A Thesis

Entitled

Effects of garlic mustard (*Alliaria petiolata*) on soil nutrient dynamics and microbial community function and structure

By

Erin L. Hammer

Submitted as partial fulfillment of the requirements for

The Master of Science Degree in Biology (Ecology-track)

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College of Graduate Studies

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An Abstract for

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Recent studies have shown that exotic plant species can have wide-ranging effects on soil ecosystem processes and biotic diversity, and those effects may be more extensive than previously thought. Garlic mustard [*Allliaria petiolata* (M. Bieb.) Cavara and Grande] is an exotic, invasive herb that poses a threat to North American forests by reducing native plant performance and recruitment. However, little is known about potential impacts to belowground communities and processes. The object of this study was to determine the effects of *A. petiolata* on soil physical, chemical, biogeochemical, and biotic properties. Soil samples were collected between October 2006 and February 2008 from plots with *A. petiolata* present or absent in three Ohio forests: the University of Toledo Stranahan Arboretum (Arboretum), Fuller Preserve (Fuller), and South Park (South). Variables measured included moisture content, pH, organic matter (SOM),

nutrient dynamics [dissolved organic carbon (DOC), microbial biomass carbon (C_{mic}), dissolved organic nitrogen (DON), microbial biomass nitrogen (N_{mic}), ammonium, nitrate, and phosphate], microbial community function [activities of acid phosphatase (PHOS), α-glucosidase (AG), β-glucosidase (BG), N-acetyl-β-glucosaminidase (NAG), β-D-cellobiohydrase (CBH), β-xylosidase (BXYL), leucine amino peptidase (LAP), urease, phenol oxidase (phenox), and peroxidase (perox)], and microbial community structure [community DNA profiles, Simpson index (*D*), Shannon index (*H'*), and richness (*S*)].

Results showed that *A. petiolata* presence had a significant affect on only approximately one third of the measured variables. Generally, the variables affected and the direction of effects (positive or negative) were inconsistent both spatially and temporally. Soil moisture, SOM, N_{mic}, DOC, phosphate, BG activity, ratio of C- to P-acquiring enzyme activities (C:P), and bacterial richness and diversity were lower with *A. petiolata* present at Arboretum, but most treatment differences were observed at only one sampling date, Fall 2006. Moisture, C_{mic}, LAP activity, C:P, and bacterial richness and diversity were impacted at Fuller with all but C:P lower in soils with *A. petiolata* present. At South, BXYL and PHOS activities and ratio of C- to N-acquiring enzyme activities were lower in soils from *A. petiolata* stands, but pH, N_{mic}, nitrate, phosphate, CBH activity, and C:P were higher.

Despite inconsistent effects of *A. petiolata* presence on bacteria community richness, diversity, and extracellular enzyme production, bacterial community composition shifts were found between treatments at all three sites. Generally, communities from soil with *A. petiolata* present were only approximately 50 percent

similar to communities from non-invaded soils. Unfortunately, it is not known which species of bacteria were impacted or if the species impacted were similar between sites. However, the observed community changes strongly suggest a pervasive and consistent impact of *A. petioloata* on soil bacteria.

It is uncertain if *A. petiolata* altered these properties or if soil properties influenced *A. petiolata* colonization. However, *A. petiolata* was associated with differences in a broad range of soil properties, most notably bacteria community composition. These changes may potentially lead to impacts on ecosystem processes such as nutrient turnover and decomposition and on plant community composition. If exotic plants benefit from these modifications, it could create a feedback loop that promotes *A. petiolata* growth or additional exotic plant invasions.

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Chapter 1: Introduction

Exotic plant species can significantly impact communities in which they are introduced. Most studies have focused on impacts of exotics on aboveground biodiversity of flora and fauna (Brussaard et al. 1997; Levine et al. 2003), but recently focus has shifted to include impacts on belowground diversity and ecosystem functions. Exotic plants can alter soil biota, physical and chemical characteristics, and nutrient availability through a variety of mechanisms including: modifying timing, quality, and quantity of plant inputs; increasing overall plant standing biomass, growth rate, and resource use; and affecting symbiotic relationships with nitrogen-fixing bacteria or mycorrhizal fungi (Ehrenfeld et al. 2001; Ehrenfeld 2003; Wolfe & Klironomos 2005). However, recent studies show that the extent and direction of effects vary between species and sites (Ehrenfeld 2003).

Garlic mustard (*Alliaria petiolata* [M. Bieb.] Cavara and Grande) is a non-indigenous, invasive herb of the mustard family (Brassicaceae). Originally native to western Eurasia, *A. petiolata* is considered one of the most rapidly expanding invasive plants in eastern North American forests (Welk et al. 2002). First reported in North America in 1868 on Long Island, New York, it has since spread across the continent and is now found in at least 34 U.S. states and 4 Canadian provinces (Cavers et al. 1979; Nuzzo 1993; Anderson et al. 1996; Welk et al. 2002). Stands are most commonly

established in shaded, mesic deciduous forests, but plants can be found in shaded coniferous forests, along partially shaded forest edges and roadsides, and even in open fields (Cavers et al. 1979; Nuzzo 1993). *Alliaria petiolata* is a biennial with germination occurring in early spring before many native woodland ephemerals have emerged (Cavers et al. 1979; Welk et al. 2002). First-year rosettes remain green throughout winter and will even continue biomass production if temperatures remain optimal (Anderson et al. 1996; Welk et al. 2002). Second-year plants flower in May, set seed in June, and die by winter.

The effects of *A. petiolata* on the growth, abundance, and diversity of native vegetation and insects have been documented. *Alliaria petiolata* presence has decreased overall plant species diversity and relative abundance, recruitment, and growth rate of several woody and herbaceous species (McCarthy 1997; Meekins and McCarthy 1999; Vaughn and Berhow 1999; Prati and Bossdorf 2004; Aminidehaghi et al. 2006; Stinson et al. 2006; Stinson et al. 2007). Effects on vegetation are generally attributed to allelopathy (Vaughn and Berhow 1999), but competitive ability may also be a factor (Meekins and McCarthy 1999). Few effects on native fauna have been reported, but negative impacts on several butterfly species have been found. *Pieris napi oleracea* and *P. virginiensis* larvae have poor survival rates on *A. petiolata* leaves due to chemicals in tissues that act as feeding deterrents (Bowden 1971; Huang et al. 1994; Porter 1994; Haribal and Renwick 1998; Haribal et al. 2001; Renwick et al. 2001).

Impacts on belowground communities are not as well understood as impacts on aboveground communities, but are also generally attributed to allelopathy (Roberts and Anderson 2001; Stinson et al. 2006). Studies found that *A. petiolata* reduces the inoculum

potential of arbuscular mycorrhizal fungi (AMF) in field soils and inhibits germination of AMF spores (Roberts and Anderson 2001; Stinson et al. 2006). Additionally, extracted glucosinolates from *A. petiolata* tissues inhibited some species of gram-negative bacteria (Rudat 1957, *in* Cavers et al. 1979). However, little is known about how *A. petiolata* impacts a broader range of soil properties. The focus of this study was to evaluate the broader effects of *A. petiolata* on soil physical, chemical, biogeochemical, and biotic properties. More specifically, the goals of the experiment were to 1) determine the effects of *A. petiolata* on soil properties, 2) determine if effects vary seasonally, and 3) determine if impacts are consistent between sites.

Chapter 2: Methods

In the present study, soil samples were collected from three deciduous forests in Ohio from plots with *A. petiolata* present or absent during different morphological stages of *A. petiolata* growth to compare differences in soil chemical properties; activity of ten extracellular enzymes involved in carbon, nitrogen, and phosphorus cycling and soil organic matter degradation; and microbial DNA profiles. By measuring enzymes, taxonomic assemblages, and nutrient dynamics, the effects of *A. petiolata* can be evaluated on scales ranging from molecular to entire ecosystems (Sinsabaugh et al. 1991).

2.1 Site Description

Three research sites were selected for this project: the R.A. Stranahan Arboretum, Fuller Preserve, and South Park. These sites were selected because all have areas with and without *A. petiolata* and a canopy dominated by maple and oak species. Soil series for each site were determined using the web soil survey provided by the United States Department of Agriculture Natural Resources Conservation Service (2008).

The Stranahan Arboretum (Arboretum) is a 54.5-acre property in Toledo, Lucas County, Ohio (41°41’44” N, 83°40’13” W). The study site was confined to the approximately 6 acre woodlot located on the property. The Arboretum is located in an

urban setting surrounded by residential housing, several high-traffic roadways, and the Ottawa River. Soils are classified as coarse-loamy, mixed, nonacid, mesic Aeric Haplaquepts (USDA 2008). Fuller Preserve (Fuller) is an 8 acre wooded lot located north of Bowling Green, Wood County, Ohio (41°26'13" N, 83°37'59" W). It is located in a rural area and is surrounded on three sides by farm fields and on the fourth by residential housing. Soils are classified as fine, mixed, active, mesic Typic Argiaquolls (USDA 2008). South Park (South) is a 46 acre public recreation area in Athens, Athens County, Ohio (39°19'16" N, 82°6'58" W). It is located in a semi-urban area with the city of Athens and the Ohio University campus to the North and East and open grassy and wooded areas to the South and West. Soils are classified as fine, mixed, active, mesic Hapludalfs (USDA 2008).

2.2 Sample Collection

Ten plots were established at each research site: five with no garlic mustard present (NM) and five with garlic mustard present (GM). Plots were selected based on the distribution of *A. petiolata* stands. However, garlic mustard and non-garlic mustard plots were similarly placed with respect to slope, woodlot edge, and micro-topography. Plots were established at Arboretum and Fuller Preserve in October 2006, but plots at South were not established until April 2007.

To determine *A. petiolata* presence or absence at a potential plot, a two-meter radius circle was measured from a central point, selected by visual evaluations of *A. petiolata* density. The plot was designated as a garlic mustard plot if there were abundant *A. petiolata* throughout the circle (at least 30 individuals). Non-garlic mustard plots were

those where *A. petiolata* was absent. *Alliaria petiolata* presence was determined by the occurrence of standing dead stalks and first-year rosettes (Arboretum and Fuller) or first- and second-year rosettes (South). The actual sampling plot was restricted to a one-meter radius from the center point. This ensured that any sample taken within a garlic mustard plot would be surrounded by *A. petiolata* to at least one meter, and any sample taken within a non-garlic mustard plot would be at least one meter away from an *A. petiolata* plant.

Beginning in October 2006 for Arboretum and Fuller and in April 2007 for South, soil samples were taken from each plot throughout the year. Samples were collected at six times at Arborerum and Fuller: Fall 2006; Early Spring, Late Spring, Summer, and Fall 2007; and Winter 2008 (N=10 per date for each site). Sampling only occurred at South during Early Spring, Summer, and Fall 2007 and Winter 2008. Sampling times corresponded to five seasonal changes in *A. petiolata* morphological life stages (see Table 1 for the sampling schedule, description of morphology at each time period, and abbreviations used).

Within each plot, a 2.5 cm soil core (5 cm deep) was taken from four different locations, picked at random. The soil cores from each plot were consolidated and placed in a pre-labeled zip-loc bag. Any excess soil on the corer was removed between sample collections. Samples were placed in a cooler and transported immediately to the lab to be analyzed. Once in the lab, each sample was homogenized by hand, and any roots, leaf litter, small rocks, and macro-fauna were removed. All samples were stored at 4 °C when not in use.

2.3 Soil Properties

Soil moisture content was measured gravimetrically by placing 5 g of each composite soil sample into a 60 °C Isotemp gravity convection oven (Fisher Scientific, Pittsburgh, PA) for 48 hours. Each sample was reweighed to determine soil dry weight, and percent moisture content was calculated as (wet soil weight-dry soil weight)÷wet soil weight×100.

Percent soil organic matter (SOM) content was determined by mass loss on ignition. Previously dried samples were placed in a muffle furnace (Thermolyne, Dubuque, IA) at 500 °C for at least 4 hours. Samples were reweighed to determine soil ash free dry weight, and SOM was calculated as (dry soil weight-ash free dry weight)÷dry soil weight×100.

To determine soil pH, 2 g of each composite sample were combined with 20 ml of Nanopure water in a 50 ml beaker. The soil-Nanopure solution was stirred, allowed to sit for ten minutes, and then re-stirred prior to measuring pH with a hand-held pH meter (Oakton Instruments, Vernon Hills, IL). After the soil pH for each plot was measured, they were averaged to get the overall pH for each site. This value was used to determine the pH of the sodium acetate buffer used in the extracellular enzyme assays (see Methods 2.5 below). Soil pH was only measured during the first sampling period.

2.4 Nutrient Pools

Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were extracted from soils following procedures outlined by Weintraub et al. (2007). Five grams of each soil sample were extracted with 25 ml of 0.5 M potassium sulfate (K_2SO_4)

for 1 hour, while being continuously shaken at 220 RPM on a multi-purpose rotator (Barnstead International, Dubuque, IA). Extracts were vacuum-filtered and then stored at -20 °C until further analysis. Dissolved organic carbon and TDN from extracted soils were analyzed with a Shimadzu total organic carbon (TOC-VCPN) analyzer with an ASI autosampler and TNM-1 total dissolved nitrogen module (Shimadzu Scientific Instruments, Inc., Columbia, MD). Procedures were similar to those described by Pan et al. (2005).

