Metacommunity organization of soil microorganisms depends on habitat defined by presence of *Lobelia siphilitica* plants

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**Abstract.** We tested regional-scale spatial patterns in soil microbial community composition for agreement with species sorting and dispersal limitation, two alternative mechanisms behind different models of metacommunity organization. Furthermore, we tested whether regional metacommunity organization depends on local habitat type. We sampled from sites across Ohio and West Virginia hosting populations of *Lobelia siphilitica*, and compared the metacommunity organization of soil microbial communities under *L. siphilitica* to those in adjacent areas at each site. In the absence of *L. siphilitica*, bacterial community composition across the region was consistent with species sorting. However, under *L. siphilitica*, bacterial community composition was consistent with dispersal limitation. Fungal community composition remained largely unexplained, although fungal communities under *L. siphilitica* were both significantly different in composition and less variable in composition than in adjacent areas. Our results show that communities in different local habitat types (e.g., in the presence or absence of a particular plant) may be structured on a regional scale by different processes, despite being separated by only centimeters at the local scale.

**Key words:** habitat type; *Lobelia siphilitica*; metacommunity; microbial biogeography; plant–microbe interactions; soil microorganisms; terminal restriction fragment length polymorphism.

**INTRODUCTION**

Explaining patterns of biodiversity and community composition is a central theme in ecology that applies to all taxonomic groups. Metacommunity theory recognizes that individual communities are linked into networks (i.e., metacommunities) by dispersal, and that communities are subject to both local and regional effects (Hubbell 2001, Leibold et al. 2004). Four models of metacommunity organization have been described (species sorting, patch dynamics, mass effects, and neutral dynamics), which differ in the types of trade-offs, levels of dispersal limitation, and variation in responsiveness to environmental conditions among species that are hypothesized to explain community distributions (Leibold et al. 2004, Chase et al. 2005). Empirical studies frequently focus on the two primary mechanisms generating spatial patterns in community composition that vary among these models (Gilbert and Lechowicz 2004, Cottenie 2005, Lekberg et al. 2007, Van der Gucht et al. 2007): (1) species sorting, where community composition is regulated by niche partitioning and environmental conditions in a habitat patch, with species abundance reflecting environmental heterogeneity, and (2) dispersal limitation, where community composition is regulated by spatial proximity to other populations, with little niche differentiation between species (Chase et al. 2005). Under neutral dynamics, dispersal limitation is the only factor generating spatial structure on ecological timescales. Mass effects and patch dynamics can be seen as intermediate processes, where population dynamics respond to environmental heterogeneity, but local extinction rates are not rapid enough to mask the effects of dispersal from source populations of a given species. Assessing metacommunity organization by focusing on species sorting and dispersal limitation is a useful approach because these hypotheses can be evaluated by testing for nonrandom spatial structure in community composition, and correlation with environmental factors (Gilbert and Lechowicz 2004, Cottenie 2005, Martiny et al. 2006).

Recent work has challenged the assumption that microbial communities do not display spatial (i.e., biogeographical) structure and, by extension, that microbes are not limited by dispersal (Martiny et al. 2006). However, the initial studies applying metacommunity theory to microbial communities have found contrasting results. Bacteria in soil, streams, and eutrophic lakes have shown evidence of species sorting at continental and regional scales (Fierer and Jackson 2006, Fierer et al. 2007, Van der Gucht et al. 2007), whereas evidence for dispersal limitation has been seen in arbuscular mycorrhizal fungal community composition (Lekberg et al. 2007), bacteria in oligotrophic lakes (Reche et al. 2005), and ascomycetes in soil (Green et al. 2004). These studies focused exclusively on regional or larger-scale phenomena, with the implicit assumption that any difference in metacommunity organization...
between local habitat types would be overwhelmed by regional patterns. However, different plant species growing in the same soil type often develop distinct microbial communities in the rhizosphere (Kowalchuk et al. 2002, Kuske et al. 2002, Garbeva et al. 2008). It is unknown whether the microbes affiliated with a particular plant species have unique metacommunity dynamics, or if they are dominated by common metacommunity processes affecting all surrounding soil habitats (i.e., bare soil and soil under other plant species at the same site).

