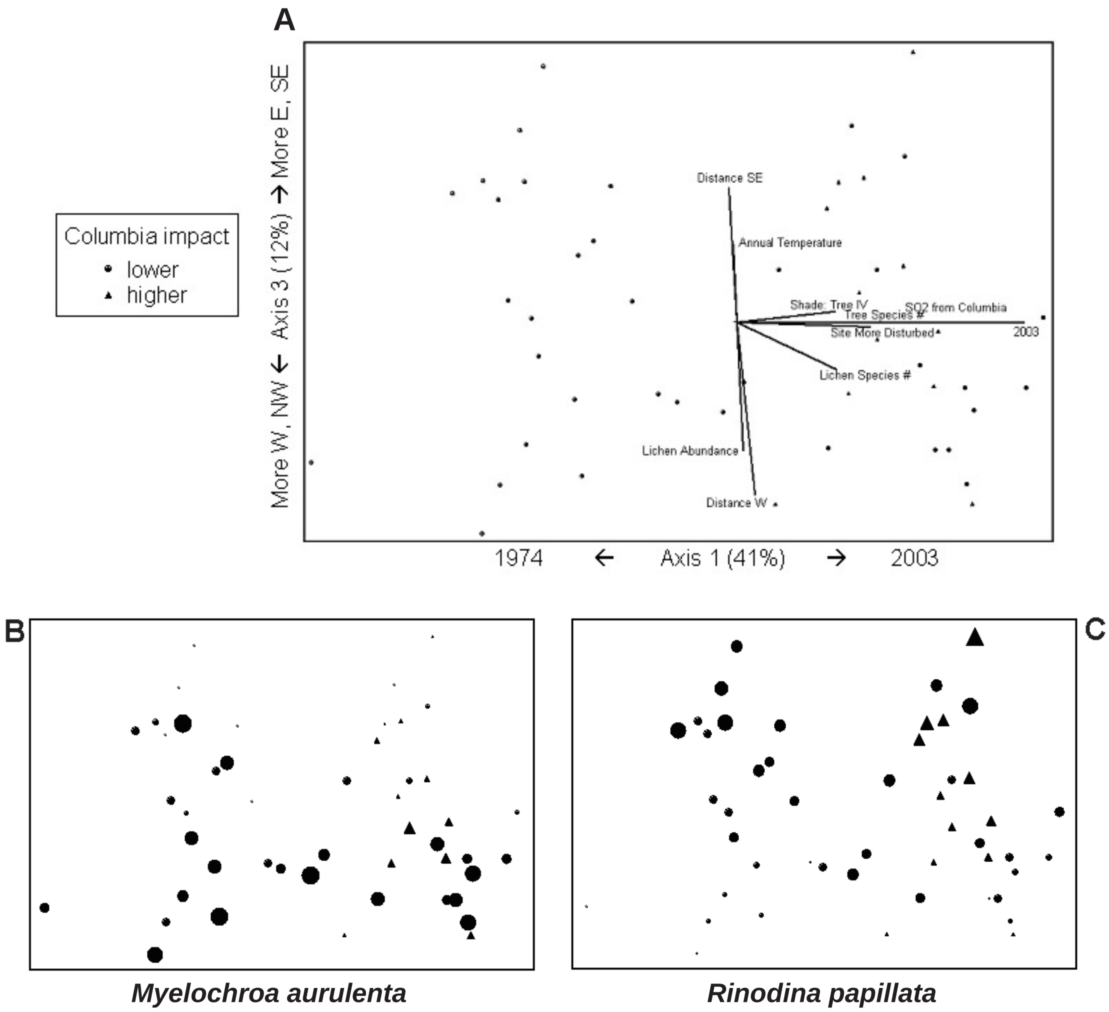

Figure 5-2. **A.** Location more E and SE vs more W and NW (Axis 3, 12% of variation), with lichen abundance higher to the W and NW, is the third most important gradient in lichen communities at 24 sites in 1974 and 2003 from the NMS ordination. Axis 1 is the same gradient of 1974-2003 change as in Fig. 5-1. All three graphs have the same two axes. Response is displayed for two common species: more abundant (larger symbols) **B**. at W and NW sites or **C.** at E and SE sites.

Impact of Columbia was not expected to be one of the major constraints on variation in lichen communities because power plant design was expected to minimize ground-level concentrations, and dispersion models of SO_2 concentrations (Will-Wolf 1980a and Ch. 2) support this expectation. Analysis of patterns of lichen community composition confirm that this is indeed the case. No Columbia impact variable was correlated with major changes in lichens 1974-78, or major variation in either 1978 or 2003 lichen communities (also see Fig. 3 of Will-Wolf 1980a). While Columbia impact is correlated with major changes in lichens 1974-2003 (Fig. 5-1A), this is expected because there was no impact in 1974 while some small 2003 impact was modeled even for sites distant from Columbia (Table 2-3). Within-site disturbance has a similar pattern, again related to study design. Neither correlation is extremely strong, and with no correlations related to 2003 community variation, these 1974-2003 correlations are not strong support from ordinations to hypothesize that either variable caused changes over time.

Measured similarities between sites based on lichen communities provide data to test hypotheses about changes in these communities suggested from the ordinations. We compared replicate sites between 1974 and 1978 (Table 5-2) and between 1974 and 2003 (Table 5-3) based on relative abundance of lichen species at a site. Individual sites were more similar between 1974 and 1978 than they were between 1974 and 2003; most of the site differences were significant by the time of the 2003 re-survey. For the 1974-1978 comparison, while average site similarity between years (Table 5-2) was almost the same for both Columbia impact groups (0.7369 for "higher" vs 0.7562 for "lower"), more changes were significant at sites modeled to have lower SO_2 levels (a difference from Will-Wolf 1980a). While 1974-2003 changes were almost all significant (Table 5-2), there was no strong difference between Columbia impact groups. Some unmeasurable component of differences between 1974 and 2003 is related to increased sample scrutiny and increased expertise in identifying lichens in 2003.

Table 5-2. Lichen community changes 1974-1978. NS= not significant.

