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Microbial biomass and nitrogen availability under the invasive plant species *Lonicera japonica* and native grasses in wetland soil

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ABSTRACT

Invasive plants decrease aboveground biodiversity and suitable wildlife habitat. Wetlands are especially valuable ecosystems because they provide habitat, floodwater control, and function as filters for urban runoff. Wetland soils also act as sinks for nutrients. This characteristic reduces levels of excess nutrients often found in adjacent aquatic systems. The importance of soil functions in wetlands necessitates further investigation of the effects of invasive species on belowground nutrient pools. Approximately 75% of a small neighborhood wetland located in Fayetteville, Ark., has been invaded by *Lonicera japonica*. The effects of *L. japonica* and its replacement with native grasses on soil microbial biomass and nutrient pools were evaluated. Eight plots were established in April 2003. Four were left vegetated with the invasive species *L. japonica* while the other four were revegetated with transplants of five native grass species: *Andropogon gerardii*, *Schizachyrium* spp., *Sorghastrum nutans*, *Panicum virgatum*, and *Tripsacum dactyloides*. Soil samples were taken three times over the growing season, once prior to the removal of *L. japonica* and twice after transplanting occurred. Microbial biomass, soil carbon and nitrogen, Mehlich III- extractable phosphorus, pH, moisture content, and inorganic nitrogen were analyzed and significance was tested using a one-way ANOVA test ($P < 0.05$). Temporal changes in the inorganic N pool and pH were significant. However, data showed no significant differences between treatments for any of the properties tested, suggesting that data need to be collected for more than one growing season before significant changes may be observed following revegetation.

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MEET THE STUDENT-AUTHOR

Kimberly R. Payne

I am from Benton, Ark., but I was able to attend The Arkansas School for Mathematics and Sciences in Hot Springs, Ark., where I graduated from high school in 2000. I will graduate in August 2004 with a B.S.A. degree in environmental, soil, and water sciences. Through the Crop, Soil, and Environmental Sciences (CSES) Department I was able to aid in restoring a wetland, conduct independent research, and make presentations at two American Society of Agronomy (ASA) meetings. These experiences have allowed me to apply techniques taught in the classroom to pertinent research problems. Dr. Mary C. Savin served as my research advisor and as one of the CSES Club's primary advisors. With assistance from Dr. Savin and Dr. Charles P. West, I was able to guide the CSES Club's restoration and fundraising activities while serving as president for the 2003 calendar year. Organizing the club's activities allowed me to gain beneficial leadership and networking skills that will be indispensable throughout my career.

While attending the University of Arkansas, I received many awards and scholarships including: the Dale and Wilhelmina S. Hinkle Scholarship, Environmental Protection Association Scholarship, C. Roy Adair Scholarship, a certificate of appreciation from the City of Fayetteville for restoration efforts at Bryce-Davis Wetland, an award of second place at the ASA 2003 National Meeting in the Undergraduate Research Symposium Oral Contest, and the ASA Outstanding Senior Award for the University of Arkansas. I also received a Dale Bumpers Undergraduate Research Grant and a SILO Undergraduate Research Fellowship. These awards allowed me to continue my research through the spring and summer of 2004.

INTRODUCTION

Invasive plant species are considered to be a problem because they out-compete native vegetation, effectively reducing plant diversity. This reduction in diversity leads to a reduction in wildlife habitat and forage. Native plant species are beneficial to native wildlife because these two groups have co-evolved. Thus, native plant species most likely provide associated wildlife with the best resources. While biodiversity is not ranked as high as resilience and adaptability when determining ecosystem health, it is still used as a major indicator of ecosystem health (Schläpfer et al., 1999). The underlying assumption is that the greater the number of species that live in an area, the more productive and efficient the ecosystem. Studies monitoring the effect of plant diversity on nutrient retention have resulted in positive correlations (Schläpfer and Schmid, 1999). Schwartz et al. (2000) found that higher levels of diversity led to more durable and sustainable ecosystems. Stable, diverse ecosystems were resilient and able to "spring back" from changes within the environment more readily than ecosystems with low diversity of species. Thus, an understanding of how invasive plant species change an environment could enhance understanding of why diversity is better for stability.