The metacommunity perspective has the potential to make important contributions to our understanding of ecological mechanisms driving distributions of soil microbes. Controlled greenhouse experiments have indicated that both plant species and “soil type” can affect soil microbial community composition (Garbeva et al. 2004, Berg and Smalla 2009). In this context, “soil type” has been used as a general term to denote soils with differing chemical or physical characteristics, and collected at differing geographic locations. It is often assumed that physicochemical soil characteristics cause communities to diverge in different soil types, and some studies have aimed to determine which specific soil characteristics contribute to the “soil type” effect on microbial assemblages (Johnson et al. 2003, Ulrich and Becker 2006, Wakelin et al. 2008). However, geographic proximity, and the possibility of microbial dispersal limitation between sites, has not been considered. Given the current debate over geographic structure in microbial communities, this factor should be assessed as a potential mechanism behind the “soil type” effect on microbial community composition.

Our goal was to test two hypotheses: (1) that microbial metacommunity organization on a regional scale is similar for different habitat types within local sites and (2) that soil microbes exhibit biogeographical scale is similar for different habitat types within local microbial metacommunity organization on a regional microbial community composition. When sampling habitat types defined by presence of a particular plant species present at each of our sampling sites, *Lobelia siphilitica*.

**METHODS**

Field collection.—Fourteen sites across Ohio and West Virginia were chosen as a regional sample for this study (Appendix: Table A1). Separation between sites ranged continuously from 2 to 508 km. Each site hosted a natural population of great blue lobelia, *Lobelia siphilitica* (Lobeliaceae), an herbaceous perennial plant native to eastern North America (Johnston 1991). This species is often found in wet soils near drainage ditches and stream banks, and occurs on a wide variety of soil types (Appendix: Table A2). Seeds germinate in late May–June, peak flowering is typically late July–September, and mature fruits can be seen in October–November.

In October 2006, we collected soil samples from each site and grouped them into two microbial “habitat types.” We collected 10 cm deep soil cores directly beneath 10 randomly selected *L. siphilitica* plants (hereafter IS habitat). We also collected ten soil cores in “interspaces,” at least 8 cm from *L. siphilitica* (hereafter IS habitat). Cores from each site were bulked by habitat type, and transported to the laboratory on ice on the day of collection. Soil was thoroughly mixed and sieved (2 mm) to remove large pieces of plant material. Soil samples were divided into three subsamples which were (1) frozen (−80°C) for molecular analysis, (2) air dried for physical and chemical characterization, and (3) oven dried overnight at 60°C to determine percent moisture.

Chemical and physical soil analyses.—Soil pH was measured in a 1:1 mixture with water. Soil texture (percent clay, silt, sand, and gravel) was determined using the soil hydrometer method (American Society for Testing and Materials Standards 2002), after sieving to remove gravel and coarse plant material (>2 mm). Cation exchange capacity (CEC) was measured by the unbuffered salt extraction method (Sumner and Miller 1996). Extractable phosphorus, potassium, calcium, magnesium, sulfur, aluminum, boron, copper, iron, manganese, molybdenum, sodium, and zinc were determined by Mehlich 3 extraction and inductively coupled plasma (ICP) analysis at the Soil Testing and Research Lab, Ohio Agricultural Research Development Center, Wooster, Ohio, USA. Percentage nitrogen and carbon of each sample was measured on a CHN Analyzer (ECS 4010 Nitrogen/Protein Analyzer; Costech Analytical Technologies, Inc., Valencia, California, USA) by K. Smemo at Holden Arboretum (Kirtland, Ohio, USA). Hot-water soluble organic carbon was measured as described by Curtin et al. (2006) on a total organic carbon analyzer (Shimadzu Corporation, Kyoto, Japan). Anaerobic mineralizable nitrogen was determined after 7-d incubation according to Keeney and Bremner (1966).
Microbial community analysis.—To perform T-RFLP, we extracted total DNA from duplicate subsamples of all IS and LP samples. DNA was isolated using the PowerMax Soil DNA isolation kit (Mo Bio Laboratories; Solana Beach, California, USA). Polymerase chain reaction was performed in 50-μL reaction volumes using bacterial 16S ribosomal primers Eub338F-0-III and 1392R (Daims et al. 1999, Blackwood et al. 2005), and general fungal ITS primers NSI1F and NLB4R (Martin and Rygiewicz 2005). Forward primers were labeled with Fam (6-carboxyfluorescein). Thermal cycling for bacterial primers consisted of an initial denaturing step of 95°C for 3 minutes, 30 cycles of 30 s at 94°C, 30 s at 57°C, and 1.5 minutes at 72°C, and a final extension step of 72°C for 7 minutes. The program for fungal primers was similar except that annealing was performed at 60°C with 35 cycles. Bacterial and fungal PCR’s included polymerase buffer (1X), dNTPs (1.6 mmol/L), MgCl2 (3 mmol/L), BSA (0.1 μg/μL), forward and reverse primers (0.1 μmol/L each), and DNA polymerase (0.025 units/μL; Gene Choice, Frederick, Maryland, USA). Duplicate PCR’s were performed for each DNA extraction, resulting in four analytical replicate PCRs for each sample (2 PCR duplicates × 2 DNA extraction duplicates), which were then pooled. PCR products were digested with restriction enzyme HaeIII (New England BioLabs, Ipswitch, Massachusetts, USA). Restriction fragments were purified using the QIAquick nucleotide removal kit (Qiagen, Hilden, Germany). Fragment analysis for T-RFLP was performed at the Plant-Microbe Genomics Facility (The Ohio State University, Columbus, Ohio, USA). Peaks which were between 50 and 600 bp in size and greater than 50 fluorescence units in height were aligned to facilitate quantitative comparison of community composition profiles.