Site number	Distance from Columbia	Lichen species 1974	Lichen species 1978	Comparison Indices: Similarity Index 74-78	Likelihood Ratio	χ^2 74-78 Degrees of Freedom	P value
Higher SO2 sites							
1	6.4 km SE	21	25	0.7056	9.922898393	6	NS
2	9.7 km SE	22	24	0.7674	10.2296128	10	NS
3	14.5 km SE	18	24	0.7678	21.55471116	9	p=0.010
4	30.6 km SE	17	17	0.7406	4.547789808	6	NS
7	7.2 km E	15	21	0.6851	6.286832697	6	NS
8	10.5 km E	21	23	0.7395	7.185350623	7	NS
9	14.5 km NE	21	23	0.6831	8.850550969	5	NS
10	30.6 km E	17	17	0.7636	3.508268915	7	NS
13	4.8 km NE	22	25	0.7267	8.038971754	11	NS
14	8.9 km NE	17	18	0.7122	1.715227488	6	NS
15	15.3 km NE	18	21	0.8242	3.151540725	8	NS
26	9.7 km W	23	20	0.7273	9.041072799	6	NS
Lower SO2 sites							
5	48.3 km SE	13	15	0.7879	3.839051776	6	NS
11	48.3 km E	13	16	0.7094	5.321443335	4	NS
12	64.4 km E	8	13	0.8155	1.423143428	4	NS
16	33.0 km NE	20	17	0.8476	4.828771755	10	NS
17	51.5 km NE	14	22	0.7729	21.50690081	8	p=0.006
19	5.6 km NNW	17	18	0.7959	4.146468356	5	NS
20	9.7 km NNW	23	26	0.7349	4.940936677	8	NS
21	17.7 km NNW	17	19	0.7312	10.03729579	8	NS
22	33.0 km NNW	27	26	0.7427	12.54738954	13	NS
23	46.7 km NNW	22	27	0.7995	7.341668242	10	NS
25	4.0 km W	20	22	0.7970	4.11643589	10	NS
27	14.5 km W	18	21	0.8314	11.63707993	11	NS
28	32.2 km W	24	22	0.6888	13.5094408	7	p=0.061
29	46.7 km W	15	17	0.7472	21.03974531	8	p=0.007
30	4.8 km SW	17	14	0.6130	47.50367558	9	p=0.000
31	9.7 km SW	17	26	0.7489	23.65598167	11	p=0.014
32	16.1 km SW	22	19	0.6917	21.6657888	11	p=0.027

Table 5-3. Lichen community changes 1974-2003. NS= not significant.

Site number	Distance from Columbia	Lichen species 1974	Lichen species 2003	Comparison Indices: Similarity Index 74-03	Comparison Indices: Likelihood Ratio	χ^2 74-03 Degrees of Freedom	P value
Higher SO2 sites							
1	6.4 km SE	21	27	0.5493	55.90278208	12	p=0.000
2	9.7 km SE	22	24	0.5393	58.47844963	14	p=0.000
3	14.5 km SE	18	25	0.5420	50.72118543	12	p=0.000
4	30.6 km SE	17	24	0.5469	27.78682116	10	p=0.002
8	10.5 km E	21	23	0.5603	17.06765899	7	p=0.017
10	30.6 km E	17	20	0.6332	32.90942672	9	p=0.000
13	4.8 km NE	22	25	0.6205	19.46262461	11	p=0.053
14	8.9 km NE	17	30	0.4264	31.31985049	6	p=0.000
15	15.3 km NE	18	19	0.5772	23.84898705	6	p=0.001
26	9.7 km W	23	13	0.4682	56.35289458	9	p=0.000
Lower SO2 sites							
5	48.3 km SE	13	18	0.5197	20.08363334	5	p=0.001
11	48.3 km E	13	16	0.4147	24.43783197	4	p=0.000
12	64.4 km E	8	13	0.5930	14.37394194	3	p=0.002
16	33.0 km NE	20	19	0.5963	54.98775365	10	p=0.000
21	17.7 km NNW	17	29	0.4206	65.51264813	9	p=0.000
22	33.0 km NNW	27	24	0.5596	62.06321532	14	p=0.000
23	46.7 km NNW	22	27	0.5174	88.97727381	11	p=0.000
25	4.0 km W	20	23	0.6355	12.65811997	8	NS
27	14.5 km W	18	24	0.7083	27.28098355	12	p=0.007
28	32.2 km W	24	20	0.5647	34.6061553	9	p=0.000
29	46.7 km W	15	24	0.5851	22.10204312	10	p=0.015
30	4.8 km SW	17	20	0.5411	52.68760224	10	p=0.000
31	9.7 km SW	17	31	0.5905	60.55392152	9	p=0.000
32	16.1 km SW	22	28	0.4960	66.99425694	14	p=0.000

Although individual sites showed differences between sample years, lichen communities as a whole became more similar to each other within a given year over time. A slight, though significant, increase in average similarity between 1974 and 1978 occurred while an even more dramatic increase occurred between 1974 and 2003 (Figure 5-3). This trend, occurring in spite of increased lichen species richness at sites, suggests that lichen communities have become homogenized across the study sites, losing their individual distinctiveness. Lichen communities could have become more similar in response to increased pollution, in response to reduced diversity of light environments driven by changes in forest plant communities, or both.

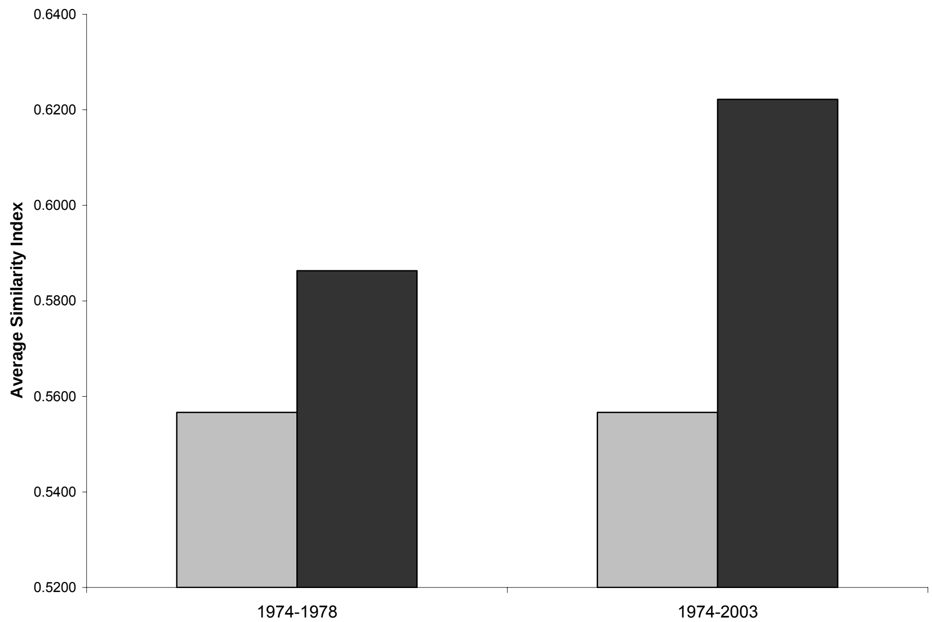

Figure 5-3. Homogenization of lichen communities 1974-1978 and 1974-2003. The increase in average similarity of the lichen community in each case is significant at the p=0.001 level.

