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THE IMPACT OF INVASION BY *IMPERATA CYLINDRICA* ON MICROBIAL ABUNDANCE AND ABIOTIC SOIL FACTORS IN *PINUS TAEDA* PLANTATIONS

by
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ABSTRACT
Cogongrass (*Imperata cylindrica*) is a significant pest in plantation forestry, agriculture and right-of ways. In belowground communities, which are notoriously difficult to quantify, *I. cylindrica* may have wide reaching effects that largely go unnoticed. We quantified carbon and nitrogen present in the mass of all living microorganisms in the soil. We also measured pool size of several key nutrients in the top 10 cm of both *I. cylindrica* present and absent plots. We analyzed these variables across spatial and temporal scales to account for seasonal variations. There were no significant reductions in soil moisture in any plot types. Ratio of microbial biomass C:N was significantly not significantly different in any *I. cylindrica* present plots. Finally, we determined that differences between nutrient pools existed between *I. cylindrica* present and absent plots. These factors have the potential to significantly affect the microbial communities. In order to determine what long term consequences will be to nutrient cycles and native plant communities additional research is necessary.

INTRODUCTION
Cogongrass (*Imperata cylindrical* (L.) Beauv.) is an invasive grass species native to Asia. In its invaded range of the southeastern United States, it influences soil moisture and is more robust on acidic soils (Bryson & Carter 1993; Jose et al. 2002). *Imperata cylindrica* is more effective at locating and exploiting nutrient patches, altering fire regimes and altering light resources compared to native grass species (Lippincott 1997). Daneshgar & Jose (2009) found that due to severe competition for nitrogen, compared to native vegetation, *I. cylindrica* reduced the growth of loblolly pine (*Pinus taeda* L.). In general, invasive plant species tend to be more dominant in an invaded range through a variety of mechanisms including the production of novel "biochemical weapons" (Callaway & Ridenour 2004). This dominance has the potential to drastically alter plant community composition.

Plant community composition along with several other factors such as availability of resources and land use determine fine scale spatial and temporal distribution of soil microbes (Stotzky 1997; Zak *et al.* 2003; Bossio et al. 2005). Soil microbial organisms largely are averse to growing in culture (Amann *et al.* 1995). The underground effect of an invader may, therefore, be difficult to quantify and go unnoticed. Since microorganism functions are an integral component of ecosystem functions such as nutrient cycling, fertilization and organic matter transformation, altered microorganism communities may have far-reaching effects (Černý *et al.* 2003). To quantify these differences we investigated how *I. cylindrica* alters some of these below ground functions. We expected that differences would be observed in soil physical properties, nutrient availability and microbial biomass of stands invaded by *I. cylindrica* due to the presence of *I. cylindrica* and microbial biomass of stands invaded by *I. cylindrica* due to the presence of *I. cylindrica* and the compounds it produces.

MATERIALS & METHODS

Site Description

Twenty plots (30 m x 30 m) were established on production loblolly pine stands in Greene County, Mississippi in 2010. Ten plots had *I. cylindrica* present and ten plots did not contain *I. cylindrica*. In 2011 percent cover of *I. cylindrica* was assessed. Plots were divided into *I. cylindrica* present (n=10) and absent (n=10), with all invaded plots exhibiting over 50 % cover of *I. cylindrica*. The sites were re-examined in 2013 and a smaller subset of plots (16 m x 16 m) were reclassified by cover of *I. cylindrica*. Plots were divided into sites that had less than 50 % cover (n=2), sites that had greater than 50 % cover (n=3) and sites in which *I. cylindrica* was absent (n=3) (referred to as present, abundant, and absent, respectively). The soil series on which these plots are located was identified as Benndale sandy loam on 8 to 15% slopes, and Benndale sandy loam or McLaurin sandy loam on 2 to 5% slopes.

Soil Moisture

Field sampling was conducted during April 2014, July 2014, October 2014 and January 2015. Four samples were collected from each site, 1 m in each cardinal direction from plot center. The top 10 cm of soil was sampled with a shovel and placed in plastic bags, samples were then stored at 4°C until lab processing. Samples were passed through a 2-mm sieve in order to remove coarse organic debris. Soil moisture was determined by calculating the percentage of weight loss in each sample after being subjected to 105 °C for a 72 hour period.