Statistical analysis.—All analyses were performed separately for bacterial and fungal community profiles. T-RFLP relative fluorescence (based on peak heights) was square-root transformed prior to analysis in order to compare communities using Hellinger distance (Legendre and Gallagher 2001, Blackwood et al. 2003). First, we tested the hypothesis that microbial community composition differed between LP and IS soil by redundancy analysis (RDA; Legendre and Legendre 1998). Adjusted $R^2$ ($R^2_{adj}$; Peres-Neto et al. 2006), correcting for the number of predictor variables relative to number of samples, is presented for all RDA analyses. We also tested the hypothesis that multivariate dispersion differed between LP and IS metacommunities (i.e., that variation among communities was greater in IS compared to LP soils). Dispersion of LP and IS communities was calculated by finding the mean pairwise Hellinger distance for each group. The difference between mean pairwise Hellinger distances was then calculated, and this was compared to a distribution generated from 999 random permutations of sample identity before recalculation of Hellinger distances for each permutation.

We then performed the two most commonly used, complementary methods to partition variance in community composition into a purely spatial component, a purely environmental component, and a spatially structured environmental “overlap” component in which the two effects cannot be separated. RDA is a method of constrained ordination based on raw data, where a model is constructed that predicts transformed taxa abundances based on linear combinations of environmental variables or spatial coordinates (Legendre et al. 2005). Mantel analysis is instead a distance-based method, in which metrics of distance are calculated from community composition, geographic position, and environmental characteristics, and then a model is constructed that predicts community composition distance from spatial or environmental distance.

The relative merits of these statistical tests are currently under debate, but Mantel analysis against spatial distances may be a more appropriate test for dispersal limitation than RDA on geographic variables (Tuomisto and Ruokolainen 2006, 2008), whereas RDA may be more appropriate for variance partitioning and analysis of large sets of environmental variables (Legendre et al. 2005, 2008). Prior to analysis, GPS coordinates were converted to the UTM coordinate system in meters. Soil characteristics are shown in Appendix: Tables A2 and A3. Environmental variables were standardized (mean = 0, variance = 1) prior to analyses. Many soil characteristics were correlated (Appendix: Table A4), so we chose variables for analysis as explanatory variables that (1) would be representative of the entire data set, (2) have been shown to be important controls on microbial community composition in previous studies, and (3) would not saturate a predictive model using the environmental variables as explanatory factors (Blanchet et al. 2008). The nine representative variables chosen include percent C, percent N, C:N ratio, pH, percent clay, percent gravel, and three principal components (PCs) derived from the twelve elements measured by Mehlich 3 extraction (Appendix: Table A5). $P$ values for all statistical tests were generated by 999 random permutations of sample identity.