Results and Discussion 2) Lichen guilds: Shifts in relative abundance of Morpho-guilds occurred at sites between 1974 and 2003: small foliose lichens, corticate crusts and granular, ecorticate crusts all increased, while large and medium foliose lichens decreased (Figure 5-4, Table 5-4). Some Morpho-guilds are strongly related to light environment: small foliose lichens increased more at shadier sites, while medium foliose lichens decreased more at shadier sites. Changes in abundance (paired t-tests, Table 5-4) of small and medium (marginally significant for one group) foliose lichens were essentially similar across clean versus polluted site groups, while abundance of large foliose lichens (decreased mostly at clean sites) and corticate crusts (increased mostly at polluted sites) shifted differentially. Granular/ecorticate crusts increased significantly at both site groups (paired t-tests), however they increased significantly more at polluted sites (Chi-square test of association). The shifts in large foliose, corticate crust, and granular/ecorticate crust guilds that show evidence of impact from Columbia are interesting in that the impact is displayed as increases in presumably more tolerant species rather than decreases in presumably more sensitive species.

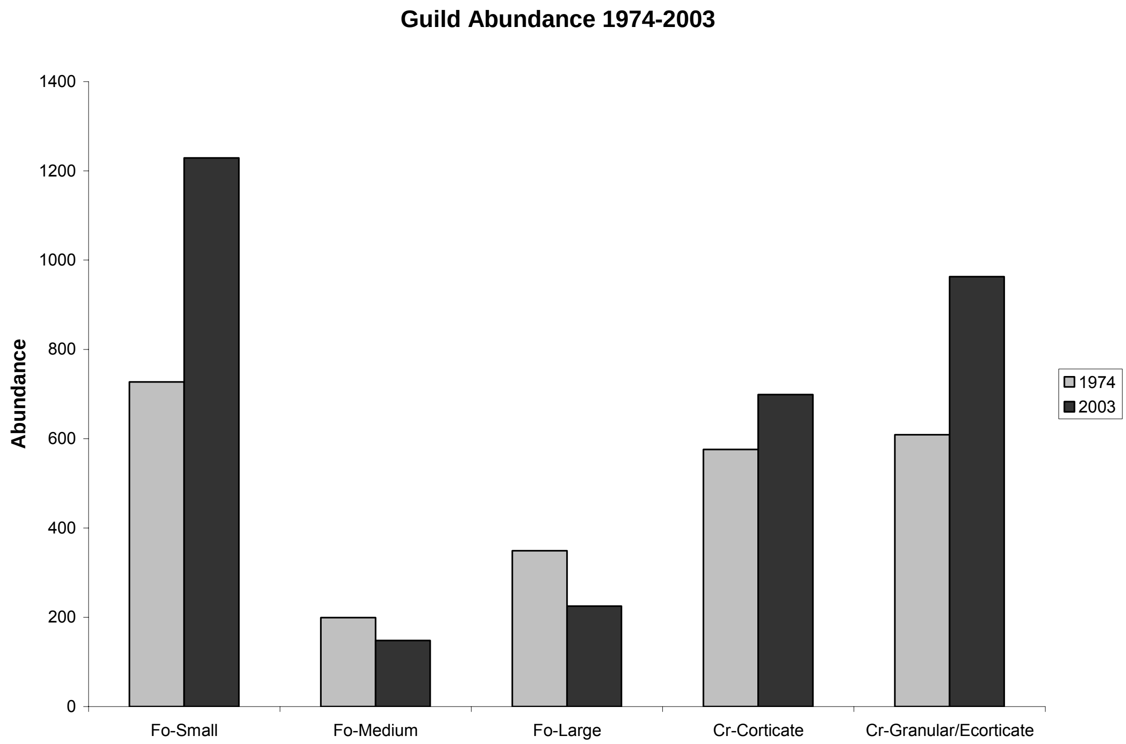

Figure 5-4. Abundance of each guild on plots in two years. For all but the Medium foliose guild, the change was significant.

Table 5-4. 1974-2003 Guild change based on paired t-tests across all sites, and within polluted and clean site groups (p-values listed). Pearson correlation coefficients and p-values are also listed for correlations with a sun variable.

Morpho-Guilds (ranked change in abundance)	Increase/decrease	Increase/decrease in polluted	Increase/decrease in clean	Sun: TIV+Shrubs
Fo-Small	increase, p=0.000	increase, p=0.000	increase, p=0.000	-0.528, p=0.008
Fo-Medium	decrease, p=0.017	decrease, p=0.048		0.471, p=0.020
Fo-Large	decrease, p=0.001		decrease, p=0.009	
Cr-Corticate	increase, p=0.007	increase, p=0.016		
Cr-Granular/ Ecorticate	increase, p=0.000	increase, p=0.000	increase, p=0.015	

For 2003, abundance of Morpho-guilds correlated with sun variables and geography but not Columbia impact. Small foliose lichens were marginally more abundant at shady sites, while large foliose, cladoniiform and granular/ecorticate crust guilds were more abundant at sunnier sites (Table 5-5). Medium foliose lichens were more abundant at more western sites. No other Morpho-guilds showed significant correlations.

Most of the Algal-guilds were found to be strongly correlated with sun variables for 2003 data (Table 5-5), while one guild showed correlations with geography and again none were correlated with Columbia impact. *Trentepohlia*-containing lichens are negatively correlated with sun variables, while *Trebouxia*- and *Asterochloris*-containing lichens tend to be positively correlated with sun variables (but note the *Trebouxia*-containing small foliose subguild prefers shade). This is consistent with earlier studies showing *Trentepohlia*-containing lichens usually occur more frequently in moist, shady habitats and many *Trebouxia*-containing lichens prefer higher light environments (Barkman 1958). The *Trebouxia*-containing lichen guild was positively correlated with Distance West; linked to the same pattern for its large + medium foliose subguild.