Concentrated C and N standard solutions were prepared by dissolving 1.0625 g potassium hydrogen phthalate (PHP; $C_8H_5KO_4$) in 500 ml Nanopure water (1000 ppm-C) and 3.6 g potassium nitrate (KNO_3) in 500 ml Nanopure water (1000 ppm-N). Three blank vials containing Nanopure water and one vial containing check standards (10 ppm-C standard and 1 ppm-N standard) were analyzed first to prime the machine. Next, calibration curves were run using one vial of 100 ppm-C standard and one vial of 10 ppm-N standard. The two vials were placed in the machine and were automatically diluted to concentrations of 0 ppm, 4 ppm, 25 ppm, 50 ppm and 100 ppm for C and 0 ppm, 1 ppm, 2 ppm, 5 ppm, and 10 ppm for N.

Soil samples, blanks, and check standards were placed in the autosampler and analyzed in three steps: 1) hydrochloric acid was automatically added to all vials to reduce pH to <2, and samples were purged with oxygen; 2) samples were combusted at 680 °C; and 3) samples were purged with ozone (O_3). Released carbon dioxide (CO_2) during the combustion stage was measured with a non-dispersive infrared detector (NDIR), and light emitted due to chemiluminescence of nitrogen (NO_2) during step three was measured with a photo-multiplier. Repeated measures were taken to achieve a 2

percent coefficient of variation or 0.1 standard deviation. The average results, coefficients of variation, and standard deviations were automatically calculated by the software and exported into Excel files. Total dissolved nitrogen concentrations were automatically determined by the software based on stoichiometric proportions between amount of light emitted and NO_2 concentrations.

All samples were scaled to check standard concentrations to account for any instrument variability, and concentrations of blanks were subtracted based on the equation: [((10×sample concentration)÷check standard concentration)×10)-blank concentration]. Next, concentrations were converted from μg ml^{-1} to μg g dry $soil^{-1}$ ([corrected sample×(volume of K_2SO_4 added+wet soil weight-dry soil weight)]÷dry soil weight). Dissolved organic nitrogen (DON) was calculated as extracted ammonium and nitrate concentrations (see methods below) subtracted from the TDN concentration.

Microbial biomass carbon (C_{mic}) and microbial biomass nitrogen (N_{mic}) were determined using the chloroform fumigation-extraction method (Brookes et al. 1985; Scott-Denton et al. 2006). For fumigated samples, 5 g of soil and 2 ml of ethanol free chloroform were added to 250 ml Erlenmeyer flasks, and flasks were stoppered. After 24 hours of incubation, stoppers were removed, and flasks were ventilated for 30 minutes. DOC and DON were extracted with 25 ml of 0.5 M K_2SO_4 and analyzed with the Shimadzu analyzer as described above. Microbial biomass carbon and N_{mic} were then calculated as TOC and TDN concentrations in the non-fumigated samples subtracted from TOC and TDN in the fumigated samples and were expressed as μg C_{mic} g dry $soil^{-1}$

and μg N_{mic} g dry $soil^{-1}$. Values were not calibrated using the extraction coefficients for microbial carbon (k_{eC}) or nitrogen (k_{eN}) biomass.

Soil ammonium content was determined from K_2SO_4 extracted samples using colorimetric assays developed by Rhine et al. (1998). Assays were conducted in clear 96-well microplates with three replicate wells for each standard, sample, and blank. Working standards were prepared by diluting a concentrated standard (0.9346 g ammonium sulfate in 0.5 M 0.5 M K_2SO_4; 200 ppm-N) with 0.5 M K_2SO_4 to concentrations of 0.00, 0.25, 0.50, 1.0, 2.0, 3.0, 4.0, and 5.0 ppm-N. Replicate wells were created with 125 μl working standard, 125 μl soil extract, or 125 μl 0.5 M K_2SO_4. Fifty microliters of citrate reagent (5.00 g trisodium citrate in 100 ml Nanopure water; pH lowered to 7.0) were added to each well. After 1 minute, 50 μl of 2-phenylphenol-nitroprusside reagent (PPS; 3.22 g phenylphenol sodium and 0.0127 g sodium nitroprusside in 100 ml Nanopure water) were added to each well, followed by 25 μl of buffered hypochlorite reagent (2.32 g sodium phosphate, 80 ml Nanopure water, 10 ml household bleach, and 10 ml 2 M sodium hydroxide [NaOH]) and 50 μl of Nanopure water. Plates were covered, shaken gently for 30 seconds with a multi-purpose rotator, and then allowed to sit for 2 hours. Absorbance was measured at 540 nm on a Bio-Tek Synergy HT microplate reader (Bio-Tek Inc., Winooski, VT). Sample and blank absorbance measurements were converted to concentrations using a regression of the working standard measurements ([sample or blank absorbance×slope of regression]-y intercept). Samples were then corrected for the blanks (standardized sample-standardized blank) and expressed as concentration of

ammonium per g soil ([corrected sample×(volume of K_2SO_4 added+wet soil weight-dry soil weight)]÷dry soil weight; μg NH_4-N g dry $soil^{-1}$).

For nitrate, colorimetric assays were also performed on K_2SO_4 extracted samples in 96-well clear microplates (Doane and Horwáth 2003). Working standards were prepared by diluting a 100 ppm-N solution (0.3609 g potassium nitrate and 500 ml Nanopure water) with 0.5 M K_2SO_4 to concentrations of 0.00, 0.05, 0.10, 0.20, 0.50, and 1.0 ppm-N. Replicate wells were created with 30 μl working standard, 30 μl soil extract, or 30 μl 0.5 M K_2SO_4. Vanadium (III) chloride reagent was prepared with 0.35 g of vanadium (III) chloride powder and 50 ml 1 M hydrochloric acid (HCl). The solution was vacuum-filtered before adding 3.3 ml of 2 percent sulfanilamide, 3.3 ml of 0.2 percent N-(1-naphthyl)-ethylenediamine dihydrochloride, and 400 ml of Nanopure water. All wells received 235 μl of vanadium (III) chloride reagent, and plates were incubated for 5 to 24 hours. Absorbance was read at 660 nm on the Bio-Tek microplate reader. Sample absorbances were corrected and converted to concentrations (μg NO_3-N g dry $soil^{-1}$) following the same methods as ammonium.

Assays for inorganic phosphorus were performed similarly to ammonium and nitrate using colorimetric methods developed by Van Veldhoven and Mannaerts (1987). Working standards were prepared by diluting 20 ppm-P solution (24.55 g sodium phosphate in 200 ml Nanopure water) with 0.5 M K_2SO_4 to concentrations of 0.00, 0.01, 0.05, 0.10, 0.25, 0.50, 0.75, and 1.0 ppm-P. Replicate wells were created with 150 μl working standard, 150 μl soil extract, or 150 μl 0.5 M K_2SO_4. Ten microliters of ammonium paramolybdate solution (8.775 g ammonium paramolybdate and 53 ml 3 M

sulfuric acid in approximately 447 ml Nanopure water) were added to each well. Plates were gently tapped to mix and allowed to sit. After 10 minutes, 30 µl of malachite green solution (1.75 g polyvinyl alcohol and 0.175 g malachite green in 500 ml Nanopure water) were added through reverse pipetting. Plates were incubated for 30 minutes, and absorbance was measured at 630 nm. Samples were standardized and corrected for the blanks, and sample absorbances were converted to concentrations (µg PO_4-P g dry $soil^{-1}$) following the same methods as ammonium and nitrate.

2.5 Microbial Community Function

Ten enzymes involved in carbon, nitrogen, and phosphorus cycling were evaluated following the procedures outlined in Saiya-Cork et al. (2002). The enzymes assayed are listed in Table 2 along with their abbreviations, functions, and corresponding substrates.

Enzyme assays were conducted on buffered soil slurries. Arboretum and Fuller soil sample slurries were prepared by combining 1 g of soil sample with 125 mL of 50 mM sodium acetate buffer (pH 6.6). Soils at South had high clay content compared to Arboretum and Fuller. Clays naturally aggregate into dense peds, so initial homogenization may be less effective at mixing clay compared to other soil types. Therefore, 1 g of soil at South may be less representative of the total plot than 1 g of soil at the other sites. To ensure an adequate representation of overall plot conditions, 3 g of South soil samples were mixed with 375 ml of buffer (pH 5.9). The solutions were homogenized with a BioSpec Tissue Tearor (BioSpec Products, Bartlesville, OK) for 1

minute, and slurries were continuously stirred using a magnetic stir plate to prevent settling of sediment during pipetting. Sixteen replicate wells with 200 μl of soil slurry were pipetted for each sample and each assay.

NAG, CBH, AG, BG, LAP, BXYL, and PHOS assays were fluorimetric and were executed in black 96-well microplates. Sample wells received 50 μl of 200 μM substrate (Table 2), and eight replicate wells were created for each blank (200 μl of soil slurry + 50 μl of buffer) and quench standard (50 μl of corresponding standard + 200 μl of sample). Eight replicate wells for negative controls (50 μl of corresponding substrate + 200 μl of buffer) and reference standards (50 μl of corresponding standard + 200 μl of buffer) were pipetted for each microplate. Ten mM 4-methylumbelliferone (MUB) was used as the reference standard for all fluorimetric enzymes, except LAP, which received 7-amino-4-methylcoumarin (MC). All microplates were covered and incubated in the dark at 20 °C. After 4 to 5 hours, 10 μl of 1.0 M NaOH was pipetted in each well to stop the chemical reaction and increase the pH because MUB and MC fluoresce better at a higher pH (Sinsabaugh et al. 1993; Saiya-Cork et al. 2002; Weintraub et al. 2007). The Bio-Tek microplate reader was used to measure fluorescence with 360 nm excitation and 460 nm emission filters. Fluorescence of each sample well was corrected based on the equation: [sample well÷(quench standard well÷reference standard well)]-(negative control well+blank well).

Phenox and perox assays were colorimetric and were performed in clear 96-well microplates. For Phenox, 50 μl of substrate were added to each sample well. Eight

replicate blank wells (200 μl of soil slurry and 50 μl of buffer) were pipetted for each sample, and eight replicate negative control wells (200 μl of buffer and 50 μl of substrate) were pipetted for each microplate. Peroxidase assays were performed similarly, except sample, negative control, and blank wells also received 10 μl of 0.3 percent hydrogen peroxide (H_2O_2). Microplates were incubated as described above, and absorbance was measured at 460 nm with the Bio-Tek microplate reader. Activities were corrected by subtracting the absorbance of the blank and negative control wells from the absorbance of the sample wells. Phenol oxidase activity was subtracted from peroxidase activity (Sinsabaugh et al. 1992) because both enzymes react with the L-DOPA substrate.

Urease assays were also colorimetric and were performed similarly to phenox. However, only 10 μl of substrate were added to sample and negative control wells. All microplates were incubated as before. Prior to reading the microplates, salicylate and cyanurate solutions were prepared using reagent packets from Hach (Loveland, CO). Packets were dissolved in Nanopure water (1 packet per 10 ml), and 40 μl of salicylate solution were added to each well. After 3 minutes, 40 μl of cyanurate solution were also added to each well. Plates were incubated for an additional 20 minutes, during which the reagents caused color development in response to ammonium released by urease activity (Sinsabaugh et al. 2000). Absorbance was then measured at 610 nm with the Bio-Tek microplate reader. Activities were corrected by subtracting the absorbance of the blank and negative control wells from the absorbance of the sample wells.

All enzyme activities were expressed as the rate of reaction product released per unit of soil (nmol h^{-1} g dry $soil^{-1}$). Prior to statistical analysis, EEA was standardized by the amount of microbial biomass carbon to approximate specific enzyme activity. Extracellular enzyme activity was divided by C_{mic} and expressed as nmol h^{-1} μg C_{mic}^{-1}. Additionally, indices of enzymatic resource allocation to nitrogen and phosphorus acquisition compared to carbon acquisition were estimated by calculating ratios of BG activity to (LAP plus NAG) activities as an indicator of C:N and BG activity to PHOS activity as an indicator of C:P (Sinsabaugh et al. 2008).

2.6 Microbial Community Composition

The microbial community composition aspect of this study was added after initial sampling had already begun. Therefore, bacterial DNA was only extracted from soil samples collected during Early Spring, Late Spring, Summer, and Fall 2007 and Winter 2008 for the Arboretum and Fuller (n=50 for each site) and Summer and Fall 2007 and Winter 2008 for South (n=30). The isolated DNA was used to create community fingerprints to detect differences in bacteria communities between treatments.

Bacterial DNA was extracted using a bead beating method of mechanical lysis combined with sodium dodecyl sulfate (SDS) chemical lysis (Sigler and Zeyer 2002). Approximately 0.5 ml of glass beads (0.10-0.11 mm diameter; Bio-Spec Products, Inc., Bartlesville, OK) and a 0.8 g soil sample from each plot were combined with 1.00 ml of extraction buffer (50 mM NaCl, 50mM Tris-HCl [pH 7.6], 50 mM EDTA, and 5 percent SDS; pH 8.0) in 2 ml microcentrifuge tubes. The tubes were processed in a Fast-Prep 120 bead-beater (Thermo Savant, Waltham, MA) at 5.5 ms^{-1} for 30 seconds and then

centrifuged at 13,000 x *g* for 3 minutes. Next, the supernatant was decanted into fresh 2ml tubes, and the extracted DNA was purified and precipitated (Ogram et al. 1987; Sambrook et al. 1989). The volume of each supernatant was determined, and half volumes of phenol and chloroform-isoamyl alcohol (24:1) were added to each tube. Tubes were briefly vortexed and centrifuged at 13,000 x *g* for 3 minutes. The aqueous phase was pipetted into new 2 ml tubes, and an equal volume of chloroform was added. The tubes were centrifuged again as before, and the aqueous phase was transferred into 1.5 ml tubes. Based on the volume of the extract, 0.1 volumes of 3 M sodium acetate solution and 0.7 volumes of isopropanol were added. DNA was precipitated by centrifugation at 13,000 x *g* for 30 minutes at 10 ºC (Eppendorf, Hamburg, Germany). The supernatant was aspirated, and the remaining pellet was washed with 0.5 ml ice-cold 70 percent ethanol (EtOH). Samples were centrifuged at 10 ºC for an additional 5 minutes. The EtOH was aspirated, and the DNA pellets were resuspended with 50 µl of DNase/RNase-free water (DNA-water). All samples were stored at -20 °C when not in use.