In the RDA approach, forward selection criteria developed by Blanchet et al. (2008) were used to select a subset of environmental and geographic variables that parsimoniously explained variance in community composition. This method first requires a “global” RDA using all explanatory variables. If the global test is significant, forward selection can proceed with the $R^2_{adj}$ (Peres-Neto et al. 2006) from the global analysis used as an additional stopping criterion. Variables are added stepwise into the model, in the order of greatest additional variance explained, until a variable is reached that is either not significant at the chosen $\alpha$ cutoff or whose addition would cause $R^2_{adj}$ of the parsimonious model to exceed $R^2_{adj}$ of the global model. This procedure...
was performed separately for geographic coordinate variables and environmental variables within each habitat type. UTM coordinates were used to calculate second-order geographic coordinate variables (Easting, Northing, Northing × Easting, Northing², Easting²) in order to model simple nonlinear spatial patterns (Legendre and Legendre 1998). Selection of environmental variables was performed using either raw environmental variables or principal components (PCs) derived from the environmental variable correlation matrix. The latter analysis was conducted to minimize the likelihood of spurious results caused by remaining correlations among raw variables (Graham 2003). If both soil characteristics and geographic coordinate variables were significant during forward selection, partial RDA was performed to assess the significance of one subset of variables when the other subset was used as covariates, and to quantify overlap in variance explained. Forward selection RDA was performed with α = 0.1 for acceptance into this model, while α = 0.05 was used to evaluate the significance of full models in the global and partial tests. RDA was performed using Canoco (Microcomputer Power, Ithaca, New York, USA).

In the Mantel analysis approach, Mantel statistics (correlation coefficients) were calculated between the community Hellinger distance matrix and the geographic and environmental distance matrices. Geographic distance matrices were calculated using both straight line distances, and using log-transformed distance (Tuomisto et al. 2003). Environmental distance matrices were calculated from both the suite of environmental variables used in the global RDA test (i.e., a fully representative but non-saturated subset of all measured soil characteristics), and the parsimonious subset of environmental variables selected during forward selection RDA. Partial Mantel tests were used to assess the significance of one set of explanatory variables after variation that can be explained by the other set is removed. Mantel analyses and multivariate dispersion tests were performed in SAS Proc IML (SAS Institute 2004).

**Results**

**Comparison of LP and IS communities.**—When LP and IS soil communities were analyzed together, habitat type explained a significant \(P < 0.01\), albeit small, portion of variation in both fungal and bacterial T-RFLP profiles (\(R^2_{\text{adj}} = 9\%\) and 6\% of total variation, respectively; Fig. 1). For fungi, multivariate dispersion was greater in IS soils than in LP soils by 28\% (mean Hellinger distance between profiles = 1.19 for IS and 0.92 for LP; \(P = 0.002\); Fig. 1). For bacteria, the difference in dispersion between IS and LP soils was not significant (mean Hellinger distance between profiles = 0.55 for IS and 0.56 for LP; \(P = 0.65\); Fig. 1). After showing that habitat type affected both fungal and bacterial community composition, we performed separate tests on LP and IS profiles to determine whether or not soil communities were organized similarly between habitat types.

**Organization of bacterial communities in LP soil.**—Among LP soils, statistically significant portions of the variance in bacterial T-RFLP profiles (\(R^2_{\text{adj}} = 9\%\) and 6\% of total variation, respectively; Fig. 1). For fungi, multivariate dispersion was greater in IS soils than in LP soils by 28\% (mean Hellinger distance between profiles = 1.19 for IS and 0.92 for LP; \(P = 0.002\); Fig. 1). For bacteria, the difference in dispersion between IS and LP soils was not significant (mean Hellinger distance between profiles = 0.55 for IS and 0.56 for LP; \(P = 0.65\); Fig. 1). After showing that habitat type affected both fungal and bacterial community composition, we performed separate tests on LP and IS profiles to determine whether or not soil communities were organized similarly between habitat types.
environmental variables, only soil pH met the significance threshold ($P = 0.031$), accounting for 13% of the variation in community composition. In the forward selection analysis of geographic variables, four of the terms were added ($P < 0.1$), accounting for 34% of community variation. Combined analysis using terms previously found to be significant by forward selection analyses accounted for 40% of variance, indicating an overlap of 7% between the variability explained by pH and the significant geographic terms (Fig. 2). Partial RDA indicated that the selected geographic variables were significant after taking pH into account as a covariate ($P = 0.013$), whereas pH was only marginally significant after taking geographic coordinate variables into account ($P = 0.077$).