Table 5-5. Pearson correlations of 2003 lichen guild abundance with sun and geographic variables, based on ranked data (only significant correlations listed). Grouping by Morpho-guilds and Algal-guilds (Table 5-1) is independent for crust lichen guilds. For the foliose and cladoniiform guilds, the grouping is identical (same correlation coefficient) or very similar between the two schemes.

Guilds (ranked abundance)	Sun:Tree BA+Shrubs	Sun: TIV+Shrubs	Distance West
Morpho-guilds			
Fo-Small		-0.367, p=0.05	
Fo-Medium			0.613, p=0.000
Fo-Large	0.683, p=0.000	0.599, p=0.001	
Cladoniiform	0.411, p=0.027	0.510, p=0.005	
Cr-Corticate			
Cr-Granular/ Ecorticate	0.461, p=0.012		
Cr-Thallus Embedded/ Absent			
Algal-guilds			
Trentepohlia	-0.547, p=0.002	-0.492, p=0.007	
Trebouxia	0.510, p=0.005		0.603, p=0.001
Cr-*Trebouxia*	0.451, p=0.014	0.486, p=0.008	
Fo-Large+Medium *Trebouxia*	0.638, p=0.00	0.467, p=0.011	0.512, p=0.004
Fo-Small *Trebouxia*		-0.367, p=0.05	
Asterochloris		0.457, p=0.013	
Cladoniiform *Asterochloris*	0.411, p=0.027	0.510, p=0.005	
Leprose *Asterochloris*			

Patterns of correlation between lichen guilds and variables are mostly consistent with lichen community patterns. Forest structure, here represented by sun variables, and geography are significantly correlated with abundance of most of the lichen guilds. Data from 1974-2003 confirm that all guilds changed significantly. Data from 1974-2003 and 2003 both link abundance of small foliose lichens with shadier sites. 2003 data link abundance of medium foliose lichens with more western sites.

In contrast to lichen community analyses, there is distinct evidence here for an impact of Columbia; two guilds changed very differently at cleaner versus more polluted sites and a third guild showed moderate differences between the site groups.

Results and Discussion 3) Lichen species: There were enough data for 30 species to test for significance in pattern of abundance. There was much shifting; 15 species changed abundance significantly over time, while another six species had marginally significant changes between 1974 and 2003 (Table 5-6). About 2/3 of large foliose species decreased, and about the same proportion of small foliose species increased over the study period. Number of crustose species changed approximately equally in either direction.

Table 5-6. Increase or decrease of abundance at sites from 1974 to 2003 for common species. Probability values for paired t-test/Wilcoxon signed rank test are reported if p≤0.05; NS = not significant.

Species	Paired T test/ Wilcoxon Signed Ranks Test		Species	Paired T test/ Wilcoxon Signed Ranks Test	
Amandinea polyspora	Increase	p=0.022/ p=0.017	*Myelochroa aurulenta*	Increase	NS/ NS
Amandinea punctata	Decrease	NS/ NS	*Myelochroa galbina*	Decrease	p=0.021/ p=0.021
Arthonia caesia	Decrease	p=0.001/ p=0.002	*Parmelia sulcata*	Decrease	p=0.003/ p=0.005
Candelaria concolor	Increase	p=0.000/ p=0.000	*Pertusaria pustulata*	Decrease	NS/ NS
Candelariella efflorescens	Increase	p=0.003/ p=0.000	*Phaeophyscia pusilloides*	Increase	p=0.046/ p=0.042
Candelariella xanthostigma	Increase	NS/ NS	*Phaeophyscia rubropulchra*	Increase	p=0.000/ p=0.000
Flavoparmelia caperata	Decrease	p=0.005/ p=0.005	*Physcia millegrana*	Decrease	p=0.000/ p=0.001
Flavopunctelia soredica	Decrease	p=0.021/ p=0.012	*Physcia stellaris*	Decrease	p=0.000/ p=0.000
Graphis scripta	Increase	NS/ NS	*Punctelia bolliana*	Decrease	p=0.003/ p=0.002
Hyperphyscia adglutinata	Increase	p=0.000/ p=0.000	*Punctelia rudecta*	Increase	NS/ NS
Lecanora hybocarpa	Decrease	p=0.000/ p=0.000	*Pyrenula pseudobufonia*	Decrease	NS/ NS
Lecanora strobilina	Decrease	NS/ NS	*Rinodina* cf. *degeliana*	Increase	NS/ p=0.024
Lecanora thysanophora	Increase	p=0.000/ p=0.000	*Rinodina papillata*	Increase	p=0.044/ p=0.051
Lepraria lobificans	Increase	NS/ NS	*Scoliciosporum chlorococcum*	Increase	p=0.005/ p=0.002
Mycobilimbia epixanthoides	Increase	p=0.000/ p=0.000	Sterile Crust 1	Increase	p=0.000/ p=0.000

The sun-shade gradient, represented for analysis by several different shade and forest structure variables, is probably the most important factor correlated with change in abundance and 2003 abundance of individual lichen species (Table 5-7). The variable from this class with the strongest correlation differed among species. Three of the variables reported in Table 5-7, Sun: Tree BA + Shrubs, Sun: Tree IV + Shrubs, and Shady Tree BA, are targeted estimators of shade at sites, while Tree BA is a total forest structure variable often related to more shade at sites (see Chapter 4). Tree Species Richness is positively correlated with more shade (Ch 4) for the particular forests in this study. While some of the correlations were in the marginally significant range (p = 0.01 to 0.05), especially for change over time, the large number of species correlated with this class of variables underscores the importance of the sun versus shade gradient. Magnitude of change in abundance between 1974 and 2003 for increaser species *Candelaria concolor* (small foliose guild) and *Mycobilimbia epixanthoides* (corticate crust guild) is positively correlated with magnitude of increase in shade (Table 5-7a). The 2003 abundance of *C. concolor* was also correlated with increasing shade (Table 5-7b).

Table 5-7a and b. Correlations of change in species abundance (2003-1974) and 2003 abundance with important variables. For Sun-Shade gradient variables, the strongest correlation of four tests is reported: [1]Sun: Tree BA+shrubs, [2]Sun: TIV+shrubs, [3]Tree BA, [4]Shady Tree BA. Pearson product moment correlations were calculated for ranked data; Spearman rank correlations were calculated for unranked data.