Impurities such as humic acids were removed from the samples with polyvinylpyrrolidone (PVPP) cleanup. Spin columns were prepared by adding 300 ml PVPP solution (10 g PVPP in 100 ml DNA-water). Columns were placed in 1.5 ml centrifuge tubes and centrifuged at 13,000 x *g* for 1 minute. After empting the liquid from the tube, centrifugation was repeated to dry the PVPP matrix. The spin column was then placed in a new 1.5 ml tube. If necessary, DNA-water was added to raise the volume of each sample to approximately 100 µl. Then, the whole sample was added to the spin column and centrifuged at 13,000 x *g* for 1 minute. PVPP cleanup was repeated until

samples were relatively clear. DNA concentration and purity of samples was then quantified spectrophotometrically. In 1.5 ml microcentrifuge tubes, 10 μl of sample were mixed with 990 μl of DNA-water, and the tubes were briefly vortexed. Concentration of samples was determined by measuring absorbance at 260 nm with a Bio RAD SmartSpec 3000 (Hercules, CA). Purity was determined by the ratio of absorbance at 260 nm to absorbance at 280 nm, and concentration was determined based on the assumption that an absorbance of 1 at 260 nm corresponds to 50 μg DNA ml^{-1} (Sambrook et al. 1989; Sigler and Zeyer 2002). If sample concentrations were higher than 100 μg DNA ml^{-1}, samples were diluted with DNA-water. Due to the large sample size (130 samples total) and time and monetary constraints, DNA samples were pooled by treatment, sampling period, and site to reduce the sample size to 26 communities.

Polymerase chain reaction (PCR) was used to amplify bacterial rDNA to estimate community diversity and characterization (Roose-Amsaleg et al. 2001). Primers BAC 341 f gc (5′-GC-clamp-CCT ACG GGA GGC AGC AG-3′) and BAC 534 r (5′-ATT ACC GCG GCT GCT GG-3′) were used to amplify genes from the 16S rDNA region in bacteria, corresponding to the 341 to 534 positions in *Escherichia coli* (Muyzer et al. 1993). The GC-clamp attached at the 5′ end of the forward primer was used to prevent complete melting of DNA fragments during later denaturing gradient gel electrophoresis (DGGE) and increase the detection of sequence variants (Myers et al. 1985; Sheffield et al. 1989). Sample tubes received 1 μl sample DNA and 49 μl master mix containing 5.0 μl 2.5 mM $MgCl_2$ buffer (5 Prime, Inc., Gaithersburg, MD), 0.8 μl bovine serum albumin (BSA), 4 μl 0.2 mM each deoxynucleotide triphosphates (dNTPs), 0.1 μl of each primer, 38.8 μl DNA-water, and 0.2 μl *Taq* DNA polymerase (5 Prime, Inc., Gaithersburg, MD).

One positive control tube received 1 μl template *E. coli* DNA and 49 μl master mix, while a negative control received 1 μl DNA-water and 49 μl master mix. Reaction tubes were capped, mixed by gently flicking the tubes, briefly centrifuged, and then placed in the thermal cycler (Eppendorf, Hamburg, Germany). Initial denaturation was performed for 5 minutes at 94 °C. Amplification was performed in 30 cycles of denaturation for 45 seconds at 94 °C, primer annealing for 45 seconds at 55 °C, and primer extension for 45 seconds at 72 °C. After the final cycle, samples were incubated for 30 minutes at 72 °C and then cooled to 5 °C.

When the process was complete, PCR products were visualized with agarose gel electrophoresis to ensure successful gene amplification. The gel was prepared by heating 0.8 g agarose in 80 ml 1 X Tris-acetate-EDTA (0.04 M Tris base, 0.02 M sodium acetate, and 1.0 mM EDTA, pH 7.4; TAE) buffer until boiling and then stirring to ensure all the agarose had dissolved. Contents were cooled to approximately 55 °C, 4 μl ethidium bromide were added, and the gel was poured into a casting tray with combs. After 30 minutes, the combs were removed, and the casting tray (with gel) was placed on a RunOne Electrophoresis cell platform (Embi Tech, San Diego, CA). Buffer was added to the electrophoresis tank until the gel was covered by 1 to 2 mm. Samples were prepared by adding 5 μl of loading dye (4 g sucrose and 3 μl bromo phenol blue in 10 ml Nanopure water) to each PCR tube and briefly vortexing until mixed. Three μl of 100 base pair DNA ladder (Promega, Madison, WI) was pippetted into the first well of each row as a reference, and the subsequent wells received 5 μl of sample. When pipetting was finished, the apparatus was covered and run at 100 V. After 20 minutes, the casting tray was removed from the tank platform, the gel was removed from the tray, and bands were

visualized under UV transillumination with a Gel Logic 200 Imaging System (Kodak, Rochester, NY).

Bacterial communities were “fingerprinted” by separating PCR-generated DNA products using DGGE (Muyzer et al. 1993). Polymerase chain reaction products were loaded onto 8 percent acrylamide gels with a vertical urea-formamide denaturing gradient from 50 to 60 percent in the direction of electrophoresis. Both denaturing concentration solutions contained 0.3 ml 50 X TAE buffer, 3.0 ml 40 percent bis-acrylamide (37.5 acrylamide:1 bisacrylamide), and 0.3 ml glycerol. Additionally, 7.0 ml DNA-water, 3.3 ml formamide, and 3.5 g urea were added to the 50 percent solution and 5.5 ml DNA-water, 3.6 ml formamide, and 3.7 g urea were added to the 60 percent solution. Solutions were syringe filtered, and then 90 µl 10 percent ammonium persulfate solution (APS, 0.1 g ammonium persulfate in 1 ml Nanopure water) and 5 µl N,N,N',N'-tetramethylenediamine (TEMED) were added so the acrylamide would polymerize after being poured.

The 50 to 60 percent gradient was produced using a gradient wheel. Two syringes, one with the 50 percent solution and the other with the 60 percent solution, were attached to the wheel and connected with a Y-shaped delivery tube. The gel was poured into a plate assembly consisting of 16 cm x 16 cm and 16 cm x 14 cm glass plates separated by 1 mm spacers by slowly and consistently turning the gradient wheel. Once poured, 2 ml of 1 X TAE buffer was added to the top of the gel to smooth the surface. The gel was allowed to polymerize for 30 minutes before the buffer was removed. Approximately, 3 ml of cap solution (50 µl 50 X TAE buffer, 650 µl bis-acrylamide, 1.8 ml DNA-water, 18 µl APS and 1 µl TEMED) was added to the top of the gel, and a comb

was inserted into the cap solution. After 2 to 3 hours, the comb was removed, and the wells were flushed with 1 X TAE buffer to remove any unpolymerized acrylamide.

Gels were run on a DCode Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA). The buffer chamber was filled with approximately 6.5 L of 1 X TAE buffer and heated to 50 ˚C. The prepared gels were attached to the core assembly and lowered into the buffer chamber. An additional 0.5 L of 1 X TAE buffer was added to fill the top reservoir, and the wells were flushed again before loading the PCR products. For each gel, the first two and last two lanes were left empty and the third, middle, and third to last lanes received 8 µl of DGGE marker as a reference. The DGGE marker contained equal amounts of PCR-amplified 16S rDNA (Muyzer et al. 1993) from *Bacillus subtilis*, *E. coli* DH5α, *Pseudomonas aeruginosa*, *Ralstonia pickettii*, and four unknown environmental bacteria. Fifty microliters of sample PCR product were pipetted into the remaining lanes. After pipetting, the buffer was re-heated to 60 ˚C and the apparatus was run at 20 V for 10 minutes. The apparatus was then run at 60 V for 16 hours.

Once electrophoresis was complete, the core assembly was removed from the buffer chamber, and the gels were detached from the core assembly. The cap portion of the gel and any unused lanes were trimmed, and the gel was stained in 50 ml of 1 X TAE with 1:10,000 dilution of GelStar nucleic acid stain (BioWhittaker Inc., Walkersville, MD) for 15 minutes. Finally, the gel was viewed under UV transillumination with a Gel Logic 200 Imaging System. Gel images were analyzed using GelCompar II software (Applied Maths, Kortrijk, Belgium). Individual marker and sample lanes were identified and normalized before manually selecting bands in each lane. Once all the bands were

identified, the software analyzed each lane to determine the position and relative intensity (band intensity÷intensity of all bands in the lane) of each band. Bands were only detected if they accounted for more than 1 percent of the cumulative lane intensity.

Dice similarity coefficients were then calculated for each lane pair possible to create a similarity matrix. Coefficients were calculated as $S_D = [2n_c \div (n_a + n_b)] \times 100$, where n_a = number of bands in lane *a*, n_b = number of bands in lane *b*, and n_c = number of common bands between lanes *a* and *b* (Dice 1945). If two lanes were completely similar, S_D would be 100 percent. Dendrograms were created based on the similarity matrices using an unweighted pair group method with arithmetic mean (UPGMA) clustering method. Additionally, DNA band counts and relative intensity were used to determine bacterial community richness and diversity. The number of band positions in a lane was used as an indicator of species richness, while the relative intensity of the band was used as an indicator of that species' relative abundance (Muyzer et al. 1993). To determine community diversity, the Shannon-Wiener index [$H' = -\Sigma (n_i \div N) \times \ln(n_i \div N)$] and Simpson index [$D = 1 - \Sigma (n_i \div N)^2$] were used with n_i being the relative intensity of each band and N being the sum of all relative band intensities for the lane (Magurran 1988).

2.7 Statistical Analysis

Prior to analyses, assumptions of normality were tested using the UNIVARIATE procedure of SAS software Version 9.1 (SAS Institute, Cary, NC). Specifically, the Shapiro-Wilk (*W*) statistic was calculated for each variable for each site separately with small values of *W* being significant and indicating non-normality (Shapiro and Wilk 1965). Although the data set was multivariate, the univariate *W* statistic was used based

on the assumption that if univariate distributions are normal, the multivariate distribution will also be normal (Scheiner 1993, Marcoulides and Hershberger 1997). When distributions were nonparametric, data were rank transformed prior to parametric analyses (Toothaker and Newman 1994). Rank transformations and all subsequent statistical analyses were performed using DataDesk software Version 6.2 (Data Description, Inc., Ithaca, NY). All post-hoc comparisons were performed using Bonferonni pair-wise comparisons, and results were considered to be significant when $P \leq 0.05$.

For pH, separate two-sample t-tests were used to evaluate differences between treatments at each site. Additional two-sample t-tests were used to evaluate overall differences between treatments for microbial community diversity (H' and D) and richness (S). Moisture content and SOM were analyzed using separate two-way analyses of variance (ANOVAs) by site to evaluate effects of treatment, sampling period, and their interaction. Alpha was set at 0.05 for each test.

Separate multivariate analyses of variance (MANOVAs) were performed by site to evaluate effects of treatment, sampling period, and possible interaction on 1) nutrient dynamics (C_{mic}, N_{mic}, DOC, DON, ammonium, nitrate, and phosphate), 2) standardized EEA (NAG, CBH, AG, BG, LAP, BXYL, PHOS, urease, phenox, and perox), and 3) ratios of nutrient acquiring enzyme activities (BG:LAP+NAG and BG:PHOS). Effects were analyzed separately by site because uneven sampling may bias results when using a factorial MANOVA design, and the Pillai's trace method of MANOVA was used because it is more robust than other parametric tests (Scheiner 1993; Finch 2005). When factor effects were significant, effects on individual variables were analyzed by separate two-

way ANOVAs. Due to the large number of statistical tests being performed, the threshold for significance was adjusted for each two-way ANOVA using a Bonferonni correction (α'=0.05÷number of tests performed) to reduce the possibility of Type I error (Scheiner 1993).

Principle components analysis (PCA) was used to reduce the total number of variables studied to determine which characteristics contributed most to factor variance. Soil moisture, SOM, pH, nutrient pools, and EEA were included in the PCAs. Bacteria community diversity and richness were not included because only one data point was available for each sampling date. Again sites were analyzed separately. Only the first two principle components for each analysis were reported in this study.

A partial Mantel test was used to determine if relationships existed among the different sets of variables studied. Distance matrices were constructed for each site separately for 1) extracellular enzyme activities (dependent), 2) moisture, SOM, and nutrient dynamics (independent), and 3) DNA band positions and intensities (independent). Prior to constructing the matrices, sample values were pooled by treatment and sampling dates to be comparable to the bacteria community dataset. Only sampling dates with data available for all the measured variables were included in the analysis. Matrices consisted of Euclidean distances calculated between pairs of observations. Where samples had values $x_{i1}, \ldots, x_{it}$, Euclidean distance between x_i and x_j (d_{ij}) were determined as $[(x_{i1}-x_{j1})^2+\ldots+(x_{it}- x_{jt})^2]^{1/2}$ (Kruskal 1964). Regression models were generated with Permute (Version 3.4; Casgrain 1998). Stepwise regression was not available with the program so the models were generated using backward elimination and

forward selection procedures. Backward elimination started with all the variables included in the model and then removed those that were not significant, whereas forward selection started with no variables and added only those that were significant. Regressions were performed for matrices 1 and 2 and for matrices 1 and 3. Residuals from each regression were then used to construct two new matrices. A Pearson correlation coefficient was calculated comparing the two residual matrices, and the coefficient was tested using permutation testing (Legendre et al. 1994). The null model was created by permutating the original dependent matrices (2 and 3) one thousand times and calculating the Pearson correlation coefficient after each permutation. Finally, the correlation coefficient for the observations was compared to the generated distribution of correlation coefficients to determine significant relationships.