Microbial Biomass

Samples were also stored at 4°C until microbial biomass was measured using the chloroform-fumigation extraction technique as described in detail by Ricker & Lockaby (2014) and Vance *et al.* (1987). Samples were divided into two subsamples, one of which was fumigated with chloroform. Soil fumigation occurred in complete darkness for a 24 h period, following which 125 ml of 0.5 M K_2SO_4 was added. The solution was shaken for 30 minutes and vacuum filtered through No. 5 Whatman filter paper before being frozen. The unfumigated samples were extracted and frozen using the same protocol.

After a minimum of 3 days samples were thawed and analyzed, using a Shimadzu TOC-V and N combustion analyzer (Shimadzu Scientific Instruments, Columbia, MD.) for total organic C (TOC) and N (TON). Organic C and N from unfumigated samples were subtracted from fumigated samples and the product used to calculate the microbial biomass C and N with an extraction efficiency coefficient of 0.45 (Jenkinson 1988; Wu *et al.* 1990).

Nutrient Analysis

Soil samples were collected in November 2011. Four soil cores (5 x 60 cm) were removed from each plot at approximately 1 m from plot center in each cardinal direction. The Soil Characterization Laboratory at the University of Missouri performed sample analyses by combustion analyzer or Mehlich-3 procedure (Mehlich 1984; Bremner 1996). Values were reported in concentrations from which pool size was derived for nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na) with bulk density values.

Statistical Analysis

R® version 3.0.2 and STATISTICA® (StatSoft 2013) were used for statistical analyses on mean soil moisture and mean microbial biomass using ImerTest package. Means were calculated from multiple samples in each plot (4) per sampling date (4). We performed repeated measure mixed model ANOVAs in which *I. cylindrica* abundance was a whole plot between subjects factor, sampling date was a within subject factor and plot was a random factor. These models included Treatment and Month and all two-way interactions between them as fixed factors.

Models were fit with the "lmer" function using REML likelihood and degrees of freedom. P-values were estimated using the Satterthwaite method. Marginal means and standard errors for significant predictors were obtained using the "lsmeans" function, and pairwise significant differences among means were estimated using the "difflsmeans" function. For significant interactions of Treatment with Depth or Month, we only conducted pairwise tests between levels of Treatment within each Depth or Month, to minimize the number of pairwise tests conducted. As such, and due to our low replication for the Treatment effect, the significance of all p-values was assessed at $\alpha = 0.05$.

SAS® version 9.3 (SAS Institute Inc. 2010) and STATISTICA® (StatSoft 2013) were used for statistical analyses of soil nutrients. A multivariate analysis of variance (MANOVA) was undertaken for N, P and K nutrient pool size and select cation pool size. Post hoc Tukey tests (α=0.05) were performed for all pairwise comparisons. Residuals were tested for normality and log transformed when data sets were found to be non-normally distributed.

RESULTS

Soil Moisture

A significant treatment effect on soil moisture was not found to be present across *I. cylindrica* absent, present and abundant plots ($F_{(2,5)}$=0.506, p=0.631) (Figure 3.1). Soil moisture was significantly different over time ($F_{(2,15)}$=15.477, p<0.0001) indicating that across treatments seasonal variations are present in the soil moisture. A significant interaction between treatment and month was not present ($F_{(6,15)}$=1.571, p=0.223).

Microbial Biomass

A significant treatment effect on soil microbial biomass was not found to be present across treatments (($F_{(2,5)}$=3.661, p=0.104). A significant time effect was present ($F_{(3,15)}$=5.371, p=0.010) indicating that across treatments microbial biomass C:N varies between seasons. No significant interaction was observed between treatment and month ($F_{(6,15)}$=2.026, p=0.125).

Nutrient Analysis

Composition of N, P and K pools varied significantly between invaded and non-invaded plots ($F_{(3,16)}$=6.05, p=0.006) (Figure 3.3). Individual comparisons showed that there was no significant difference between *I. cylindrica* abundant and absent plots for the total N pool ($F_{(1,18)}$=2.88, p=0.11) or P pool ($F_{(1,18)}$=0.40, p=0.53). *Imperata cylindrica* abundant plots had significantly higher levels of total K pools compared to *I. cylindrica* absent plots ($F_{(1,18)}$=17.23, p=0.0006). Additional soil structure related cations (Ca, Mg and Na) pools did not vary significantly between invaded and non-invaded plots ($F_{(3,16)}$=2.21, p=0.127). Individual analyses did, however, reveal that the Na pool significantly varied between *I. cylindrica* present and absent plots where it was found at higher levels ($F_{(1,18)}$=5.80, p=0.03) (Figure 3.4).