Damage to ecosystems by invasive plant species can sometimes be measured economically; for instance, the annual cost in the United States associated with the losses due to invasive plant species and measures implemented to control these invasive plants is approximately $25,000,000 (Pimentel et al., 1999). While pasture and food crop producers absorb the majority of this cost, the Bureau of Land Management also spends millions of dollars trying to control invasive species (Bureau of Land Management, 2004). Although much is known about the interactions among plants, crops, and wildlife, and although progress is being made, an effective pro-

gram for preventing the spread of invasive plants appears to be far from reach. In 1999, President Bill Clinton created the Invasive Species Council, whose duties include preventing the spread of invasive species and restoring areas disturbed by invasive plants ("Invasive Species," 1999).

One example of an invasive species is *Lonicera japonica*. *Lonicera japonica* is an invasive plant vine found throughout eastern North America, including Arkansas, that out-competes native vegetation by producing expansive root systems and dense aboveground stands, with which native plants may be unable to compete for sunlight (Nuzzo, 1997). *Lonicera japonica* is able to outcompete other plants above- and belowground by producing rhizomes that allow for very rapid growth (Nuzzo, 1997). *Lonicera japonica* has invaded a small neighborhood wetland in Fayetteville, Ark. This wetland clearly shows the above ground adverse effects associated with *L. japonica*. Approximately 75% of the vegetation is *L. japonica*, and removal of the *L. japonica* from trees at the wetland reveals deep scars on the cambium. Dense mats of growth prevent sunlight from reaching the ground. This wetland is currently undergoing restoration by the University of Arkansas undergraduate Crop, Soil, and Environmental Sciences Club in conjunction with the Fayetteville Parks and Recreation Department and other local organizations. Restoration efforts aim to remove *L. japonica* and restore native vegetation that will provide habitat and forage for wildlife. The ultimate goal of this restoration effort is to utilize the wetland as a recreational area and an outdoor classroom. In this study, the objectives were to determine the effects *L. japonica* has on nutrient levels, soil pH, and microbial biomass, and how these properties change following revegetation with native grasses.

Little is known about the response of soil microbial communities to the displacement of native plants due to invasive species (Kourtev et al., 2002). The removal of invasive species in order to promote the growth of native species and restore plant biodiversity is often a challenging task. Since plant and soil microbial communities are so interwoven, it seems insight into interactions between invasive species and soil microbes, and the resultant soil changes, could provide a better understanding of that which is required to promote biodiversity and prevent infestations by invasive species.

MATERIALS AND METHODS

Site Description

The 0.2-hectare wetland being investigated is located in a neighborhood park in Fayetteville, Ark. Eight plots (2 m x 2 m) were established in April 2003. In May 2003, the aboveground portion of *L. japonica* was removed manually from four of the plots, which were then revegetated a week later with transplants of five native grass species: *Andropogon gerardii*, *Schizachyrium* spp., *Sorghastrum nutans*, *Panicum virgatum*, and *Tripsacum dactyloides*. Plots received no irrigation water or fertilizer.

Soil Samples

Eight random surface soil samples (10-cm depth) were collected and combined within each plot. Initial soil samples were collected in April 2003, while the dominant vegetation on all plots was *L. japonica*. Soil samples were again collected in June and October 2003. Sterile soil cores with a diameter of 1 cm were used to collect the samples for April and October. Sterile soil cores with a diameter of 5 cm were used to collect samples in June. All samples were stored on ice for transport to the laboratory. The samples were sieved through stacked 4-mm and 2-mm mesh sieves and stored at 4°C. All extractions were conducted within 4 d of collection. In April soil samples were analyzed for total carbon and nitrogen and Mehlich III-extractable phosphorus. Additionally, soil samples were analyzed for moisture, microbial biomass carbon and nitrogen, pH, and inorganic nitrogen on all sampling dates.

Gravimetric Water Content

Gravimetric water content was calculated after oven drying moist soil (5 g) for 24 h at 105°C. Results for all nutrient analyses are reported on per gram of dry soil basis.

Total Carbon (C) and Nitrogen (N) and Mehlich III-Extractable Phosphorus (P)

The Soil Testing Lab at the University of Arkansas-Fayetteville conducted total C and N, and Mehlich III-extractable P analyses. Total C and N were measured on oven-dried, ground soil combusted at 1100°C (LECO CN2000, Joseph, Mich.). Oven-dried, ground soil was extracted with Mehlich III solution at a 1:10 (wt:vol) ratio and analyzed by inductively coupled plasma spectroscopy (SPECTRO CIROS ICP, Fitchburg, Mass.).

