

AUBURN UNIVERSITY

FOREST HEALTH COOPERATIVE

RESEARCH REPORT 16-02

IMPERATA CYLINDRICA IMPACTS COLONIZATION BY MYCORRHIZAL FUNGI ON *PINUS TAEDA* IN COMMERCIAL STANDS

by

Adam M. Trautwig and Lori G. Eckhardt

ABSTRACT

Cogongrass (*Imperata cylindrica*), an invasive grass species native to Asia, has been shown to reduce tree vigor in loblolly pine (*Pinus taeda*) plantations, which comprise over 50 % of growing stock in commercial forests of the United States. *Imperata cylindrica* produces exudates with possible allelopathic effects that may influence abundance of *P. taeda* symbionts such as soil microbes and ectomycorrhizal fungi. Soil microbial communities and root colonization by mycorrhizal fungi were sampled in intensively managed *P. taeda* stands in Greene County, Mississippi, in *I. cylindrica* present and absent plots. *Imperata cylindrica* present plots had a reduction in abundance of ectomycorrhizal colonization of pine fine feeder roots in the top 40 cm of soil in comparison to *I. cylindrica* absent plots. Abundance of pine fine feeder roots in the 21–40 cm and 41–60 cm layers of the soil profile was also reduced in *I. cylindrica* present plots. Vegetative diversity was negatively correlated with *I. cylindrica* abundance (% cover) which probably contributed to the reduced microbial diversity in *I. cylindrica* present plots. Due to the variety of roles these microorganisms play, the changes associated with the invasion of *I. cylindrica* in commercial forestry plantations are likely to alter nutrient cycling and reduce *P. taeda* growth as well as site productivity.

INTRODUCTION

Economic losses attributable to the nearly 50,000 non-indigenous species in the United States are estimated to be US\$ 120 billion per year (Pimentel *et al.* 2005). A large portion of the impacts of invasive species is ecological in nature and often unnoticed, although they can contribute to ecosystem or community transformation over time (Simberloff *et al.* 2013). In addition to direct impacts on above ground flora and fauna, effects on below ground symbionts and other organisms that co-occur with native species can magnify the impact of an invasive species. While a few instances of compounding effects of co-invasion by plants and their symbionts have been well documented (Richardson *et al.* 2000; Pasternak *et al.* 2007; Dickie *et al.* 2010), principles that outline how an invader can affect pre-existing communities of mutualists are poorly defined.

Cogongrass (*Imperata cylindrica* (L.) Beauv) is classified as a noxious weed in over 70 countries and represents a tangible threat to biodiversity and resource management (Burrell *et al.* 2015; Estrada & Flory 2015). In areas where it has successfully invaded it often forms dense monotypic stands (MacDonald 2004). *Imperata cylindrica* was introduced accidentally from Japan to the United States in 1912, but later was intentionally introduced for forage crop trials and erosion control (Bryson & Carter 1993; Holzmüller & Jose 2011). Due to its high silica content, it was ultimately not selected for use as a forage crop (MacDonald 2004). *Imperata cylindrica* is a C₄,

rhizome producing, perennial plant that can reach heights of 3 m but typically grows to heights of 1.2 m (Koger & Bryson 2004). It spreads both sexually through seeds and asexually through rhizomes. Seed production can be prolific with most seeds generally dispersed within 15 m of the plant (Daneshgar *et al.* 2008). *Imperata cylindrica* can grow on a variety of soils, from nutrient-poor, coarse sands to nutrient-rich, sandy loam soils (Jose *et al.* 2002). It is particularly difficult to eradicate because it produces a vast network of underground rhizomes from which the plant regenerates. Rhizomes can reach densities of 89 m.m⁻² of soil (MacDonald 2004).

Daneshgar & Jose (2009) found that competition for nitrogen (N) between *I. cylindrica* and loblolly pine (*Pinus taeda* L.) seedlings resulted in a greater reduction in pine seedling growth than competition with native vegetation. *Imperata cylindrica* also may impact growth of timber species such as *P. taeda* through production of exudates that may have an allelopathic effect (Hagen *et al.* 2013; Holzmüller & Jose 2011; Koger & Bryson 2004; Hussain & Abidi 1991). Compounds isolated from exudates of *I. cylindrica* with possible allelopathic effects include seven phenolic acids, two aromatic acids, one trihydroxy anthraquinone, and one meta dihydroxyl phenol (Hagen *et al.* 2013). In addition to direct impacts on plant growth, compounds present in *I. cylindrica* exudates may affect growth through influence on mycorrhizae and other soil microbial taxa (Roberts & Anderson 2001; Kourtev *et al.* 2002). Boufalis *et al.* (1994) found that the growth of two ectomycorrhizal fungal species, *Cenococcum geophilum* and *Laccaria laccata*, were affected by the applications of several phenolic acids similar to those produced by *I. cylindrica*. For this study, we hypothesized that *I. cylindrica* present *P. taeda* plots would have reduced mycorrhizal and fine *P. taeda* feeder root abundance, a reduction in microbial biomass (N) (mg.L⁻¹) and *P. taeda* growth rate (mm.yr⁻¹), as well as reduced vegetation diversity when compared to *I. cylindrica* absent plots.