Chapter 3: Results

3.1 Soil Physical Properties

At Arboretum, soil moisture content did not differ between treatments (27.9±1.02 percent), but an interaction with sampling period occurred (P=0.0179). This was mostly due to significantly higher moisture content in NM plots (31.8±2.81 vs. 23.1±0.82 percent) during Fall 2006 (Figure 1). Differences between sampling periods were also significant (P≤0.0001). At Fuller, moisture content was significantly higher in plots without garlic mustard than plots with garlic mustard (42.4±2.91 and 37.2±2.53 percent respectively; P=0.0013). There was also a significant effect for sampling period (P≤0.0001; Figure 1). No significant treatment differences were present for South (21.1±1.27 percent), but there was a sampling period effect (P≤0.0001; Figure 1).

At Arboretum, SOM was significantly higher in NM plots (16.9±1.98 percent) than GM plots (11.5±1.44 percent; P=0.0231). Also, the interaction effect approached significance (P=0.0739), possibly due to higher SOM in GM plots (11.9±4.30 vs. 8.82±0.61 percent) during Summer 2007 (Figure 2). The large treatment differences that occurred during the Early Spring 2007 sampling period (35.0±6.71 in NM plots vs. 8.28±3.52 percent in GM plots) was likely due to a sampling error in one NM plot which contained 51.4 percent SOM. Treatments at Fuller were not significantly different (21.4±1.04 percent), but differences by sampling period approached significance

(P=0.0992; Figure 2). Soil organic matter did not differ by treatment at South (11.2±0.75 percent), but a significant sampling period effect occurred (P=0.0047; Figure 2).

There were no significant differences due to treatment for pH at either the Arboretum (6.4±0.1; P=0.3897) or Fuller (6.9±0.1; P=0.2040). At South, a significant difference between treatments was found (P=0.0249), with pH being higher in GM (6.4±0.1) than NM (5.4±0.1) plots.

3.2 Nutrient Pools

Nutrient data were not available at Arboretum and Fuller during Early Spring 2007 because K_2SO_4-extracted samples were lost. Therefore, sample size was reduced to 50 at each site. The threshold for significance was adjusted to P≤0.0071 for individual two-way ANOVAs.

Pillai's trace revealed significant general effects at Arboretum for treatment (P=0.0220), sampling period (P≤0.0001), and interaction (P=0.0156). Likewise, at Fuller, treatment (P=0.0042), sampling period (≤0.0001), and interaction (P=0.0478) effects were significant. South also had significant general treatment (P=0.0004) and sampling period differences (P≤0.0001). However, South did not have a significant interaction effect (P=0.1791).

Two-way ANOVAs revealed no significant treatment effects for any individual nutrients at Arboretum. However, there were significant interaction effects for N_{mic} (P=0.0047), DOC (P≤0.0001), and phosphate (P=0.0017; Figure 3). Post-hoc comparisons revealed that interaction effects were due to treatment differences being significant only during Fall 2006 with all three nutrients being higher in NM plots

(27.9±12.5 vs. 10.7±4.80 μg N g dry soil^{-1} for N_{mic}, 102.8±12.1 vs. 9.59±1.40 μg C g dry soil^{-1} for DOC, and 0.41±0.22 vs. 0.004±0.004 μg P g dry soil^{-1} for phosphate). Additionally, significant sampling period effects were present for each individual nutrient (Table 3). At Fuller, treatment effects for individual nutrients were only significant for C_{mic} (P=0.0004; Figure 3), with levels being higher in NM plots (844.6±124.8 vs. 600.6±62.0 μg C g dry soil^{-1}). Sampling period effects were significant for all nutrient pools (Table 3). Treatment effects at South were significant for N_{mic} (P=0.0007), nitrate (P=0.0014), and phosphate (P≤0.0001; Figure 3). All three pools were higher in GM plots (63.2±6.66 for N_{mic} μg N g dry soil^{-1}, 2.91±0.57 μg NO_3-N g dry soil^{-1} for nitrate, and 0.27±0.08 μg P g dry soil^{-1} for phosphate) than NM plots (41.5±3.37 μg N g dry soil^{-1}, 1.29±0.36 μg NO_3-N g dry soil^{-1}, and 0.01±0.01 μg P g dry soil^{-1}). Additionally, there were significant sampling period effects for each individual nutrient, except phosphate (Table 3).

3.3 Microbial Community Function

Microbial biomass carbon data was not available at Arboretum or Fuller during Early Spring 2007 because K_2SO_4-extracted samples were lost so EEA could not be standardized by C_{mic}. Therefore, sample size for standardized EEA was also reduced to 50 at each site. The threshold for significance was adjusted to P≤0.0050 for standardized EEA individual ANOVAs and P≤0.0250 for enzyme ratios.

MANOVA results for standardized EEA at Arboretum showed a significant sampling period effect (P≤0.0001), and treatment effect approached significance

(P=0.0660). There was no significant overall interaction (P=0.2184). At Fuller, there was a significant sampling period effect (P≤0.0001), and interaction effect approached significance (P=0.0682). Overall treatment effect was not significant (P=0.2690). An overall treatment effect was significant for South (P=0.0002), and sampling period was also significant (P≤0.0001). However, the interaction was not significant (P=0.7378).

Two-way ANOVAs for individual enzyme activities revealed a significant treatment difference only for BG (P=0.0048) at Arboretum. Activity of BG was higher in NM plots (3.73±1.11 vs. 3.69±1.17 nmol h^{-1} μg C_{mic}^{-1}; Figure 4). Sampling period effects were significant for all enzymes (Table 4). At Fuller, treatment effects were not significant for any individual enzyme activities. However, treatment effects for LAP approached significance (P=0.0096) with activity higher in NM plots (2.06±0.78 vs. 2.01±0.72 nmol h^{-1} μg C_{mic}^{-1}; Figure 4). Additionally, there were significant sampling period effects for all enzymes assayed (Table 4). At South, differences between treatments were significant for BXYL (P≤0.0001) and PHOS (P≤0.0001) and approached significance for CBH (P=0.0060). Plots with garlic mustard had higher CBH activity (0.27±0.04 vs. 0.18±0.03 nmol h^{-1} μg C_{mic}^{-1}) while plots without garlic mustard had higher BXYL (0.16±0.02 vs. 0.06±0.01 nmol h^{-1} μg C_{mic}^{-1}) and PHOS (1.67±0.10 vs. 1.03±0.12 nmol h^{-1} μg C_{mic}^{-1}; Figure 4) activities. Effects of sampling period were significant for all enzymes except for PHOS (P=0.1316), Urease (P=0.0231) and Perox (P=0.0396; Table 4).

Pillai's trace showed that ratios between carbon and nitrogen acquiring enzymes (C:N) and carbon and phosphorus acquiring enzymes (C:P) only approached significance

for treatments (P=0.0924), but were significant for sampling periods (P≤0.0001) at Arboretum. At Fuller, treatment effect approached significance (P=0.0607) and sampling period effect was significant (P≤0.0001). At South, treatment effect (P≤0.0001) and sampling period effect (P=0.0022) were both significant.

At Arboretum, two-way ANOVAs for enzyme ratios revealed a treatment effect approaching significance for C:P with GM plots having a lower ratio (0.66±0.04 vs. 0.77±0.04; P=0.0282). No treatment difference was found for C:N (1.55±0.11; P=0.5696). However, there was a significant effect of sampling period for C:N (P≤0.0001; Figure 5). At Fuller, treatment effect approached significance for C:P (1.22±0.08 in GM plots vs. 1.13±0.05 in NM plots; P=0.0400) but not C:N (1.36±0.07; P=0.1013; Figure 5). Additionally, sampling period effect was significant for C:N (P≤0.0001) and C:P (P=0.0126). Significant treatment effects did occur at South for both C:N (P=0.0004) and C:P (P≤0.0001). Carbon to nitrogen EEA ratio was higher in NM plots (2.30±0.13 vs. 1.86±0.18), but C:P was higher in GM plots (0.85±0.12 vs. 0.76±0.09; Figure 5). Also, an interaction effect for C:N approached significance (P=0.0468), possibly due to large treatment differences in Fall and Summer 2007. Sampling period effect was only found for C:N (P≤0.0001).

3.4 Microbial Community Structure

A total of 118 distinct bands were detected in soil samples collected from Arboretum. Of those, only 50 bands were common to communities from both soils in NM and GM plots. Communities in NM plots had a significantly higher number of bands (44.8±2.35 vs. 31.8±5.92; P=0.0347) when compared to GM plots. Additionally, NM

plots had higher *H′* (3.57±0.07 vs. 3.15±0.17; P=0.0226) and *D* (0.96±0.003 vs. 0.95±0.007; P=0.0246). At Fuller, 105 different bands were detected with only 57 bands in common between NM and GM communities. Soil communities had significantly higher richness in NM plots with 43.2±0.97 separate bands present compared to only 33.6±2.01 bands in communities from GM plots (P=0.0056). Additionally, diversity was significantly higher in NM plots (3.55±0.04 vs. 3.31±0.06 and P=0.0122 for *H′*; 0.97±0.002 vs. 0.96±0.002 and P=0.0255 for *D*). One hundred thirteen distinct bands were detected in DGGE profiles from South soils. Only 38 were found in communities from both treatments. Contrary to the other two sites, richness and diversity at South were slightly higher in GM plots, but there were no significant differences between treatments (P= 0.1338 for *S*, P=0.1279 for *H′*, and P=0.1814 for *D*).

Dendrograms indicated that bacterial communities were more similar within treatments than between treatments, but in most cases, similarities were relatively low. At Arboretum, bacterial communities clustered separately with communities from plots without garlic mustard being less than 50 percent similar to communities from plots with garlic mustard (Figure 6). However, the Winter 2008 GM community did not fall into either cluster and had 0 percent similarity with all other communities (not shown). Two distinct clusters also formed at Fuller (Figure 6). Bacterial communities from four of the five GM plots clustered together with 76 percent similarity. The Early Spring 2007 GM community was more similar to NM communities, which formed a cluster with 63 percent similarity. Both cluster were approximately 54 percent similar. At South, bacterial communities from NM plots were 59 percent similar, forming a distinct cluster to which GM bacterial communities were 47 percent similar (Figure 6).

3.5 Principal Components Analysis

The first two principal components explained 55.2 percent of overall variance at Arboretum. The first PC explained 40.4 percent of the variation and was negatively correlated with NAG, CBH, AG, BG, LAP, BXYL, and PHOS. Principle component 2 explained 14.8 percent of the variation, was positively correlated with moisture content, N_{mic}, DOC, and DON, and was negatively correlated with urease and nitrate. Overall, treatments were not distinct from one another along PC 1 or PC 2 (Figure 7). At Fuller, the first two PCs explained 57.9 percent of total variance. Principle component 1 explained 43.1 percent of the variation and was negatively correlated with NAG, CBH, BG, and PHOS. Principle component 2 explained 14.8 percent of the variation and was negatively correlated with urease, moisture, DOC, nitrate, and phosphate. There was no overall distinction between treatments along either PC1 or PC 2. The first two PCs at South explained 52.3 percent of the total variance. Component 1 explained 32.5 percent of the variation, was positively correlated with DOC, and negatively correlated with NAG, CBH, AG, and LAP. The second component explained 19.8 percent of the variation, was positively correlated with pH, C_{mic}, N_{mic}, and phosphate, and was negatively correlated with PHOS. Overall variation between treatments occurred only in relation to PC 2 with GM plots tending to have higher pH, C_{mic}, N_{mic}, and phosphate but lower PHOS activity (Figure 7).

3.6 Partial Mantel Analysis

At Arboretum, the regression accounted for only 2 percent of the variance, and no significant correlations were found between EEA and bacteria community composition

(P=0.427) or soil physical and biogeochemical properties (P=0.235). However, there were significant correlations at both Fuller and South. At Fuller, the regression accounted for 61 percent of the variance, and there was a significant relationship between EEA and soil properties (P=0.008), but the relationship with community composition only approached significance (P=0.091). The opposite trend was seen at South. Extracellular enzyme activity was significantly related to bacteria community composition (P=0.047), but only approached significance for soil properties (P=0.072), with the regression accounting for 45 percent of the variance.

Chapter 4: Discussion

Alliaria petiolata poses a threat to North American woodlands. It is able to invade relatively undisturbed areas that were previously considered unsusceptible to invasions (Nuzzo 1993; Nuzzo 1999). Additionally, its presence can cause decreases in overall plant species diversity as well as abundance and growth rate of several native woody and herbaceous plant species (Meekins and McCarthy 1999; Vaughn and Berhow 1999; Prati and Bossdorf 2004; Aminidehaghi et al. 2006; Stinson et al. 2006; Stinson et al. 2007). It also decreases the survival of certain butterfly species (Bowden 1971; Huang et al. 1994; Porter 1994; Haribal and Renwick 1998; Haribal et al. 2001; Renwick et al. 2001). The present study indicates that *A. petiolata* impacts can also extend to belowground systems. Both microbial community composition and enzyme activity were significantly altered as were other soil physical and chemical characteristics (carbon, nitrogen, and phosphate concentrations, SOM, moisture, and pH). However, treatment differences were not consistent between study sites or in some cases, even sampling periods.