RDA conducted on LP bacterial community profiles using soil environmental PCs was consistent with the results for individual environmental variables. When environmental PCs were subjected to forward selection, four PCs were included in the model ($P < 0.1$), accounting for 25% of variance (Table 1). In the combined analysis with selected geographic variables, 48% of variance was accounted for, indicating an overlap of 11% (Fig. 2). The geographic variables were significant after taking environmental PCs into account ($P = 0.011$), whereas the selected environmental PCs were not significant after taking selected geographic variables into account in the partial RDA ($P = 0.104$).

 Mantel statistics showed similar results (Table 2). The correlation between the community profile distance matrix and log-transformed geographic distance was significant ($P = 0.007$; Fig. 3A). The correlation between the environmental and community distance matrices was significant using all nine environmental variables ($P = 0.047$), although the effect was only marginally significant if the effects of geographic distance were first partialled out ($P = 0.077$). During RDA, pH was selected as the most important environmental variable determining LP soil bacterial community composition, but the Mantel correlation was only marginally significant when the environmental distance matrix was calculated from pH alone ($P = 0.093$; Fig. 3B). The correlation between community distance and geographic distance remained significant when the effects of the environmental matrices were partialled out.

**Organization of bacterial communities in IS soil.**—For bacterial communities in IS soils, the global RDA test using nine environmental variables was significant ($P = 0.037$; Table 1). However, the global RDA test using five geographic coordinate variables was not significant ($P = 0.55$). The forward selection procedure was therefore performed on environmental variables, but not geographic variables. This analysis resulted in selection of pH and percent clay ($P = 0.018$ and 0.071, respectively), explaining 21% of the variance in bacterial community composition. When environmental PCs were subjected to forward selection, three PCs were included in the model ($P < 0.05$), explaining 32% of the variance in bacterial community composition (Fig. 2).

The Mantel correlation between community distance and log geographic distance was not significant for bacterial communities in IS soil ($P = 0.41$; Fig. 3C).
Using all nine environmental variables, the correlation between community and environmental distance matrices was marginally significant ($P = 0.084$). However, using only the environmental variables selected during RDA (pH and percent clay), the correlation between community and environmental distance matrices was significant ($P = 0.005$; Fig. 3D).

**Discussion**

We have shown that microorganisms residing in different local habitat types can be structured by different metacommunity phenomena, refuting our first
hypothesis. Beneath *L. siphilitica* plants (LP), regional-scale variation in soil bacteria communities was consistent with dispersal limitation. However, in interspaces between *L. siphilitica* plants (IS), we found strong support for species sorting in bacteria across our sampling region. These divergent results were obtained despite the fact that each LP and IS sample was collected from within ~20 cm of a sample of the other type. Our second hypothesis, that the influence of “soil type” on microbial communities could be derived from dispersal limitation rather than soil characteristics, is supported, but only for bacterial taxa in the presence of *L. siphilitica*. We did not find any case where pure spatial structure and environmental variables were both significant after excluding overlap. Hence, among the four models of metacommunity organization, we only found patterns suggestive of species sorting (IS bacteria) and neutral dynamics (LP bacteria; Cottenie 2005). We cannot completely exclude the possibility of mass effects or patch dynamics using correlative data, and the modest number of sites investigated in this study implies that our analyses only had the power to detect relatively strong patterns. However, the general consistency between RDA of raw data and Mantel analyses of distance matrices indicates that the patterns we observed supporting species sorting and neutral dynamics are robust and important.

Similar to our results for IS communities, other studies have shown that soil bacterial communities sampled from multiple plant species and ecosystem types are often structured by soil pH, a result consistent with species sorting (Fierer and Jackson 2006, Wakelin et al. 2008, Jesus et al. 2009). Soil pH is postulated to affect soil bacteria directly by altering biochemical structures and maintenance of cytoplasmic pH, and indirectly by regulating a variety of other soil characteristics, such as solubility of mineral nutrients (Lauber et al. 2009). However, the interaction between soil properties and plant species composition in determining metacommunity organization of bacterial communities on a regional scale has not been previously addressed. We found that combining soils associated with multiple plant species may mask the metacommunity structure of bacterial communities associated with a given plant species. More generally, our results imply that conclusions about regional-scale metacommunity organization among sites may be highly dependent on the number and types of local habitat types present at each site, and whether or not they are combined prior to analysis. It is possible that, because the difference between the size of an individual microbe and regional spatial scales is so extreme, consideration of local habitat types is more important for microorganisms than other types of organisms. However, recent evidence indicates that local habitat types affect conclusions about macroorganism metacommunities as well (Chase 2007, Ellwood et al. 2009), and should be considered in all studies examining metacommunity organization.