MATERIALS AND METHODS

Site Description

The research site was located east of Leakesville, Greene County, Mississippi, an area under commercial *P. taeda* production. The area receives an average annual rainfall of 1,677 mm (www.idcide.com). The dominant mid-story vegetation includes yaupon (*Ilex vomitoria* Ait.), scrub oak (*Quercus* spp.), and *P. taeda*. Much of the area has also been invaded by *I. cylindrica*. The soil series was identified as Benndale sandy loam on 8 - 15% slopes, and Benndale sandy loam or McLaurin sandy loam on 2 - 5% slopes. Benndale soils are classified as a coarse-loamy, siliceous, semi-active, thermic Typic Paleudults and McLaurin soils are classified as a coarse-loamy, siliceous, sub-active, thermic Typic Paleudult. These soils are well drained and generally nutrient poor due to low amounts of organic matter and clay (Soil Survey Staff 2012).

Eight study plots (16 m X 16 m) total were established within two similar stands of *P. taeda* that have received slightly different management regimens (Table 2.1). The plots were situated within the northeast quadrant of larger plots (30 m x 30 m), which were initially established for a study in 2010 (Brunson 2013). Four plots were located within areas of the stands that were not invaded with *I. cylindrica*, the remaining four in areas that were invaded with *I. cylindrica*.

Over the course of this study, *I. cylindrica* invaded one of the non-*I. cylindrica* plots and it was determined that *I. cylindrica* density in one of the *I. cylindrica* invaded plots was lower than in the original larger plot as a whole. Therefore, for analyses the plots were characterized by whether *I. cylindrica* was present (< 50% cover, n=2), abundant (>50% cover, n=3), or absent (n=3) (Figure 2.1).

Sites were all in close proximity and represent a high degree of homogeneity with the exception of *I. cylindrica* being present.

Mycorrhizae

Field sampling of roots for mycorrhizae was performed in November 2014 and May 2015. Preliminary studies have shown that May is the most reliable time to survey active mycorrhizal communities in this region (J. Hoeksema pers. obs.). Another sampling in November was included to account for seasonal variation (Brundrett *et al.* 1996). At each plot, nine evenly distributed soil cores (10 cm dia x 60 cm ht) were removed across each of the eight plots using a pneumatic coring device. This method of soil core sampling assured that both the O and A soil horizons were sampled for mycorrhizae. As it is unclear how deep *I. cylindrica* exudates may leach into the soil, an analysis of multiple soil layers was necessary. Each soil core was taken approximately 2.5 m from the next core and each plot contained several trees. Due to limitations in space and accessibility more sampling was not feasible. Individual cores were sealed and immediately stored at 4°C until processing.

Soil cores were cut into 20 cm increments and roots were gently washed free of soil with water through a 0.5 mm sieve (Horton & Bruns 1998; Roberts & Anderson 2001). Roots were pooled within each plot by soil core increment (0–20, 21–40 and 41–60 cm). Pine roots were separated and kept in a 2% cetyl trimethyl ammonium bromide (CTAB) lysis buffer (Tedersoo *et al.* 2006). As ectomycorrhizal tips are abundant ($7\text{--}72 \times 10^4 \cdot \text{m}^{-2}$) (Taylor 2002) we arbitrarily sampled 100 1-cm root segments for a total of 100 cm of fine roots per 20 cm soil core increment except when there were not enough roots present, in which case we examined all roots (Anderson *et al.* 2010) to measure the percent of fine root segments that were colonized. The length of fine feeder roots (<2 mm) was measured (cm) for all plots. Mycorrhizal structures on subsampled roots were identified and quantified at 10–40x magnification using the gridline intercept method (Brundrett *et al.* 1996).

Vegetation Survey

A vegetation analysis was undertaken in July 2014 to quantify percent cover of vegetation on *I. cylindrica* invaded and non-invaded plots (Table 2.2). Vegetation was assessed using a 10% scale by the line transect method, where ten adjacent 1 m² quadrats were sampled on a diagonal transect within each plot (Krebs 1989). Plants that were present but in lower abundance than 10% were given a classification of 5% to ensure they were accounted for in diversity estimates. Vegetation that could not be identified in the field was collected and pressed for later identification by plant taxonomists at Auburn University.