Site-specific treatment differences are likely due to inherent differences in soils between sites. If differences in soil abiotic factors, microbial community composition, or microbial community function existed between sites preinvasion, those factors will likely react differently once the exotic is introduced (Wolfe and Klironomos 2005). Background values (when *A. petiolalta* absent) for soil properties, nutrient availability, extracellular

enzyme activities, and microbial community diversity naturally varied between sites so it is not surprising that the effect of *A. petiolata* on these variables would also differ by site. Additionally, density of *A. petiolata* stands varied between sites, with South Park having the highest densities.

Because this study was conducted in the field and were not manipulative, observed differences cannot conclusively be attributed to *A. petiolata*. It is possible that *A. petiolata* may be preferentially colonizing microsites where differences in soil factors existed preinvasion (Ehrenfeld et al. 2001). If site preference is occurring, it may also explain the inconsistency in treatment effects. It is however more likely that many of the observed treatment differences are due to *A. petiolata* presence rather than natural microsite differences, because results from studies of exotic plant effects involving greenhouse experiments or exotic removal verify that differences in soils can be attributed to the exotic plant itself (see Ehrenfeld 2003 for a review).

4.1 Soil Properties

Moisture content was significantly lower in plots with *A. petiolata* at Fuller Preserve and during Fall 2006 at Stranahan Arboretum (Figure 1). Lower moisture content in invaded plots is generally attributed to exotic plants having deeper or larger root systems or higher evapotranspiration rates due to greater leaf area (Gordon 1998; Ehrenfeld 2003). However, moisture differences were more likely due to *A. petiolata* microsite preference. Plots without *A. petiolata* at Arboretum and Fuller were more frequently flooded following rain events or snow melts compared to plots with *A. petiolata* stands (personal observation).

Soils in this study generally contained between 10 and 30 percent organic matter (Figure 2). Differences between treatments only occurred at Arboretum where SOM content was significantly lower under *A. petiolata*, especially in Early Spring 2007 (Figure 2). Soil organic matter content is determined by the relationship between inputs through plant litter decomposition and losses due to SOM decomposition (Swift et al. 1979; Smith and Paul 1990; Bot and Benites 2005). Therefore, lower SOM in GM plots could indicate that either lower plant litter inputs, greater SOM decomposition, or both are occurring in invaded plots. Then again, treatment differences could have been due to the presence of introduced Asiatic earthworms, which can greatly decrease litter and humus layers (Bohlen et al. 2004; Norden 2008). Additionally, Asiatic earthworms were abundant at the Arboretum and appear to avoid wetter areas (Norden 2008), which may explain why SOM was higher in the more frequently flooded NM plots at that site.

Soil pH was generally unaffected by *A. petiolata* but was higher under *A. petiolata* stands at South. Exotic species including *Berberis thunbergii* and *Microstegium vimineum* have also been found to increase pH in forest ecosystems (Kourtev et al. 1998; Kourtev et al. 1999; Ehrenfeld et al. 2001; Kourtev et al. 2003). Elevated pH is generally attributed to higher rates of N mineralization because hydroxyl ions (OH^-) are released when organic N is converted to ammonium (Haynes and Swift 1988; Ehrenfeld 2003). Differences in pH can also be attributed to differences in litter quality as some litter types can alter pH during decomposition (Swift et al. 1979; Ehrenfeld et al. 2005). However, pH differences between treatments could also be due to *A. petiolata* microsite preference. *Alliaria petiolata* has been found to benefit from higher soil alkalinity and nutrient content and may therefore colonize areas with those characteristics (Cavers et al. 1979).

4.2 Nutrient Pools

In general, C, N, and P cycling are stabilized by different mechanisms so they will be affected by *A. peitiolata* independently (Eviner and Chapin 2003). Nitrogen and phosphorus mineralization rates will depend on the availability of inorganic N and P respectively, while immobilization will depend on plant or microbial nutrient demand (Swift et al. 1979). However, complex relationships exist between nutrient cycles. Higher C and P availability can stimulate N mineralization, and higher C availability can stimulate P mineralization (Pastor et al. 1984; Haynes and Swift 1988; Tate and Salcedo 1988).

Carbon cycling is mostly associated with shifts in soil biota, which can alter microbial energy (C) demand (Ehrenfeld 2003; Kourtev et al. 2003). However, despite bacterial community shifts that occurred at all sites, only the amount of carbon immobilized in microbial biomass at Fuller and available as DOC at Arboretum during Fall 2006 varied between treatments (Figure 3). In this case, differences may be related to the higher soil moisture content in NM plots at Fuller and in NM plots during Fall 2006 at Arboretum. Organic carbon and microbial biomass carbon can increase as moisture increases due to increased C flushes through leaching from organic matter or root inputs (Smith and Paul 1990; Eaton 2001).

Nitrogen cycling is strongly related to litter C:N ratio and is driven by microbial C demand (McGill and Cole 1981; Eviner and Chapin 2003). Unfortunately, the nutrient content of *A. petiolata* tissues was not measured in this study and has not been quantified in other studies. However, in general, most exotic plants have relatively high N content in their litter, resulting in higher nitrogen mineralization and nitrification rates in invaded

areas (Ehrenfeld et al. 2001; Ehrenfeld 2003; Kourtev et al. 2003). On the other hand, higher nitrate levels in plots with *A. petiolata* at South may have been due to higher pH. In forest soils, very little nitrification occurs at low levels of pH (5.3 or less), and the greatest nitrification occurs at 6.5 and higher (Ste-Marie and Paré 1999). Additionally, at South, N_{mic} was higher in GM plots at South so there was also greater N immobilization. In contrast, N_{mic} was lower in GM plots at Arboretum during Fall 2006.

In studies of exotic plant effects, impacts on P availability are rarely studied. This study only measured extractable inorganic orthophosphate so results do not indicate the full extent of *A. petiolata* effects on P availability. Phosphorus mineralization is driven by the availability of inorganic P and plant and microbial demand for P (Swift et al. 1979; McGill and Cole 1981; Tate and Salcedo 1988; Eviner and Chapin 2003). Extractable phosphate availability was higher overall in GM plots at South but was higher in NM plots at Arboretum during Fall 2006 (Figure 3). Higher extractable P may be due to higher plant uptake of P. Although this seems counterintuitive, decomposition of litter with low C:P ratio stimulates greater mineralization of P from SOM, and as long as mineralization rates are higher than immobilization rates, inorganic P will increase (Swift et al. 1979; McGill and Cole 1981; Parton et al. 1988). However, at South, increased P availability may have been more likely due to higher soil pH. Phosphate availability is affected by pH with availability being highest around 6.5 and decreasing as pH decreases (Swift et al. 1979).

4.3 Microbial Community Function

Extracellular enzyme activity can be used as an indicator of soil ecological change

(Dick and Tabatabai 1992). Nevertheless, it cannot be assumed that changes in individual enzyme activities will result in changes in ecological processes because these are complex processes that involve the interaction of multiple enzymes (Sinsabaugh et al. 1991; Sinsabaugh 1994). However, activities of carbon, nitrogen, and phosphorus acquiring enzymes are inversely related to the availability of C, inorganic N and P respectively and can therefore be used as indicators of relative microbial nutrient limitations (Sinsabaugh et al. 1993; Sinsabaugh 1994; Sinsabaugh and Moorhead 1994; Kourtev et al. 2002).

The relationship between enzyme activity and nutrient availability was only clearly evident in one instance at South Park. Higher phosphate availability in GM plots (Figure 3) was accompanied by lower acid phosphatase activity (Figure 4). Alternatively, the difference in PHOS activity may be due to the higher pH in GM plots rather than P availability. As soil pH increases, the activity of PHOS decreases (Acosta-Martínez and Tabatabai 2000; Dick et al. 2000). However, because the optimal pH range for PHOS activity is 6.5 or less (Acosta-Martínez and Tabatabai 2000), and the pH of both GM and NM plots fell within that range, it is more likely that P availability was the primary cause of differences.

Additional differences occurred in EEA, but they did not correspond with differences in nutrient availability. At Arboretum, β-glucosidase activity was lower in GM plots (Figure 4), but DOC availability only decreased in GM plots during Fall 2006 (Figure 3). However, BG can also be highly correlated with SOM availability (Sinsabaugh et al. 2008), and SOM was significantly lower in GM plots at Arboretum (Figure 2). Additionally, β-D-cellobiosidase and β-xylosidase activities at South and

leucine aminopeptidase activity at Fuller (Figure 4) varied between treatments despite there being no differences in available C or N respectively. This was unexpected considering partial Mantel tests showed correlations between enzyme activity and nutrient availability at both Fuller and South. Then again, correlations were also seen between EEA and bacteria community composition at both sites so changes in enzyme activity may have been more related to shifts in microbial community composition than nutrient availability.

Principal components analysis at all three sites showed that NAG, CBH, and PHOS activities were correlated with either PC 1 or PC 2. In all cases, NAG, CBH, and PHOS were negatively correlated. Despite these similarities, treatments did not consistently separate along any of these variables. In fact, no treatment differentiation was found along any principal components except along PC 2 at South, where GM plots were associated with lower PHOS activity (Figure 7).

Ratios of enzyme activities can also be used as indicators of resource limitation because microbial communities maximize their productivity by allocating resources between C, N, and P acquiring enzymes based on which nutrients are needed most (Sinsabaugh et al. 1993; Sinsabaugh and Moorhead 1994). Both Arboretum and South had higher overall productivity of P-related enzyme than C-related enzyme (ratio < 1:1) indicating relatively P-limited microbial conditions, but no sites exhibited microbial N-limited conditions (Figure 5). Furthermore, ratios of C- and P-related enzymes differed between treatments at all sites, although differences only approached significance at Arboretum and Fuller. At Fuller and South, microbial communities in NM plots were more P-limited than communities in GM plots, but this trend was reversed at Arboretum

(Figure 5). Ratios of C- and N-related enzymes differed significantly between treatments only at South, where more energy was directed toward N-related enzymes than C-related enzymes in GM plots compared to NM plots, indicating more N-limited conditions in GM plots. The C:N ratios of microbial biomass (C_{mic}:N_{mic}) at the three sites were analyzed to determine why the ratio of C- to N-related enzymes only differed between treatments at South. No difference between treatments occurred at Arboretum (P=0.6270) or Fuller (P=0.4996). However, C_{mic}:N_{mic} was significantly lower in GM plots (5.45±0.30) compared to NM plots (6.58±0.41) at South (P=0.0012). The higher microbial N demand in GM plots is likely the driving force behind higher allocation of resources to N acquisition in GM plots.

4.4 Microbial Community Composition

The soil community plays essential roles in ecosystem properties and processes. If the abundance or presence of certain species is altered, it can affect the functional properties and processes above- and below-ground (Bot and Benites 2005; Wolfe and Klironomos 2005). Although multiple microbial species may perform the same functions, diversity loss can still alter ecosystem functions because different species may have different environmental tolerances, substrate preferences, or C, N, and P stoichiometric ratios (Eviner and Chapin 2003; Bot and Benites 2005).

Exotic plants can affect microbial composition by shifting bacteria:fungi and gram positive:gram negative bacterial ratios and interfering with mycorrhizal associations (Kourtev et al. 2002; Kourtev et al. 2003; Belnap et al. 2005). Results from this study show that *A. petiolata* can cause shifts in the bacteria community as well as losses in the

overall number of species present and community diversity, despite having relatively little affect on microbial function. Shifts in microbial community composition can have large impacts on plant community structure and diversity (Bever et al. 1997; Bever 2003; Klironomos 2002) and may result from allelopathy. *Alliaria petiolata* root, leaf, and shoot tissues contain glucosinolates, including sinigrin and glucotropaeolin, which produce highly toxic isothiocyanates when hydrolyzed (Vaughn and Berhow 1999). Allelochemicals in *A. petiolata* are known to prevent germination of arbuscular mycorrhizal fungi, reduce AMF diversity, and inhibit AMF associations with plants (Roberts and Anderson 2001). Sinigrin hydrolysis products have also been found to greatly inhibit the growth of several bacterial species, especially gram-negative bacteria (Rudat 1957; Brabban and Edwards 1995).

There are several limitations that are involved when using PCR-DGGE to determine bacterial community diversity, which may have affected results. By calculating diversity using relative band intensities, it was assumed that the final band intensity was related to the initial abundance of that bacterial strain. However, preferential amplification or different amplification efficiencies could have occurred (Rainey et al. 1994; Heuer and Smalla 1997). Additionally, some bacterial strains posses multiple *rrN* operons and form multiple bands, while other strains comigrate due to similar electrophoretic mobilities (Nübel et al. 1996; Heuer and Smalla 1997). This could have resulted in over- or underestimating diversity. Despite these limitations, band intensity is related to initial cell and relative phylotype abundances (Gelsomino et al. 1999; Konopka et al. 1999) and can provide an approximate indication of diversity.