Microbial Biomass C and N

Microbial biomass C and N were determined using a chloroform-fumigation-extraction method as described by Vance et al. (1987). Moist soil (10 g each) was obtained for fumigated and unfumigated analysis. The unfumigated soil samples were extracted immediately with 0.5 M K_2SO_4 (20 ml). Samples were shaken on a reciprocating shaker (30 min) and filtered through Whatman #42 filter paper. The fumigated soil samples were placed in a desiccator that had been lined with moist paper towels and contained ~25 ml of chloroform. A vacuum was then used to seal the desiccator and boil

the chloroform. After a fumigation period (24 h), chloroform was removed by evacuating the air within the desiccator six times for 3 min each. Fumigated soils were then extracted as described for unfumigated samples. Total organic carbon (TOC) was measured using a Rosemount Analytical Inc. DC-190 High Temperature TOC Analyzer (Tekmar-Dohrmann, Cincinnati, Ohio). Microbial biomass N was determined using persulfate oxidation as described by Cabrera and Beare (1993). Biomass C and N were each calculated as the difference between fumigated and unfumigated values.

Inorganic N

Moist soil (1g) was extracted at 1:10 (wt:vol) ratio with 2M KCl. Extracts were shaken (1 h) and solutions were filtered through Whatman #40 filter paper and stored at 4°C until further analysis. Colorimetric analysis of ammonium concentrations (NH_4^+, modified Berthelot reaction) and nitrate concentrations (NO_3^-, cadmium reduction) were conducted on a Skalar autonutrient analyzer (Norcross, Ga).

Soil pH

Moist soil (1 g) was mixed with double deionized water (10 ml) and allowed to sit (1 h). The pH was then recorded using a calibrated, combination pH electrode (VWR Scientific Products, Westchester, Penn).

Statistical Analysis

A one-way analysis of variance test ($P<0.05$) was conducted on both treatments for all sampling times for each of the properties evaluated.

RESULTS AND DISCUSSION

Gravimetric Water Content

There were no significant differences in gravimetric water content between treatments or sampling times. Mean values for gravimetric water content ranged from 0.27 to 0.36 g water g^{-1} soil for all sampling dates.

Total C and N and Mehlich III-Extractable P

Initial levels of total soil C and N and Mehlich III-extractable P did not show significant differences between treatments. Soil C levels ranged between 2.30 and 2.56%. Assuming soil organic matter is twice the amount of soil carbon (Brady and Weil, 2002), the soils were approximately 5% organic matter. While this might be considered high in surface agricultural soils, it is not a high amount for wetland soils. Histosols can have organic matter contents greater than 20% (Brady and Weil, 2002). Saturated soils tend to have greater accumulation of organic matter than upland soils. *Lonicera japonica* prefers soil that is not completely inundated by water for extended periods of time (Nuzzo, 1997). Thus, *L. japonica* surrounds the wetland but has not penetrated the wettest areas. Background soil N levels were not significantly different between treatments and were in the range of 0.16 to 0.20 percent. Soil C:N ratios typically fall between 8:1 to 15:1 for surface soils (Brady and Weil, 2002). The soil C:N ratios of the two treatments were not significantly different and ranged between 13:1 and 15:1. Mehlich III-extractable P levels were not significantly different between treatments and ranged from 12.04 to 16.99 μg P g^{-1}.

Microbial Biomass C and N

Microbial biomass C ranged from 124 to 199 μg C g^{-1} soil, and microbial biomass N values were between 11 and 31 μg N g^{-1} soil for all sampling dates and treatments (Table 1). Microbial biomass C:N ratios were calculated to be in the range of 7:1 - 14:1 (Fig. 1). Assuming that C:N ratios for bacteria are 3:1 - 5:1 and C:N ratios for fungi are 5:1 - 15:1, these ratios indicate that fungi were the dominant biomass (Sylvia et al., 1998). There were no significant differences between treatments or among sampling dates for microbial biomass C, N, or C:N ratios.

Inorganic Nitrogen

No significant difference in the inorganic N pool (Fig. 2) was found between April and October. However, inorganic N levels increased between April and June and decreased between June and October. Most of the inorganic N pool was attributed to NH_4^+, which ranged from 1 - 12 μg N g^{-1} soil. Nitrate was low throughout the growing season and no significant changes were measured among sampling dates. The NO_3 pools ranged from 0.5 - 1.5 μg N g^{-1} soil.

Soil pH

As with inorganic N, pH increased between April and June and decreased between June and October (Fig. 3). No significant differences between treatments were observed for any sampling date.