The pool size for both Ca ($F_{(1,18)}$=1.83, p=0.19) and Mg ($F_{(1,18)}$=3.41, p=0.08) were not statistically different between *I. cylindrica* present and absent sites.

DISCUSSION

No significant treatment effects were observed for either soil moisture or microbial biomass C:N. Significant seasonal differences do, however, exist in both soil moisture and microbial biomass C:N. Considering the extremely low replication size (n=3 in *I. cylindrica* absent and abundant plots and n=2 in *I. cylindrica* present plots) in conjunction with a near significant treatment effect on microbial biomass (p=.105) we recommend a more complete sampling regime during the summer months (June, July and August), when microbial biomass C:N was most variable, in order to get a more complete analysis of belowground processes. Carbon and N cycles encompass several trophic levels and these measurements provide a snapshot of availability of C and N to each respective group. Due to the importance of these nutrients, the variety of factors that affect them and their high spatial variability additional testing is warranted (Blagodatskaya & Kuzyakov 2008; Eisenhauer *et al.* 2010).

Composition of nutrient pools of N, P and K varied significantly between invaded and non-invaded plots. Pool size of N, P and K were analyzed together because utilization of these macronutrients by plants are linked in plant usage. Higher levels of nutrients were typically found in invaded plots, contrary to our hypothesis. This is, however, not unprecedented. Some invasive plants are known to, initially and temporarily, increase biomass, net primary productivity (NPP) and produce litter that decomposes more readily relative to native plants (Ehrenfeld 2003).

Difference existed between cation pools in different plot types (*I. cylindrica* present and absent). Sodium was more prevalent at sites on which *I. cylindrica* was present ($F_{(1,19)}$=5.80, p=0.03). In addition a near significant difference was observed in Mg with higher levels observed in *I. cylindrica* present sites ($F_{(1,19)}$=3.41, p=0.08), especially considering the low replication rate (n=20). The excess of Na relative to Ca and Mg may result in more dispersed soil, due to Na role as a poor flocculator relative to Ca and Mg.

A possible explanation for the abundance of K and Na may exist in some of the compounds produced by *I. cylindrica*. Hagan *et al* 2013 found that several compounds were found in plots invaded by *I. cylindrica* at higher concentrations than in plots without *I. cylindrica*, including the compound emodin. Emodin has been known to be produced by members of the family poacea and has a worldwide distribution (Izhaki 2002). It has been observed that emodin is consistent with increases in K and Na as well as decreases in Mg (Inderjit & Nishimura 1999).

CONCLUSION

Several chemical incongruities were noted between *I. cylindrica* present and absent plots that have the potential to impact tree and other vegetative growth. Differences were not observed between soil moisture in the top ten cm of the soil profile in *I. cylindrica* present plots compared to *I. cylindrica* absent and *I. cylindrica* abundant plots. The levels of organic C and organic N in microbial biomass were also not significantly different across the sampled invasion gradient of *I. cylindrica*, during any time. Different pool sizes of vital nutrients N, P and K were present as were some cation pool sizes; but not cations in general. From this data we are able to confidently conclude that there are some differences in inorganic nutrient pools consistent with *I. cylindrica* invasion.