Chapter 5: Conclusions

Alliaria petiolata presence impacted soil physical, biogeochemical, chemical, and biotic characteristics. However, differences varied in both magnitude and direction between sites and across sampling dates. The majority of treatment differences occurred at South Park and Stranahan Arboretum sampling sites. Of the 26 variables measured at each site, 11 differed between treatments at South Park, 11 differed at Stranahan Arboretum, and 8 differed at Fuller Preserve. When differences between treatments did occur, the measured variables generally decreased in the presence of *A*. *petiolata* (19 cases out of 30). The majority of exceptions occurred at South Park where values were generally elevated in plots with *A*. *petiolata*. The results from this study are not surprising considering that other studies of exotic plant impacts on soil properties have also found inconsistent effects along spatial (Kourtev et al. 1998; Kourtev et al. 1999; Scott et al. 2001; Hook et al. 2004; Chapuis-Lardy et al. 2006; Pritekel et al. 2006) and temporal (Ehrenfeld et al. 2001; Evans et al. 2001) scales.

Although many of the observed effects were found to be site-specific, they can still result in ecosystem changes. Changes in nutrient availability, microbial biomass, and microbial community composition can lead to changes in decomposition, SOM and nutrient turnover rates, and competition (Swift et al. 1979; Smith and Paul 1990; Hobbie and Vitousek 2000). For example, a main output of microbial biomass activity is mineral N and P from SOM decay (Smith and Paul 1990). If the size of microbial biomass pools

decrease, it can result in lower nutrient availability and greater competition for nutrients between plants and microbes. These changes could additionally alter the composition of current plant communities and future communities that develop at that site (Bever 2003; Kourtev et al. 2003; Wolfe and Klironomos 2005). If changes benefit *A. petiolata* or other exotic plant species, it could create a feedback loop that enhances the invasiveness of the site (Ehrenfeld et al. 2001; Ehrenfeld 2003). For example, *A. petiolata* may benefit itself in locations with relatively low nutrient availability. South Park had the lowest organic nitrogen, inorganic nitrogen, and phosphate availability in non-invaded plots of the three study sites, but it was also the only site where N and P pools increased with *A. petiolata* presence. Positive feedback has been implicated as one cause of increased abundance for several exotic, invasive plant species including *A. petiolata* (Klironomos 2002).

It may also be important to include the possible effects of *A. petiolata* on soils in management or restoration strategies. If changes to soil biotic community composition or ecosystem processes are long-lasting, more intensive rehabilitation methods may be required beyond just removing the exotic species population. Strategies may also need to be customized by location as exotic plant impacts vary spatially. It is also important to consider potential *A. petiolata* impacts because effects may become more complex due to interactions of multiple exotic species, increases in atmospheric carbon dioxide or nutrient inputs, and global climate change (Wolfe and Klironomos 2005).

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Chapter 7: Tables

Table 1. Schedule for soil sample collection with main morphological stages of *Alliaria petiolata* during each sampling period. Code indicates the abbreviation for each sampling period used in table headings and figure axes.

Sampling Period	Morphological Stage	Code
October 2006	Complete senescence of second-year plants	F06
March-April 2007	Seed germination	ES07
	Bolting of second-year plants	
May 2007	First-year rosette formation	LS07
	Flowering of second-year plants	
June 2007	Seed set	S07
October-November 2007	Complete senescence of second-year plants	F07
January-February 2008	Over-wintering basal rosettes	W08

Table 2. Extracellular enzymes assayed with the abbreviations used in this study, function, enzyme commission numbers (E.C.), and corresponding substrates (MUB=methylumbelliferyl and L-DOPA=L-3,4-dihydroxyphenylalanine). Information is taken from Pettit et al. 1976, Saiya-Cork et al. 2002, and Weintraub et al. 2007.

Enzyme	Abbreviation	Function	E.C.	Substrate
β-1,4-glucosidase	BG	Catalyzes hydrolysis of β-D-glucose from β-D-glucosides	3.2.1.21	4-MUB-β-D-glucoside
α-1,4-glucosidase	AG	Catalyzes hydrolysis of α-D-glucose, releasing α-D-glucose	3.2.1.20	4-MUB-α-D-glucoside
β-1,4-Xylosidase	BXYL	Degrades short xylan chains into xylose	3.2.1.37	4-MUB-β-D-xyloside
β-D-1,4-cellobiosidase	CBH	Catalyzes hydrolysis of β-D-glucoside, releasing cellobiose	3.2.1.91	4-MUB-β-D-cellobioside
β-1,4-N-acetyl-glucosaminidase	NAG	Catalyzes hydrolysis of N-acetyl-beta-D-glucosaminide in chitin-derived oligomers	3.1.6.1	4-MUB-N-acetyl-β-D-glucosaminide
Leucine amino peptidase	LAP	Catalyzes hydrolysis of leucine and other amino acids from peptides	3.4.11.1	L-Leucine-7-amino-4-methylcoumarin
Acid Phosphatase	PHOS	Mineralizes organic P into phosphate	3.1.3.2	4-MUB-phosphate
Urease	Urease	Catalyzes hydrolysis of urea, releasing ammonium	3.5.1.5	Urea
Phenol oxidase	Phenox	Oxidizes aromatic C compounds with O_2	1.10.3.2	L-DOPA
Peroxidase	Perox	Catalyzes oxidation through H_2O_2 reduction	1.11.1.7	L-DOPA

Table 3. Nutrient dynamics for sampling periods at Stranahan Arboretum, Fuller Preserve, and South Park. Values reported are overall mean±standard error in μg C, N, or P g dry soil^{-1}and probability (P). P-values in bold are significant (P≤0.008), P-values in italics are approaching significant (0.017≥P≥0.008), and significant differences between sampling periods are indicated with different letters.

Arboretum	F06	ES07	LS07	S07	F07	W08	P
C_{mic}	38.0±3.8(a)	ND	305.0±21.8(b)	325.7±13.1(b)	283.3±13.1(b)	517.9±40.5(c)	**≤0.0001**
N_{mic}	68.2±9.5(a)	ND	62.4±4.1(ab)	52.2±2.8(ab)	44.8±2.2(b)	90.7±7.0(c)	**≤0.0001**
DOC	56.2±17(a)	ND	414.8±15.9(b)	47.7±9.7(a)	61.8±3.4(a)	636.4±51.7(c)	**≤0.0001**
DON	8.77±2.2(ac)	ND	71.1±3.7(b)	6.86±1.5(ac)	1.89±0.7(a)	14.3±2.2(c)	**≤0.0001**
NH_4	2.38±1(ab)	ND	0.52±0.4(a)	2.04±0.9(ab)	1.39±0.2(a)	3.88±0.3(b)	**0.0005**
NO_3	2.46±0.5(a)	ND	0.17±0.1(b)	0.12±0.02(b)	4.32±0.7(a)	3.80±0.8(a)	**≤0.0001**
PO_4	0.21±0.1(a)	ND	0.00±0.0(ab)	0.00±0.0(b)	0.01±0.0(ab)	0.41±0.1(c)	**≤0.0001**
Fuller	F06	ES07	LS07	S07	F07	W08	P
C_{mic}	68.2±4.4(a)	ND	849.6±52(bc)	846.0±5(bc)	693.4±43.8(b)	1155.9±241(c)	**≤0.0001**
N_{mic}	119.0±9(ab)	ND	159.2±4.1(ac)	140.7±3(abc)	110.1±2.2(b)	219.1±43.0(c)	**0.0005**
DOC	14.5±1.3(a)	ND	984.9±49.9(b)	67.1±8.1(c)	84.4±3.4(c)	1500.9±264(b)	**≤0.0001**
DON	2.32±0.7(a)	ND	172.8±9.8(b)	6.75±0.7(c)	8.81±1.1(c)	52.7±21.6(d)	**≤0.0001**
NH_4	9.70±0.6(a)	ND	0.80±0.5(b)	2.04±0.5(c)	1.39±0.3(b)	15.8±10.1(ac)	**≤0.0001**
NO_3	5.15±0.6(a)	ND	0.91±0.3(bc)	0.48±0.1(b)	1.41±0.2(c)	9.13±1.4(a)	**≤0.0001**
PO_4	0.05±0.0(a)	ND	0.19±0.04(bc)	0.07±0.1(a)	0.07±0.02(ab)	1.80±0.9(c)	**≤0.0001**
South	F06	ES07	LS07	S07	F07	W08	P
C_{mic}	ND	395.5±40(a)	ND	253.0±21.0(b)	271.3±25 (ab)	241.4±22.1(b)	**0.0017**
N_{mic}	ND	76.6±10 (a)	ND	46.1±5.0(ab)	36.0±5.4(b)	50.8±5.5(ab)	**0.0006**
DOC	ND	9.36±5.1(a)	ND	92.9±9.4(b)	73.5±8.5(b)	318.2±23.9(c)	**≤0.0001**
DON	ND	8.75±2(ab)	ND	9.95±0.9(a)	5.13±0.8(b)	9.76±1.2(a)	**0.0068**
NH_4	ND	0.83±0.5(a)	ND	1.59±0.3(a)	0.86±0.2(b)	1.84±0.3(b)	**≤0.0001**
NO_3	ND	1.10±0.3(a)	ND	0.19±0.1(b)	4.38±0.8(ab)	2.73±0.6(b)	**0.0018**
PO_4	ND	0.09±0.07	ND	0.13±0.1	0.11±0.1	0.24±0.1	0.0206

Table 4. Standardized extracellular enzyme activities for sampling periods at Stranahan Arboretum, Fuller Preserve, and South Park. Values reported are overall mean±standard error in nmol h^{-1} μg $C_{mic}^{-1}$and probability (P). ND represents sampling periods when data was not available. P-values in bold are significant (P≤0.005), P-values in italics are approaching significant (0.010≥P≥0.005), and significant differences between samplilng periods are indicated with different letters.

Arboretum	F06	ES07	LS07	S07	F07	W08	P
NAG	9.71±1.2(a)	ND	0.59±0.06(b)	0.78±0.05(b)	0.40±0.01(c)	0.26±0.04(c)	**≤0.0001**
CBH	6.40±1.1(a)	ND	0.43±0.06(b)	0.42±0.03(b)	0.26±0.01(c)	0.16±0.01(c)	**≤0.0001**
AG	1.53±0.3(a)	ND	0.06±0.01(bc)	0.08±0.01(c)	0.06±0.002(b)	0.00±0.00(d)	**≤0.0001**
BG	18.7±1.8(a)	ND	1.26±0.17(b)	1.23±0.06(b)	0.85±0.02(c)	0.64±0.07(c)	**≤0.0001**
LAP	12.1±1.5(a)	ND	0.54±0.05(b)	0.24±0.02(c)	0.11±0.01(d)	0.01±0.01(e)	**≤0.0001**
BXYL	3.45±0.5(a)	ND	0.19±0.03(b)	0.18±0.02(b)	0.06±0.004(c)	0.05±0.01(c)	**≤0.0001**
PHOS	28.1±1.5(a)	ND	1.80±0.14(b)	1.74±0.22(b)	1.37±0.03(b)	0.84±0.07(c)	**≤0.0001**
Urease	49.7±12(a)	ND	0.00±0.00(b)	0.00±0.00(b)	3.53±0.26(ac)	1.15±0.31(c)	**≤0.0001**
Phenox	174.5±60(a)	ND	21.4±5.48(a)	6.75±2.58(ab)	10.9±0.75(ab)	0.90±0.57(b)	**0.0019**
Perox	169.5±36(a)	ND	13.0±4.36(b)	16.5±2.23(b)	19.1±1.43(ab)	3.92±1.19(b)	**0.0001**
Fuller	F06	ES07	LS07	S07	F07	W08	P
NAG	4.98±0.3(a)	ND	0.31±0.03(bc)	0.37±0.03(c)	0.20±0.04(b)	0.29±0.05(bc)	**≤0.0001**
CBH	5.52±0.4(a)	ND	0.32±0.04(b)	0.31±0.02(b)	0.22±0.05(b)	0.33±0.04(b)	**≤0.0001**
AG	1.29±0.1(a)	ND	0.06±0.01(bc)	0.10±0.01(c)	0.07±0.01(bc)	0.06±0.01(b)	**≤0.0001**
BG	12.9±1.3(a)	ND	0.72±0.05(bc)	0.83±0.06(c)	0.54±0.08(b)	0.78±0.07(bc)	**≤0.0001**
LAP	9.24±0.2(a)	ND	0.41±0.04(b)	0.25±0.03(c)	0.16±0.01(d)	0.14±0.02(d)	**≤0.0001**
BXYL	2.93±0.2(a)	ND	0.15±0.01(b)	0.16±0.01(b)	0.06±0.01(c)	0.12±0.01(b)	**≤0.0001**
PHOS	13.9±1.5(a)	ND	0.67±0.09(b)	0.66±0.05(b)	0.51±0.09(b)	0.69±0.08(b)	**≤0.0001**
Urease	3.24±1.9(a)	ND	0.67±0.39(a)	0.08±0.08(a)	1.15±0.42(ab)	4.19±0.80(b)	**0.0005**
Phenox	102.9±20(a)	ND	7.20±1.82(b)	8.73±2.63(b)	8.58±4.40(bc)	1.98±1.62(c)	**≤0.0001**
Perox	128.9±25(a)	ND	11.5±2.39(b)	7.18±1.67(b)	6.77±2.47(b)	10.2±3.37(b)	**0.0002**