5-7a

	Inc/Dec	**Correlation of change in abundance (2003-1974) with:**		
Species	Occurrences 1974-2003	Change in Sun-Shade Variables†	Ranked Distance West§	Change in Tree Species Richness†
Amandinea punctata	Decrease		-0.413, p=0.009	
Candelaria concolor	Increase‡	0.502, p=0.013[1]		
Flavoparmelia caperata	Decrease‡	0.433, p=0.035[1]		
Hyperphyscia adglutinata	Increase‡	0.416, p=0.043[1]	-0.447, p=0.029	
Mycobilimbia epixanthoides	Increase‡			0.432, p=0.035
Myelochroa aurulenta	Increase			-0.507, p=0.012
Parmelia sulcata	Decrease‡	0.413, p=0.045[1]		
Pertusaria pustulata	Decrease	-0.419, p=0.042[4]		
Physcia stellaris	Decrease‡			0.469, p=0.021
Punctelia bolliana	Decrease‡			0.419, p=0.041
Punctelia rudecta	Increase			
Pyrenula pseudobufonia	Decrease			-0.499, p=0.013
Sterile crust 1	Increase‡		0.577, p=0.003	

‡ Significant increase/decrease, see table 5-6.
† Correlation indicates greater change in variable correlated with greater change in abundance.
§ Correlation indicates increase in west.

5-7b

	Inc/Dec	**Correlation of 2003 ranked abundance with:**		
Species	Occurrences 1974-2003	Ranked Sun-Shade Variables†	Ranked Distance West§	Ranked Tree Species Richness†
Candelaria concolor	Increase‡	-0.432, p=0.035[3]		-0.640, p=0.001
Candelariella efflorescens	Increase‡	0.471, p=0.020[1]	0.576, p=0.003	
Candelariella xanthostigma	Increase			0.501, p=0.013
Flavoparmelia caperata	Decrease‡	0.687, p=0.000[1]		0.693, p=0.000
Graphis scripta	Increase	-0. 714, p=0.000[1]		
Hyperphyscia adglutinata	Increase‡	-0.720, p=0.000[2]	-0.447, p=0.029	-0.543, p=0.006
Lecanora hybocarpa	Decrease‡	0.463, p=0.023[2]		0.491, p=0.015
Lecanora strobilina	Decrease	0.417, p=0.043[2]		0.433, p=0.035
Lecanora thysanophora	Increase‡		0.508, p=0.011	
Mycobilimbia epixanthoides	Increase‡		0.530, p=0.008	-0.553, p=0.005
Myelochroa aurulenta	Increase	0.584, p=0.003[3]	0.637, p=0.001	
Phaeophyscia pusilloides	Increase‡		0.501, p=0.013	
Phaeophyscia rubropulchra	Increase‡		0.454, p=0.026	
Physcia millegrana	Decrease‡			0.422, p=0.040
Punctelia rudecta	Increase	0.687, p=0.000[2]		0.738, p=0.000
Pyrenula pseudobufonia	Decrease		0.438, p=0.032	
Rinodina papillata	Increase‡		-0.408, p=0.048	
Scoliciosporum chlorococcum	Increase‡	0.492, p=0.015[1]		
Sterile Crust 1	Increase‡	0.552, p=0.005[3]	0.577, p=0.003	

‡ Significant increase/decrease, see table 5-6.
† Correlation indicates increase in sunnier conditions.
§ Correlation indicates increase in west.

Magnitude of change in abundance for two of the large foliose species, *Flavoparmelia caperata* and *Parmelia sulcata,* was negatively correlated with magnitude of increase in shade (Table 5-7a); these two species decreased in abundance as shade increased. The 2003 abundance of *F. caperata* is correlated with sunnier conditions (Table 5-7b). 2003 abundance of *F. caperata* was also correlated with Tree Species Richness (another sun-shade variable; Table 5-7b), as was the change in abundance of another large foliose species, *Punctelia bolliana* (Table 5-7a). Both species decreased over time (Table 5-1). The findings for these large foliose species are in concordance with analyses of the lichen community and the large foliose guild in showing large foliose species mostly prefer sun and mostly declined over the study period.

However, individual species do not always follow the pattern of their Morpho-guild. Another large foliose species, *Punctelia rudecta,* did not change much in abundance over time (weak increase), although it did show preference for sunnier sites (Table 5-7a&b). While the small foliose guild increased over time and was positively correlated with increasing shade overall, there are individual species such as *Physcia stellaris* that decreased over time and preferred sunnier sites (Table 5-7a&b).

Another variable with numerous strong correlations to 2003 abundance or change in abundance is geographic location, represented by variable Distance West. Three species that changed over time changed more at more western sites (Table 5-7a). Many species (from various Morpho-guilds) that changed significantly over time had 2003 abundance correlated with geography; most increased over time and were more abundant at western sites (Table 5-7b).

1974-2003 change in *Punctelia rudecta* is negatively correlated with Model SO_2-2003 (Pearson correlation -0.418, p=0.042). This is the only example for an individual species of correlation (marginally significant) between Columbia impact and change over time or 2003 abundance.

Results and Discussion 4) Impact of Columbia: From all analyses it is clear that impact of Columbia is a less important correlate of lichen community, guild, and species patterns across sites and time than are the sun versus shade gradient and the geographic gradient. Partial correlations of several common lichen species and guilds with Columbia impact were significant when a shade variable and a geographic variable (Table 5-8) were accounted for, indicating that Columbia impact is being masked by the stronger patterns. For these analyses, we chose a single variable, Tree CI, to represent a sun versus shade gradient and Distance West to represent a geographic gradient.

Table 5-8. Partial correlations of lichen variables with Columbia impact variables controlling for Tree CI and Distance West (all data ranked). For 1974 and 2003, original data, rather than change estimates (as in Table 5-7) are used. Species with values in **bold** have a negative correlation with Columbia impact.