Incongruent organizational patterns between habitat types, such as those found here for bacteria, highlight the importance of interpreting regional-scale patterns in the context of local (i.e., within-site) community dynamics. Differences between habitat types could be explained by limited interactions between the communities inhabiting them. However, organisms in a community can generally cross boundaries between adjacent habitat types (e.g., Baxter et al. 2005, Rantalaïnen et al. 2008, Tscharntke et al. 2008). In our case, LP microbes are most likely a subset of IS microbes that are specifically stimulated by *L. siphilitica* rhizosphere exudates (Berg and Smalla 2009). Species sorting on a regional scale may have been evident for bacteria in IS soils but not LP soils if rhizosphere exudates moderate the effects of other soil characteristics, creating a more homogeneous environment and narrower range of niches in which bacteria can proliferate. In addition, composition of the surrounding plant community at our study sites could covary with soil characteristics and affect IS soil microbial community composition. While the geographic structuring of LP soil bacteria seems to indicate dispersal limitation, an alternative hypothesis is suggested by work showing the importance of plant genetic variability in structuring communities of dependent ecological guilds (Whitham et al. 2003, Crutsinger et al. 2009). For some species, plant genotype can affect rhizosphere community composition (Macdonald et al. 2006, Schweitzer et al. 2008), implying that geographic structure in LP soil bacteria could be derived from species sorting in response to geographic structure of genetic diversity in *L. siphilitica*. More detailed investigations into local-scale microbial community dynamics and plant characteristics are necessary to test these hypotheses.

In contrast to the species sorting and dispersal limitation evident for bacteria, essentially none of the variability in soil fungal communities was explained by geographic coordinates or soil characteristics. It is possible that fungal taxa respond to environmental characteristics that we did not measure, and/or that they are geographically structured at spatial scales smaller than the regional scale captured in this study. Territorial competition between patchily distributed mycelia may make it more difficult to obtain a representative fungal sample for a habitat type at a given site (Boddy 2000). This is apparent in previous comparisons of bacterial and fungal community profiles across a range of scales from individual soil samples to large geographic regions, where patterns in fungal profiles are often difficult to discern due to high variability overall and low consistency between replicate samples (Johnson et al. 2003, Ranjard et al. 2003, Costa et al. 2006, Wakelin et al. 2008). We also found variation among fungal communities to be higher than among bacterial communities. However, in contrast to previous studies (Johnson et al. 2003, Costa et al. 2006, Mougel et al. 2006), variation among fungal communities was significantly reduced in...
soil associated with a single plant species (LP soil). This implies that _L. siphilitica_ generated a consistent rhizosphere habitat, excluding some unpredictable taxa that contributed substantial variability to fungal community composition in the IS soil. We hypothesize that fungi responded to _L. siphilitica_ to a greater extent than other plants in previous studies, because we sampled natural, perennial populations where soil fungi interact with plants over multiple growing seasons. Because of their hyphal growth form, fungi associated with _L. siphilitica_ may be better able to dominate the rhizosphere of an individual plant compared to rhizosphere bacteria. In addition, IS samples may have presented a greater diversity of soil conditions and other plant types than is typically found in bulk soil in other studies, although this did not translate into discernible patterns given the environmental characteristics that we measured.

We have shown that treatment of qualitatively different habitats within sites will have a large effect on conclusions about regional-scale organization. Understanding why this is the case, and under what circumstances species sorting or dispersal limitation will be more important, will require further investigations into local-scale community distributions and relationships among habitats. Metacommunity theory has the potential to provide enormous insight into the biogeography of microorganisms. Our results are especially important because, given the multitude of interactions between plants and microbes, biogeographical patterns in microbes associated with a given plant species could have important consequences for the demographics of plant populations and diversity of plant communities (Bever 2003, Reinhart et al. 2003; S. R. Hovatter, C. B. Blackwood, and A. L. Case, *unpublished manuscript*).

**LITERATURE CITED**


APPENDIX

Site coordinates and soil characteristics at Lobelia siphilitica population sites (Ecological Archives E092-006-A1).