South	F06	ES07	LS07	S07	F07	W08	P
NAG	ND	0.59±0.03(a)	ND	0.35±0.04(b)	0.41±0.05(b)	0.27±0.04(b)	**≤0.0001**
CBH	ND	0.39±0.05(a)	ND	0.19±0.02(b)	0.27±0.03(ab)	0.07±0.03(c)	**≤0.0001**
AG	ND	0.07±0.01(a)	ND	0.00±0.00(b)	0.03±0.01(c)	0.00±0.00(b)	**≤0.0001**
BG	ND	1.10±0.09(a)	ND	0.83±0.11(ab)	0.95±0.10(ab)	0.62±0.05(b)	**0.0017**
LAP	ND	0.16±0.03(a)	ND	0.03±0.01(b)	0.05±0.01(b)	0.00±0.00(c)	**≤0.0001**
BXYL	ND	0.18±0.03(a)	ND	0.10±0.02(b)	0.13±0.03(ab)	0.04±0.02(c)	**≤0.0001**
PHOS	ND	1.45±0.20	ND	1.49±0.24	1.45±0.12	1.00±0.15	0.1316
Urease	ND	0.24±0.12	ND	0.00±0.00	1.43±0.75	0.00±0.00	0.0231
Phenox	ND	0.27±0.27(a)	ND	11.02±3.62(ab)	23.82±2.85(bc)	37.7±7.70(c)	**≤0.0001**
Perox	ND	3.71±1.72	ND	10.1±3.63	2.28±1.44	19.9±6.66	0.0396

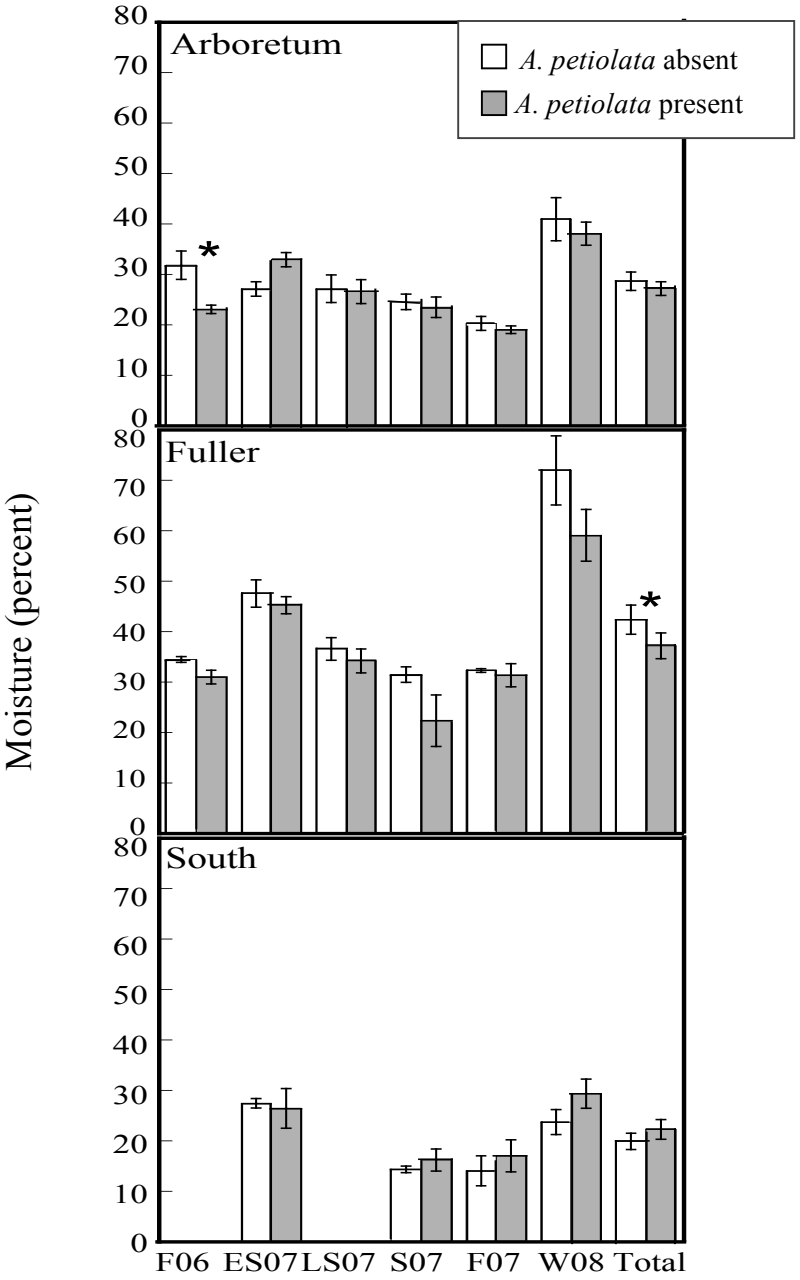

Figure 1. Moisture dynamics between treatments at the three sample sites. Bars are means with ± standard error. Sampling periods with significant differences between treatments ($P \leq 0.05$) are indicated with an asterisk. Data are represented as percent water g dry soil^{-1}.

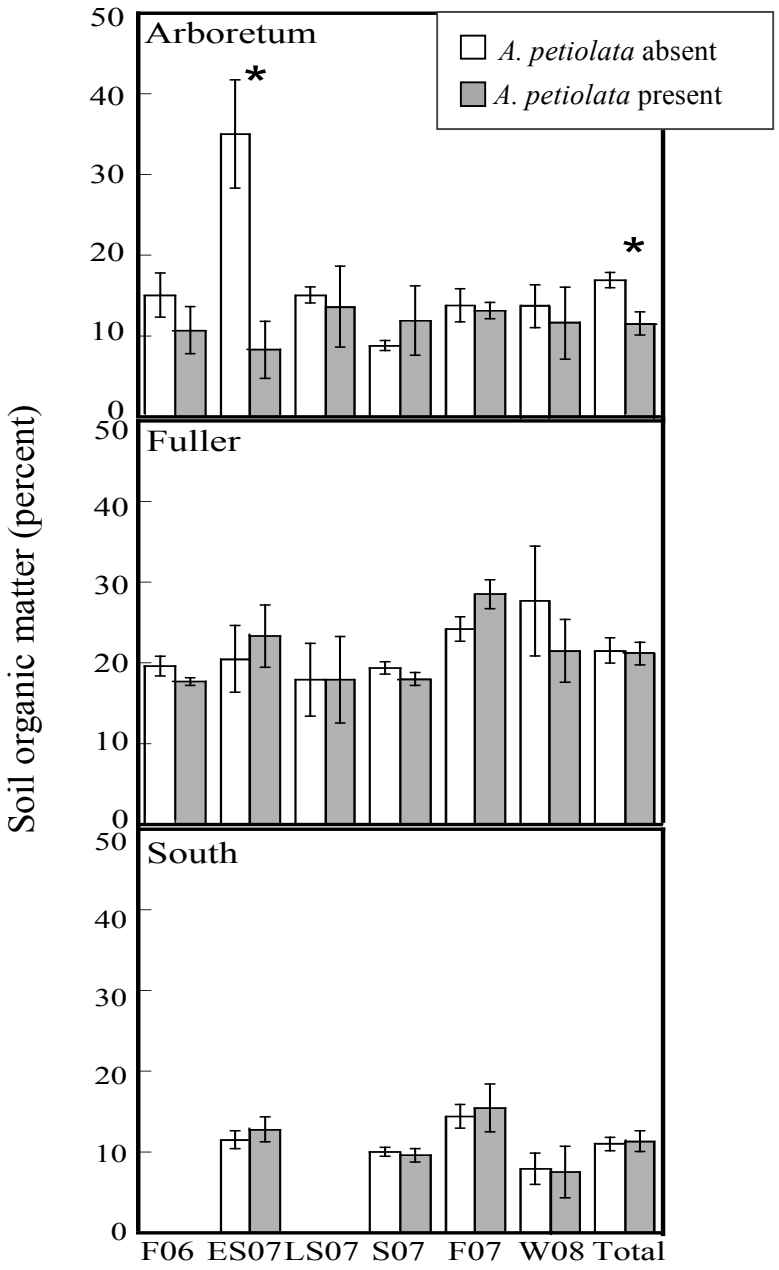

Figure 2. Soil organic matter dynamics between treatments at the three sample sites. Bars are means with ± standard error. Sampling periods with significant differences between treatments ($P \leq 0.05$) are indicated with an asterisk. Data are represented as percent organic matter g dry soil^{-1}.

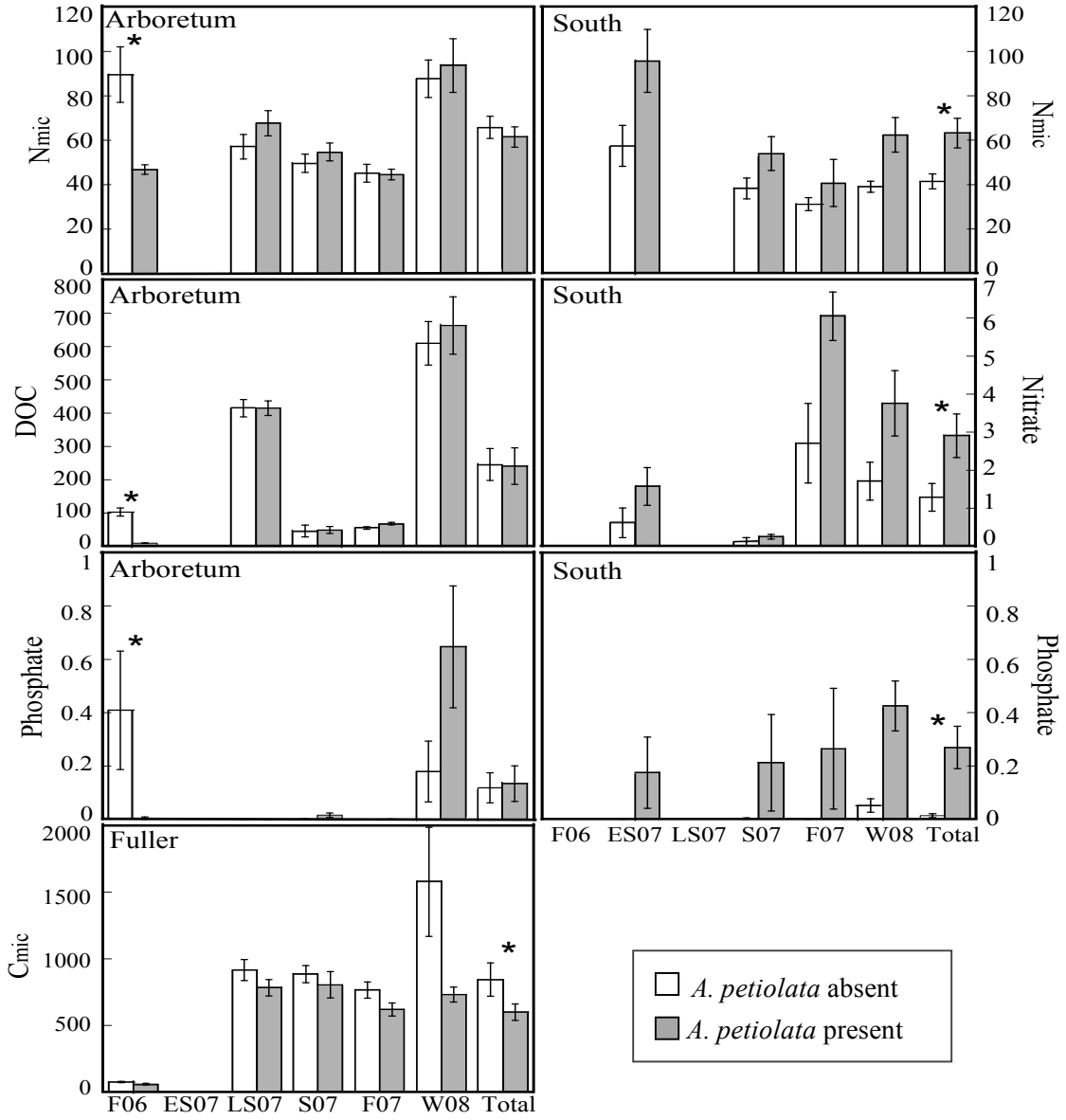

Figure 3. Nutrient dynamics between treatments at the three study sites. Bars represent mean ± standard error. Significant differences ($P \leq 0.0071$) between treatments are indicated with an asterisk. DOC and C_{mic} are expressed as μg C g dry soil^{-1}, N_{mic} and nitrate are expressed as μg N g dry soil^{-1}, and phosphate is expressed as μg P g dry soil^{-1}.