Species	Model $S0_2$-2003 partial correlation	Model$S0_2$-74&03	Model$S0_2$-74&03 partial correlation
Arthonia caesia		**-0.302 p=0.037**	**-0.352 p=0.015**
Flavoparmelia caperata		**-0.308 p=0.033**	
F. caperata + Myelochroa galbina		**-0.334 p=0.020**	**-0.304 p=0.040**
Phaeophyscia pusilloides	0.491 p=0.009		0.305 p=0.039
Phaeophyscia rubropulchra		0.769 p=0.000	0.818 p=0.000
Small foliose guild		0.689 p=0.000	0.747 p=0.000

Correlations are stronger, and partial correlation is always stronger than simple correlation, for the small foliose species (Table 5-8, third group) and guilds that have higher abundance where Columbia impact is greater. In contrast, correlations are relatively weak, and partial correlations are often no better, for the species that have lower abundance where Columbia impact is greater (Table 5-8, first and second groups). This pattern suggests the primary impact of Columbia on lichens may be to minimally reduce establishment success of many species, especially those less tolerant of pollution. Consequently, relative abundances for the few weedy and very pollution tolerant species, whose establishment success remains relatively good, become significantly higher over time compared to all other species.

Conclusions

All lichen analyses, of communities, guilds, and species, support the conclusion that the major variables affecting patterns of lichen abundance and change in abundance on black oak group trunks are a sun versus shade gradient related to forest composition, and an east-west geographic gradient. Variation in lichen communities between sunnier and drier versus shadier and more moist sites has been maintained over time, paralleling a major gradient in tree species composition. An east-west geographic gradient was important for lichen tissue Sulfur and for tree species composition as well as for lichen species richness (higher to the west) and community composition. It is at least partially correlated with forest fragmentation (forests less fragmented to the west), and with two distinctive pathways for tree species change. Whether all these east-west patterns truly have the same underlying cause, or some appear correlated just by chance is not certain.

There has been significant change from 1974 to 2003 in lichen communities at resampled sites. In general lichen communities have become significantly more similar to one another, with concomitant loss of distinctive communities at different sites. Sun-loving lichens have mostly become less abundant and shade-loving lichens have increased over time. These changes are correlated with estimates of increased shade on trunks derived from variables related to forest composition and structure. *Candelaria concolor* is an example of a lichen species that increased significantly over time whose abundance was correlated with shadier conditions. This individual species pattern was discernible as well in analysis of communities (ordination) and analysis of the small foliose guild including this species; thus was consistent at all scales of analysis.

Impact of Columbia has had a minor demonstrable effect on lichens in the study area, as evidenced by correlations of lichen variables with Columbia impact variables (present study; Will-Wolf 1980a,b). Reanalysis of the original 1974-1978 study with improved estimation of Columbia impact shows impact then was probably less than reported in Will-Wolf (1980a). Effects do not seem to have increased over time. However, for 1974-1978, for 1974-2003, and for 2003 some significant impacts of Columbia on lichens are demonstrated. No losses or major reductions in more pollution sensitive species appear to have occurred in the subset of lichen communities we studied. Rather increases in relative abundance of a few weedy and pollution-tolerant species have occurred. Most of the lichen species encountered in this study with established pollution tolerances are tolerant to intermediate; no known sensitive species were encountered. Potentially sensitive species are likely to have been reduced in abundance in the southern Wisconsin study region well before 1974 (Will-Wolf & Nelsen in press). The Columbia facility was expected to generate relatively small amounts of ground-level SO_2, and with no pollution-sensitive lichen species found even before operations started, it is not surprising to find only a minor detectable impact of Columbia on the remaining lichen communities.

Homogenization of lichen communities has probably occurred through interaction of several factors. Pollution from Columbia appears to have favored the increase of a few widespread, weedy, and pollution-tolerant lichen species. Increase in shade at most study sites resulted in lower abundance on trunks of sun-loving species, and the same increase in shade probably reduced the variety of sampled microhabitats available within a site. Forest fragmentation has probably favored immigration to the shadier trunk habitats of more widespread shade-loving species with better dispersal capacities.

Future Goals

Investigation of patterns of association of lichen species in individual trunk plots (not yet started) will allow us to estimate within-site variation of lichen communities and how that has changed over time. This will contribute to general understanding of patterns of local distributions of lichen species and guilds, and may shed light on the way in which homogenization of lichen communities between site has occurred. Differentiation of lichen communities based on which of the black oak group species is the substrate has not appeared to be as strong as differentiation based on characteristics of the entire forest environment, from current analyses. This hypothesis can be tested when data are segregated by individual substrate tree.

References

ArcGIS Desktop 9.0. 2004. ESRI, Redlands, CA, US.

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

Barkman, J.J. 1958. *Phytosociology and ecology of cryptogamic epiphytes*. Van Gorcum, Assen, NL.

Brodo, I.M. 1973. Substrate ecology. In: Ahmadjian, V. & Hale, M.E. (eds.) *The Lichens*, pp. 401-442. Academic Press, Inc., New York, NY, US.

Curtis, J.T. 1959. *The Vegetation of Wisconsin*. University of Wisconsin Press, Madison, WI, US.

Esslinger, T.L. 1997. Website: <http://www.ndsu.nodak.edu/instruct/esslinge/chcklst/chcklst7.htm>. A cumulative checklist for the lichen-forming, lichenicolous and allied fungi of the continental United States and Canada. Update 14 June 2005, North Dakota State University, Fargo, ND, US.

Ferretti, M. & Erhardt, W. 2002. Key issues in designing biomonitoring programmes. Nimis, P.L., Scheidegger, C. & Wolseley, P. (eds.) *Monitoring with Lichens – Monitoring Lichen*s, pp.111-142. NATO Science Series. Kluwer Academic Publishers, The Hague, NL.

Ferry, B.W., Baddeley, M.S. & Hawksworth, D.L. 1973. *Air pollution and lichens*. University of Toronto Press, Toronto, Ontario, CA.

Flora of North America Editorial Committee, eds. 1997. *Flora of North America North of Mexico*. Vol. 3. New York, NY, US.

Hale, M.E. 1955. Phytosociology of corticolous cryptogams in the upland forests of southern Wisconsin. *Ecology* 36:45-63.