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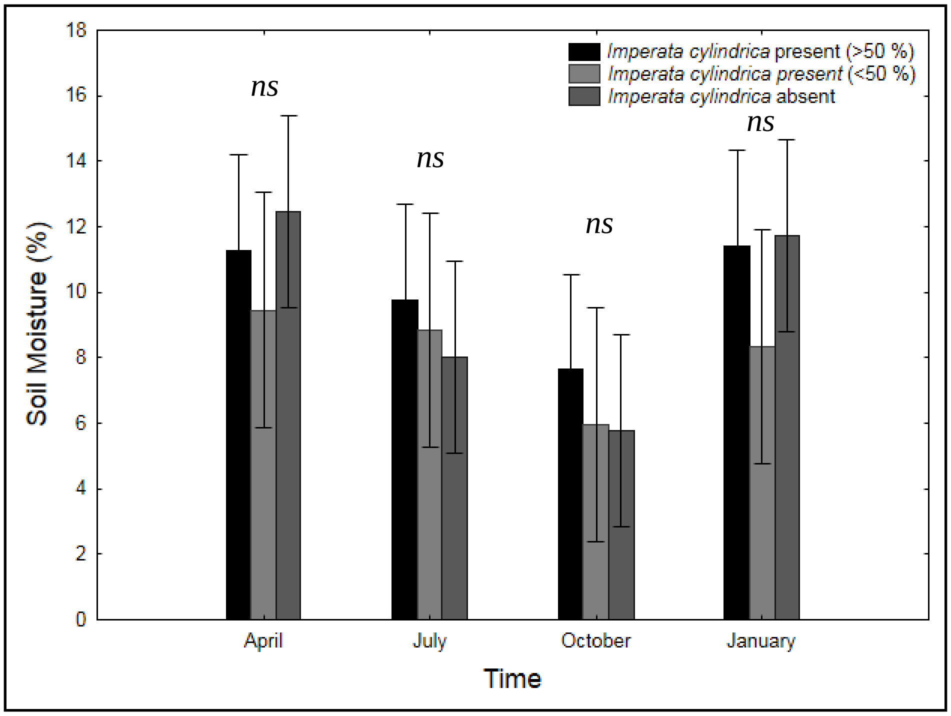

Figure 3.1 Mean values of soil moisture in *P. taeda* stands over the course of four collection periods. Comparisons were made between sites within the same collection period. Error bars represent standard error; n=8.

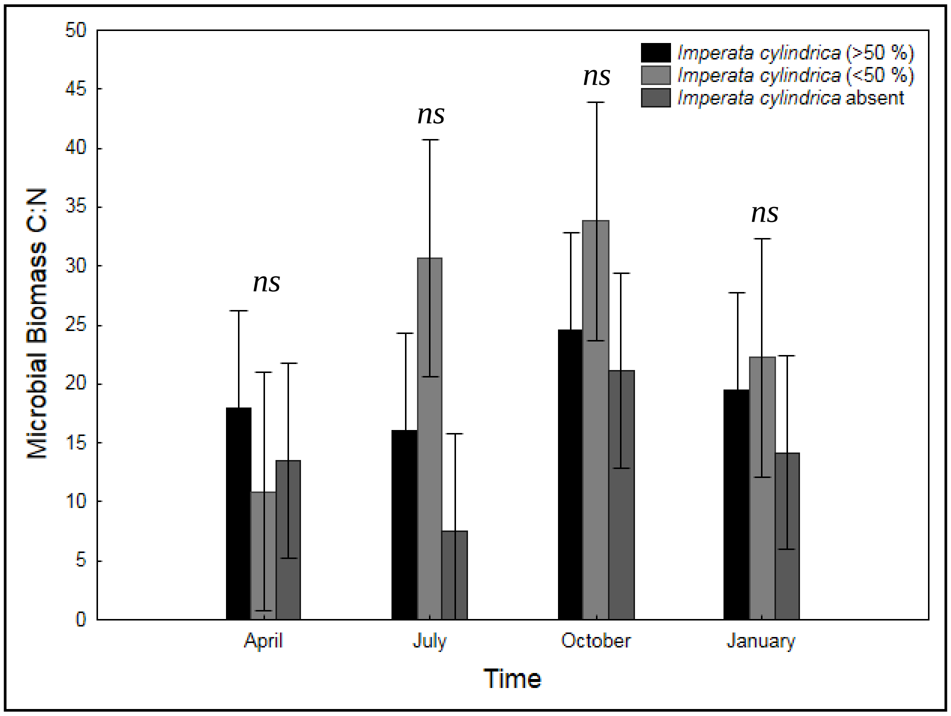

Figure 3.2. Mean values of microbial biomass C:N ratio measured during four collection periods. Comparisons were made between sites within the same collection period. Error bars represent confidence intervals; n=8.