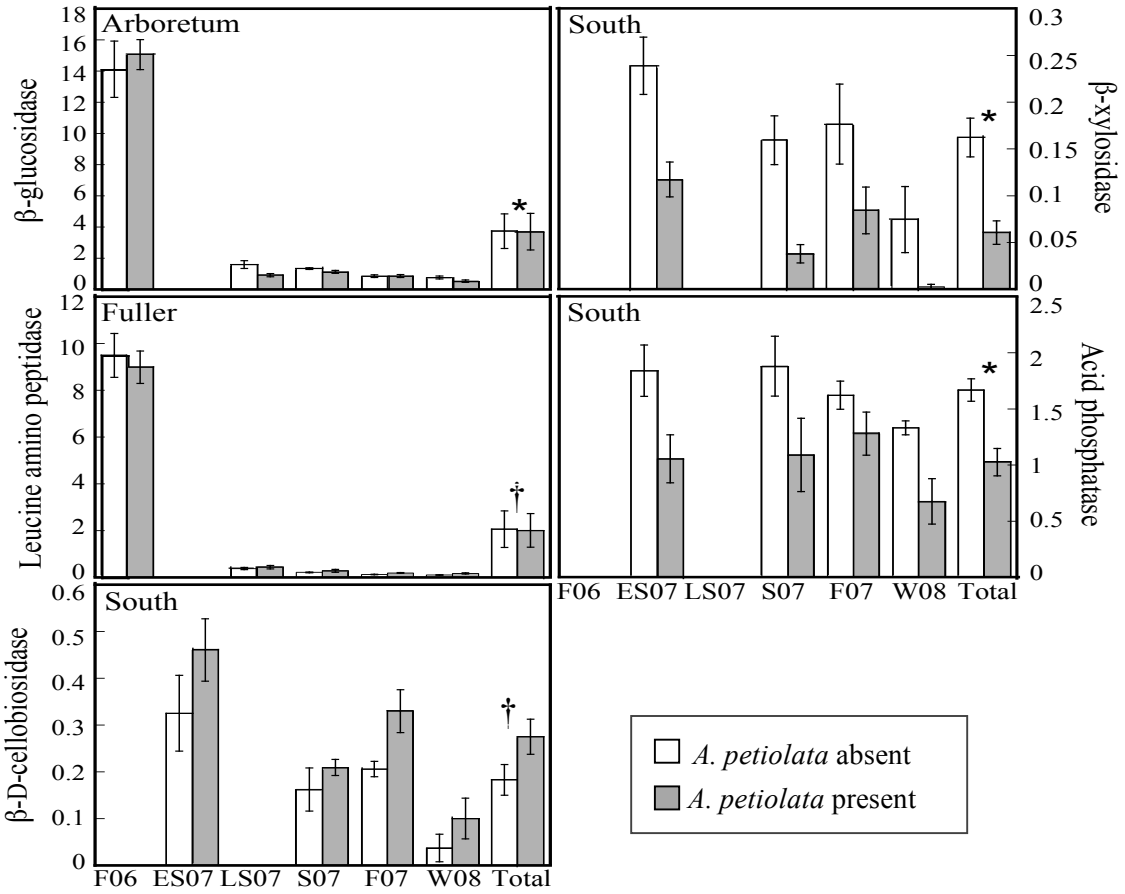

Figure 4. Standardized extracellular enzyme activity differences between treatments at the three study sites. Bars represent mean ± standard error. Significant differences (P≤0.005) between treatments are indicated with an asterisk, while differences approaching significant (0.01≤P≥0.005) are indicated with a †. All activities are reported as nmol h^{-1} μg C_{mic}^{-1}.

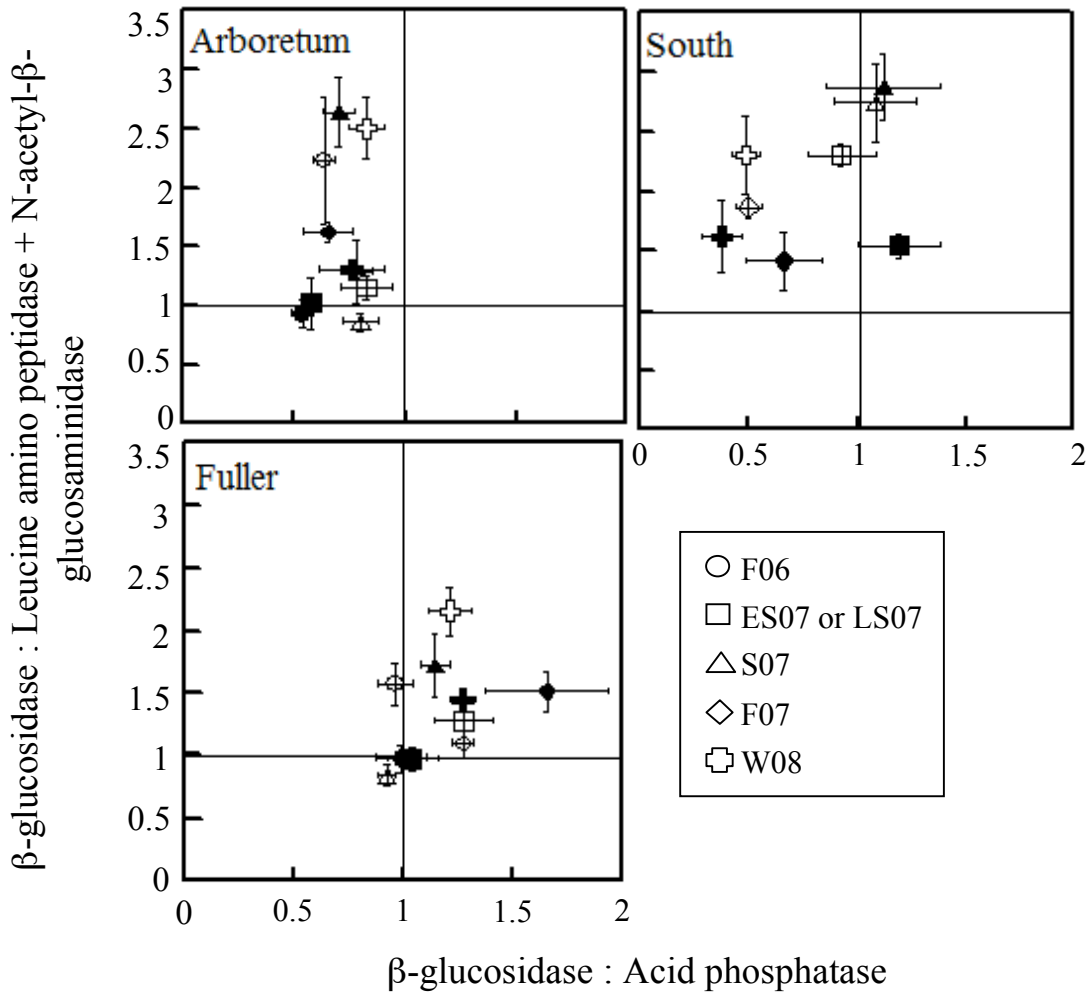

Figure 5. Ratios of carbon-acquiring enzyme activity (β-glucosidase) to phosphorus-acquiring (acid phosphatase) and nitrogen-acquiring (leucine amino peptidase + N-acetyl-β-glucosaminidase) enzyme activities at the three study sites. Symbols represent treatment [*A. petiolata* absent (open symbols) and *A. petiolata* present (closed symbols)] means ± standard errors by season.

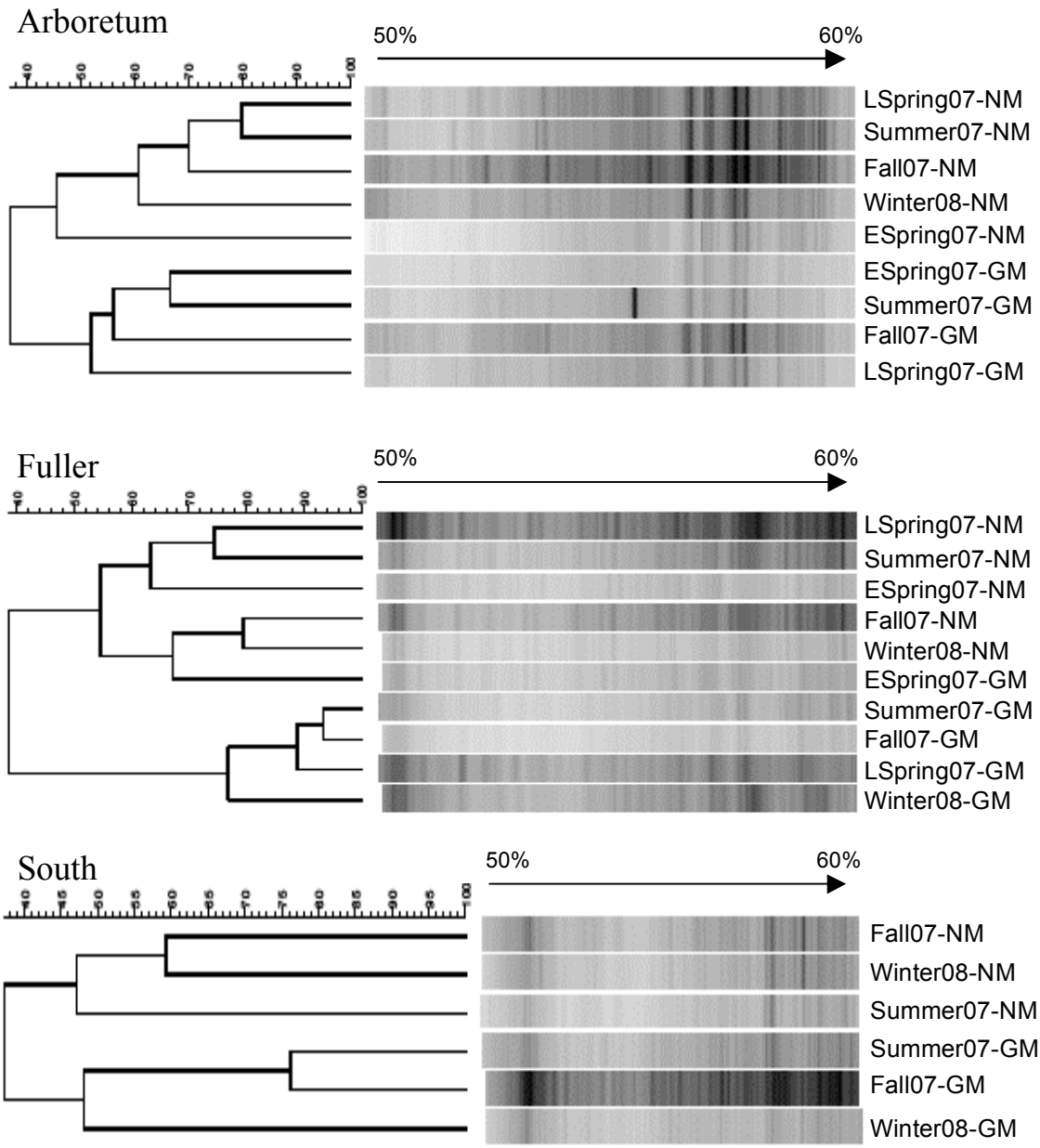

Figure 6. Cluster analysis results of polymerase chain reaction products separated through denaturing gradient gel electrophoresis at the three sites. Each lane negative image represents the community fingerprint for the treatment and sampling period indicated. Arrows indicate the direction of denaturing gel gradient from 50 percent concentration to 60 percent concentration.

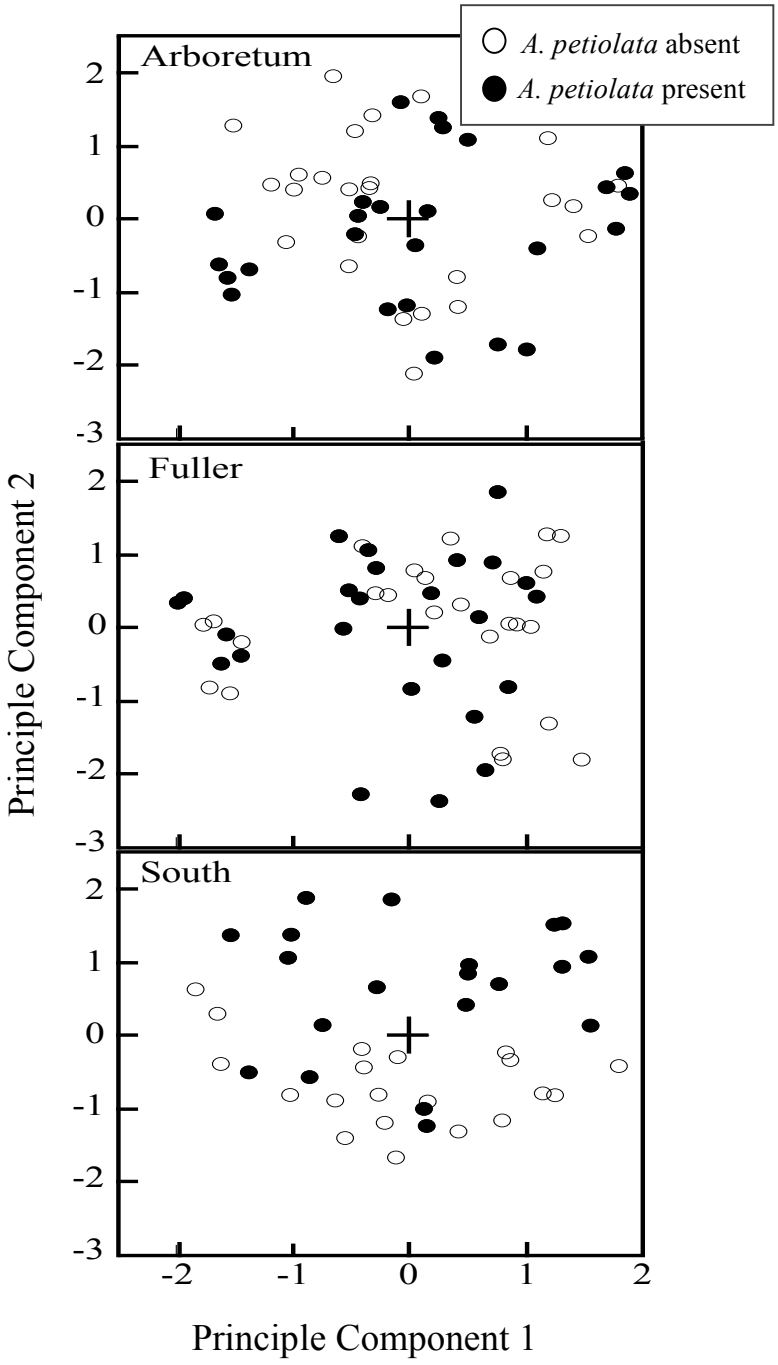

Figure 7. Principle components analysis results for the three study sites. Analyses included all of the measured variables except bacterial community richness and diversity. Only the two components, which explained the greatest variance, are shown. Crosshairs mark the origin.