It is necessary to study soil microbial interactions with higher plants in order to understand ecosystem processes on a functional level (Wardle, 2002). For example, many papers have been published in soil science that report different nitrogen mineralization under different vegetation regimes (as cited in Wardle, 2002). Scientists have been evaluating plant-soil-microbe interactions in order to determine if there is a link between microbial communities and invasive plant species. Plant species' effects on microorganisms are thought to be caused by differences in the organic compounds that are excreted by plant roots (Marschner et al., 2001). Plants influence soil microbial communities by either increasing activity by carbon substrate addition (root exudates) or decreasing activity by depleting nutrients and resources from the soil (Wardle, 2002).

Kourtev et al. (2002) analyzed the soil microbial communities under three species of plants: two invasive and one native species. Microbial communities were found to be different under native and invasive species, and the greatest differences in community structure and function were found in the rhizosphere soil (i.e., the soil located in close proximity to the root zone) suggesting that plants have a direct effect on soil microbial communities (Kourtev et al., 2002). Erhenfeld et al. (2001) suggested that the establishment of invasive species causes changes in the structure and function of the soil biota. They proposed that understanding the changes that occur within the soil microbial communities could explain how invasive plants change their surroundings and grow at such alarming rates.

Recently in a 10-week study, the growth rate of *Centaurea maculosa*, an invasive plant species in North America, was compared in four soils from its native region and six from North America, before and after soil sterilization (Callaway et al., 2004). The results showed sterilization of the native soils caused an average increase of 166% in total plant biomass, whereas sterilization of the foreign North American soils caused only a 24% increase in total plant biomass (Callaway et al., 2004). These results indicate soil microorganisms play a role in controlling the growth rate of higher plants. Callaway et al. (2004) state that once a plant invades an area, it often experiences positive relationships with soil biota due to a lack of effective soil pathogens, whereas in its native habitat negative relationships with soil biota occur. Callaway et al. (2004) suggested that this might be the reason invasive plants are so successful.

In contrast to the immediate effects on plant growth in the study by Callaway et al. (2004), differences between treatments were not observed in this study during the first growing season. Labile nutrient pools—microbial biomass C and N and inorganic N—were chosen for evaluation because they are dynamic pools that respond in the short-term to changes in the environment. Microbial biomass can serve as an indicator of soil health, which is essential for a productive ecosystem. However, the results of this study may not be surprising. The sampling times may have been too soon after transplanting for changes within the soil to be apparent. The native grasses had been planted for less than a month at the June sampling time and less than 4 months at the October sampling. The roots of the *L. japonica* were not removed from the soil and may need to be degraded before changes are observed. The Kourtev et al. (2001) experiment was carried out on plots where native plants stands were already well established.

The results from the current study indicate that changes caused by the native grasses during the first year of stand development may not have been significant enough to indicate modifications within the bulk soil nutrient pools. Thus, soil sampling and analysis for the aforementioned parameters will continue in 2004, having allowed time for some root degradation and establishment of the native plant community. In addition to monitoring microbial biomass and nutrient cycling, soil-microbial community composition will be examined using polymerase chain-reaction amplification and denaturing gradient gel electrophoresis techniques. These techniques will allow us to examine the changes within soil communities at a greater level of resolution to determine if diversity within targeted soil communities changes with respect to vegetation cover even if the total microbial biomass does not change. This study found that significantly measurable changes due to revegetation might not occur within a single growing season. We suggest that data need to be collected for at least another year before differences among treatments may be observed.

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Table 1. Mean values (standard deviation) of soil microbial biomass carbon (C) and nitrogen (N) in wetland soil growing *Lonicera japonica* and soil plots revegetated with five native grass species in the combined months of April, June, and October, 2003 (n = 4)

Treatment	Microbial biomass C (μg g^{-1})	Microbial biomass N (μg g^{-1})
Lonicera japonica	152.09 (10.51)a[1]	18.56 (2.96)a
Native grasses	171.82 (40.96)a	19.89 (10.26)a

[1]Different letters within column indicate significant differences between treatments and among sampling dates (P < 05)