Heller-Zeisler, S.F., Zeisler, R., Zeiller, E., Parr, R.M., Radecki, Z., Burns, K.I. & De Regge, P. 1999. Report on the intercomparison run for determination of trace and minor elements in lichen material IAEA-336. International Atomic Energy Agency, P.O. Box 100, A-1400 Vienna, AT. <http://www.iaea.org/programmes/aqcs/pdf/al_079.pdf>

Jackson, L.L., Ford, J. & Schwarztman, D. 1993. Collection and chemical analysis of lichens for biomonitoring. In: Stolte, K., Mangis, D., Doty, R. & Tonnessen, K. (eds.) *Lichens as Bioindicators of Air Quality*, pp. 96-115. USDA-Forest Service, Rocky Mountain Forest and Range Experiment Station General Technical Report RM-224, Fort Collins, CO, US.

Legendre, P. & L. Legendre. 1998. *Numerical Ecology.* 2nd English edition. Developments in

Environmental Modelling, 20. Elsevier Science, B.V., New York, NY, US.

Makholm, M.M. 2003. *Assessing air pollution impacts: biomonitoring with lichens and mosses*. Ph.D. Thesis, University of Wisconsin-Madison, Madison, WI, US.

Makholm, M.M. & Mladenoff, D.J. 2005. Efficacy of a biomonitoring (moss bag) technique for determining element deposition trends on a mid-range (375 km) scale. *Environmental Monitoring and Assessment* 104:1-18.

Martin, M.H. & Coughtrey, P.J. 1982. *Biological Monitoring of Heavy Metal Pollution*. Applied Science Publishers, London, UK.

McCune, B., Dey, J.P., Peck, J.E., Heiman, K. & S. Will-Wolf. 1997. Regional gradients in lichen communities of the Southeast United States. *The Bryologist* 100: 40-46.

McCune, B. & Grace, J.B. 2002. *Analysis of Ecological Communities*. MJM Software, Gleneden Beach, OR, US.

McCune, B. & Mefford, M.J. 1999. *Multivariate analysis on the PC-ORD system.* Version 4.3. MjM Software, Gleneden Beach, OR, US.

Minchin, P.R.1987.An evaluation of the relative robustness of techniques for ecological ordination. *Vegetatio* 69:89-107.

National Atmospheric Deposition Program (NRSP-3). 2005. Website: <http://nadp.sws.uiuc.edu/>. SO_4 concentration maps. Isopleths. Champaign, IL,US.

Nash III, T.H. (ed.). 1996. *Lichen biology*. Cambridge University Press, Cambridge, UK.

Nash III, T.H. & Gries, C. Lichens as bioindicators of sulfur dioxide. *Symbiosis* 33:1-21.

Nash III, T.H. & Wirth, V. (eds.). 1988. Lichens, bryophytes and air quality. *Bibliotheca Lichenologica* 30:231-267.

Nimis, P.L. & Purvis, O.W. 2002. Monitoring lichens as indicators of pollution. In: Nimis, P.L., Scheidegger, C. & Wolseley, P. (eds.) *Monitoring with Lichens – Monitoring Lichen*s, pp.7-10. NATO Science Series. Kluwer Academic Publishers, The Hague, NL.

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

Peet, R.K. & Loucks, O.L. 1977. A gradient analysis of southern Wisconsin forests. *Ecology* 58:485-499.

Puckett, K.J. 1988. Bryophytes and lichens as monitors of metal deposition. In: Nash III, T.H. & Wirth, V. (eds.) *Lichens, Bryophytes and Air Quality*. *Bibliotheca Lichenologica* 30:231-267.

Ragland, K.W., Goodell, B.D. & Coughlin, T.L. 1980. Air pollution studies near a coal-fired powerplant. Wisconsin Power Plant Impact Study. EPA-600/3-80-048. US EPA, Environmental Research Laboratory, Duluth, MN, US.

Rodrigo, A., Avila, A. & Gomez-Bolea, A.1999. Trace metal contents in *Parmelia caperata* (L.) Ach. compared to bulk deposition, throughfall and leaf-wash fluxes in two holm oak forests in Montsenty (NE Spain). *Atmospheric Environment* 33: 359-367.

Smith, C., Geiser, L., Gough, L., McCune, B., Ryan, B. & Showman, R. 1993. Species and communities. In: Stolte, K., Mangis, D., Doty, R., Tonnessen, K. & Huckaby, L.S. (eds.) *Lichens as Bioindicators of Air Quality* pp. 41-66. USDA-Forest Service, Rocky Mountain Forest and Range Experiment Station General Technical Report RM-224, Fort Collins, CO, US.

Sokal, R.R. & Rohlf, F.J. 1995. *Biometry*. 3rd ed. W.H. Freeman & Co., New York, NY, US.

SPSS 12.0 for Windows. 2003. Release 12.0.0. SPSS, Inc., Chicago, IL, US.

Spatial Climate Analysis Service. Web site: <http://www.ocs.oregonstate.edu/prism/>. Created 4 Feb 2004. 1971-2000 Normals data downloaded Winter 2004-2005. Oregon State University, Corvallis, OR, US.

TopoZone. Website: <http://www.topozone.com/>. Maps a la carte, Inc., North Chelmsford, MA, US. Data extracted Summer 2005.

Turner, D.B. 1994. *Workbook of Atmospheric Dispersion Estimates*. 2nd ed. Lewis Publishers, Ann Arbor, MI, US.

Van Haluwyn, C. & Van Herk, C.M. 2002. Bioindication: the community approach. In: Nimis, P.L., Scheidegger, C. & Wolseley, P. (eds.) *Monitoring with Lichens – Monitoring Lichen*s, pp. 39-64. NATO Science Series. Kluwer Academic Publishers, The Hague, NL.

Will-Wolf, S. 1977. Lichens for bioassay of SO_2 emissions. *Institute for Environmental Studies. Report 75*. University of Wisconsin – Madison, Madison, WI, US.

Will-Wolf, S. 1980a. Structure of corticolous lichen communities before and after exposure to emissions from a "clean" coal-fired generating station. *The Bryologist* 83:281-295.

Will-Wolf, S. 1980b. Effects of a "clean" coal-fired generating station on four common Wisconsin lichen species. *The Bryologist* 83:296-300.

Will-Wolf, S. 1988. Quantitative approaches to air quality studies. In: Nash III, T.H. & Wirth, V. (eds.) *Lichens, Bryophytes and Air Quality*. *Bibliotheca Lichenologica* 30:109-140.

Will-Wolf, S. & Nelsen, M.P. In press. Wisconsin's lichen communities – how have they changed? In: Waller, D.M. & Rooney, T.P. (eds.) *The Vanishing Present: Ecological Change in Wisconsin.* Expected publisher University of Chicago Press, Chicago, IL, US.