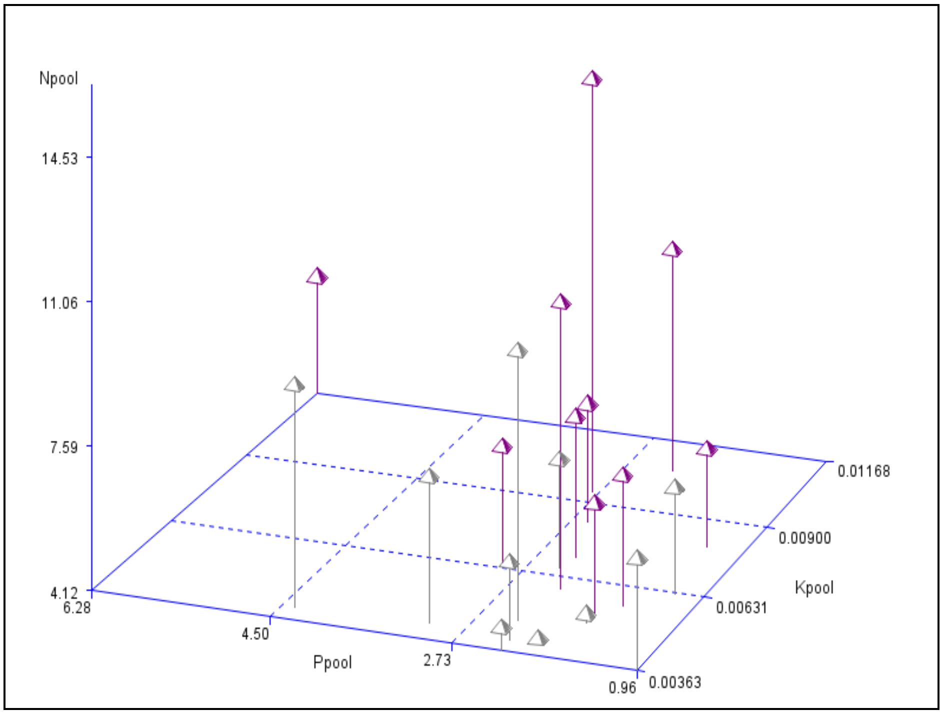

Figure 3.3 A 3 dimensional scatter plot of N, P and K pools. Darker shaded points are sites in which *I. cylindrica* was present and lighter shaded points are sites in which *I. cylindrica* was absent.

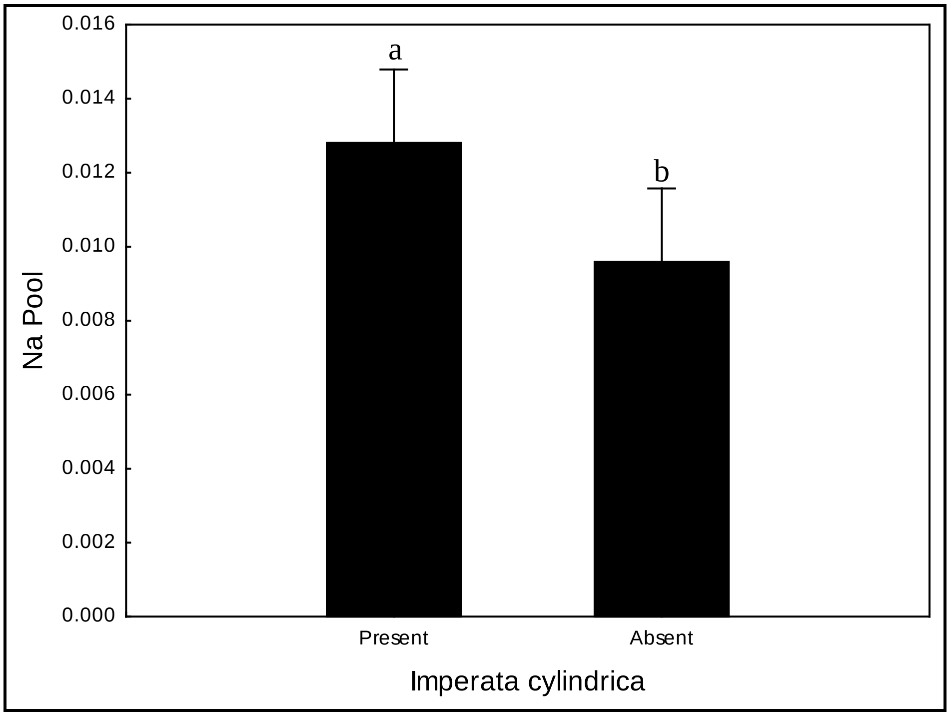

Figure 3.4. Mean pool size of Na found in *I. cylindrica* present absent sites. Significant differences are shown with Tukey variables, error bars represent significant error; n=20.