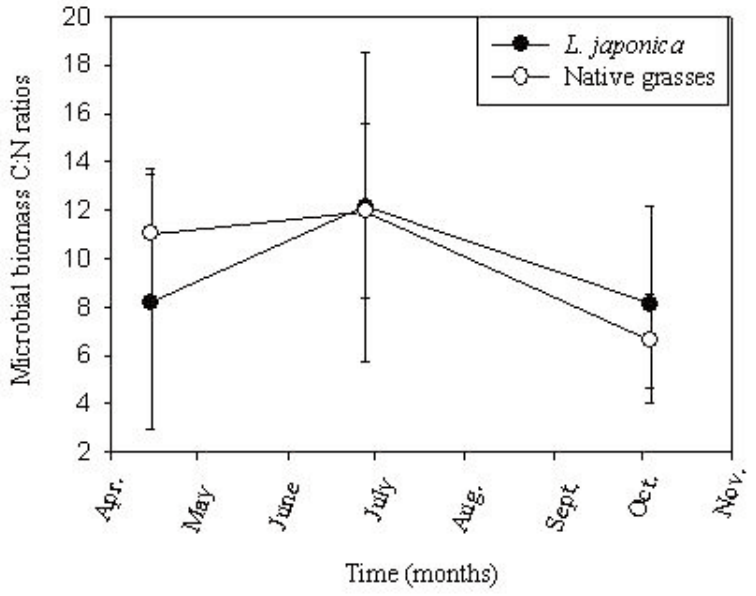

Fig. 1. Mean values of microbial biomass carbon-to-nitrogen (C:N) ratios for wetland soils growing *Lonicera japonica* and five native grass species in April, June, and October, 2003 (n = 4); error bars represent one standard deviation.

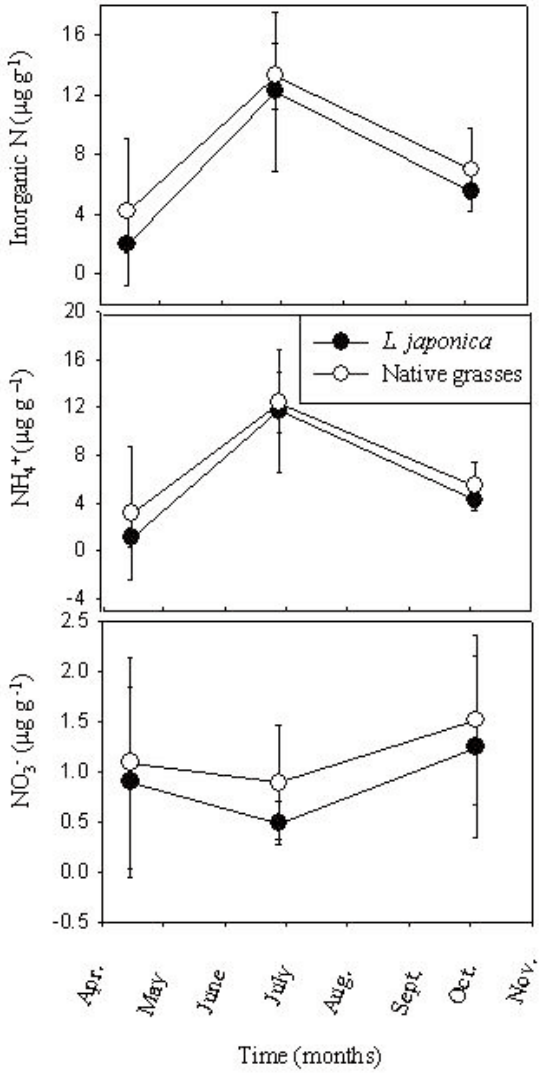

Fig. 2. Mean concentrations of inorganic nitrogen (N), ammonium (NH4+), and nitrate (NO3-) in wetland soil growing *Lonicera japonica* and native grass species in April, June, and October, 2003 (n = 4). Error bars represent one standard deviation.

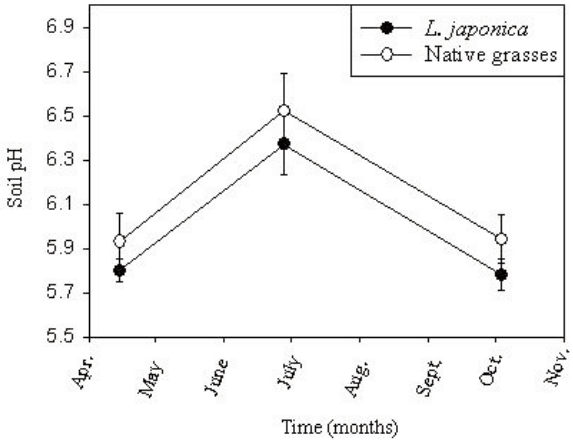

Fig. 3. Mean values (standard deviation) of soil pH in wetland soil plots growing *Lonicera japonica* and five native grass species in April, June, and October, 2003 (n = 4). Error bars represent one standard deviation.