Will-Wolf, S. & Scheidegger, C. 2002. Monitoring lichen diversity and ecosystem function. In: Nimis, P.L., Scheidegger, C. & Wolseley, P. (eds.) *Monitoring with Lichens – Monitoring Lichen*s, pp.143-146. NATO Science Series. Kluwer Academic Publishers, The Hague, NL.

Will-Wolf, S., Scheidegger, C. & McCune, B. 2002. Methods for monitoring biodiversity and ecosystem function. In: Nimis, P.L., Scheidegger, C. & Wolseley, P. (eds.) *Monitoring with Lichens – Monitoring Lichen*s, pp.147-162. NATO Science Series. Kluwer Academic Publishers, The Hague, NL.

EXECUTIVE SUMMARY

Date of Report: OCTOBER 2005

Title of Project:

LICHEN BIOACCUMULATION AND BIOINDICATOR STUDY NEAR ALLIANT ENERGY – WPL COLUMBIA ENERGY CENTER

Principal Investigator Susan Will-Wolf, Dept of Botany, University of Wisconsin-Madison

Project Period: October 1, 2002 – June 30, 2005

Object of Research:

Lichens (algae and fungi live together to form these ‘organisms) are known worldwide as excellent indicators of air pollution effects; tolerant lichens accumulate pollutants in their tissues and sensitive lichens decline with pollution, thus changing lichen community composition. Forest lichen community composition is also a useful indicator of general ecosystem conditions, varying with both general environment and with tree species composition of the forest. Will-Wolf surveyed forest lichen communities in 1974 and 1978, primarily to assess the impact of the new (1975)Alliant Columbia coal-fired electric power generating facility near Portage, Wisconsin. We repeated the surveys in 2003 to assess the long-term impact of the on these lichen communities.

Our new project combines 1) mathematical modeling of modern and historical concentrations of SO_2 from the Alliant Columbia Facility pollution point source, 2) measurement of lichen community composition (species presence and abundance) at 29 sites around the point source, and 3) measurement of mercury, sulfur, and heavy metals concentrations in tissue of selected lichen species at most of those same sites. 24 of the 29 lichen community survey sites are the same as those surveyed in 1974 and 1978, while 5 are close. This provides the opportunity to (1) assess long-term impact of pollution from Columbia on nearby lichen communities, (2) assess long-term changes at "background" sites farther from the facility, and (3) evaluate biological responses in light of relative pollution levels indicated by modeling and lichen tissue element concentrations. Results of this study are applicable to other areas in Wisconsin.

Summary of Results/Accomplishments:

- A modern dispersion model successfully estimated ground –level concentrations of SO_2 from the Alliant Energy – WPL Columbia Energy Center coal-fired generators. These concentrations provide an excellent estimate of relative impact of Columbia emissions in the south-central Wisconsin region of the study, but not of absolute impact because calibration against measured SO_2 at monitoring stations was not completed.
- Tissue samples from two common lichen species were found to provide reliable data on concentration of Sulfur, Mercury, and other elements. Mercury data showed similar low concentrations across the study area. Data for Sulfur and heavy metals clearly indicated that Columbia is a source of enrichment for these elements in lichen tissue. Analysis showed there is an additional pattern of higher Sulfur in the western part of the study area apparently unrelated to Columbia or to other known pollution sources.
- Oaks decreased from 1974 to 2003 and other tree species increased at the oak forest

lichen community study sites. Forests became shadier at most sites. Important variation from drier oak forests to more moist oak forests has been maintained over time. Forests at two groups of sites changed in different ways: A group of more isolated woodlots at more eastern sites had increases of disturbance-adapted tree species. A group of more western sites in more continuously forested landscapes had changes consistent with natural succession patterns. Differences between these two groups of sites became more important over time.

- Lichen communities on black oak group trunks at the study sites have shown much change over time, with the most important changes related to forest changes rather than to pollution. Sun-loving lichens mostly decreased and shade-loving lichens mostly increased from 1974 to 2003. Lichen communities differ at sunnier, drier sites vs shadier, more moist sites in both 1974 and 2003, in parallel to variation of tree communities. The strong distinction of two site groups based on tree species did not appear to be as important for lichen communities, but lichen communities at more east and southeast sites have become more different over time from those at more west and northwest sites.
- An east-west gradient was important for lichen tissue Sulfur concentrations, for tree species composition, and for lichen species composition. It is at least partially correlated with forest fragmentation, and for lichen and tree communities the pattern has strengthened over time, but the causes for this gradient remain obscure.
- Impact of pollution from Columbia on lichen communities has been relatively minor over the time of its operation, and effects do not seem to have increased over time. Reanalysis of the original 1974-1978 study with improved estimation of Columbia impact from modeling shows impact then was probably less than reported in Will-Wolf (1980a). However, for 1974-1978, for 1974-2003, and for 2003, some significant impacts of Columbia on lichens are demonstrated. We cannot relate this minor impact on lichen communities to absolute pollution concentrations at this time.

Future Directions/Activities:

- The Columbia dispersion model pollution estimates should be calibrated with other dispersion models and with existing measured SO_2 data from monitoring stations to establish absolute concentrations of SO_2 from Columbia in the study area. This would allow calibration between concentrations of elements from Columbia, lichen tissue element concentrations, and lichen species and community responses. Such calibration is very useful for establishing thresholds for effects; it also facilitates better comparison of this study with others and facilitates extension of impact assessment to other areas.
- Our findings that forest communities are changing in different ways possibly linked to land use, and that lichen communities appear to be affected more by forest change than by current pollution, should be investigated further. Causes for the east-west gradient in lichen tissue Sulfur concentration and its possible links to land use and forest and lichen community composition also need to be investigated. The potential linkage of these patterns to each other and to human land use has important implications for forest change and ecosystem integrity throughout southern Wisconsin.

Investigators and Institutions:
Susan Will-Wolf, Ph.D., Associate Scientist, UW-Madison, Department of Botany
Martha M. Makholm, Ph.D., Air Management Compliance Data Specialist, WI-DNR, Bureau of Air Management
John A. Roth, Stationary Source Modeling Team Leader, WI-DNR, Bureau of Air Management
Matthew P. Nelsen, **Anne H. Reis***, and **Marie T. Trest**, Associate Research Specialists, UW-Madison, Department of Botany, ***current address WI-DNR