Microbial Biomass N

Four separate soil samples were collected from each plot to determine organic N levels (mg/L) that were used to calculate microbial biomass N. Soil samples were collected 2 m apart in each

cardinal direction, in the southwest corner of each plot or the center of the original plot. Prior to sampling, the top layer of loose organic debris (or duff) was removed. As target microbial communities are more abundant higher in the soil profile, only the upper layer of soil (≤ 10 cm) was sampled. Samples were placed in plastic bags and transported within eight hours to the laboratory where they were refrigerated. All tests were performed within three days.

Soil samples were sieved using a 2 mm sieve. Each soil sample was separated into two 18.5 g samples and was processed using the chloroform-fumigation extraction (CFE) technique (Vance *et al.* 1987). This method measures only the organic N in microbial organisms in the soil by lysing cell walls in one sample (using chloroform) and not disturbing the second sample. Organic N levels were measured utilizing a TNM-1 Total N measuring Unit (Shimadzu Scientific Instruments, Columbia, MD). The organic N levels from the non-disturbed samples are subtracted from the organic N levels in the fumigated sample. The subtracted value, divided by the efficiency of extraction constant, yielded the microbial biomass N. These values for the four soil samples collected per plot were averaged prior to plot comparisons.

Tree Radial Growth

Within each of the original 30 m x 30 m plots, six dominant or co-dominant trees closest to plot center were evaluated for tree growth rate. An increment borer was used to remove a tree core at breast height (1.37 m). Cores were mounted onto blocks and sanded down to show clear annual growth rings. Total radial growth over the last five and ten years was determined by measuring from the early wood of the fifth and tenth annual rings, respectively, to the bark. In order to account for differences in growth due to relative density of trees on each plot, growth was expressed as a function of percent stocking. Percent stocking standardizes available growing space across plots with trees of various ages and sizes and differing basal area (Gingrich 1967).

Statistical Analysis

Analyses examining mean percent colonization and mean fine root length as response variables were conducted in R[®] version 3.0.2, using lmerTest package. Graphs were constructed in STATISTICA[®] (StatSoft 2013). Mean values were calculated from multiple cores (9) in each plot on each sampling date. We performed split-plot, repeated measures mixed model ANOVAs in which *I. cylindrica* abundance was a whole plot between-subjects factor, sampling depth was a split-plot between-subjects factor, sampling date was a within-subject factor, and plot was a random factor. These models included Treatment, Depth, Month, and all two-way interactions between them as fixed factors. The three-way interaction among all three was excluded from final models, as it was always highly non-significant.

Models were fit with the *lmer* function using REML likelihood. Degrees of freedom and 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 between means were estimated using the *diffsmeans* 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 one-way analysis of variance (ANOVA) on microbial biomass with *I. cylindrica* invasion as the predictor variable. In addition, a factorial ANOVA examining the effect of *I. cylindrica* presence and loblolly stocking percentage was conducted on radial growth measurements. Post hoc Tukey's HSD test ($\alpha = 0.05$) was performed to compare means in pairwise tests. Residuals were tested for normality and response variables log transformed when found to be non-normally distributed.

Species richness and diversity for each plot was calculated using both the Shannon-Wiener and Simpson's nonparametric measurements of diversity (Magurran 2004; Colwell 2013). A student's t-test was undertaken to compare mean richness and diversity across invasion category. Percent cover of *I. cylindrica* was regressed against both the Shannon-Wiener and Simpson's diversity index to determine if the two were correlated.

RESULTS

Mycorrhizae

Mycorrhizal fungi colonization was affected by a significant interaction between *I. cylindrica* abundance and month ($F_{(2,29)}=5.53$, $p=0.009$) (Figure 2.2). *Imperata cylindrica* exercised a different influence on mycorrhizal fungi colonization in November than May. Overall there is a trend towards decreased colonization by mycorrhizal fungi in the presence of *I. cylindrica*, but in November mycorrhizal colonization was significantly lower in *I. cylindrica* present plots compared to *I. cylindrica* abundant plots. In May, the comparison of mycorrhizal colonization in plots with no *I. cylindrica* versus *I. cylindrica* present plots was nearly significant ($p<0.1$), despite very low replication ($n=3$ plots each). Mycorrhizal fungi colonization was also significantly different across depth classes (Figure 2.3). Specifically, fine roots at depths of 0-20 cm were more heavily colonized than those 21-40 cm ($p<0.0001$) or 41-60 cm ($p<0.0001$). Similarly, roots in the 21-40 cm depth class were more heavily colonized than those in the 41- 60 cm depth class ($p=0.041$).

Amount of fine roots recovered was significantly affected by an *I. cylindrica* abundance by month interaction. In both November and May *I. cylindrica* absent plots yielded significantly more *P. taeda* fine roots than *I. cylindrica* present ($p=0.038$ and $p<0.0001$) and *I. cylindrica* abundant plots ($p<0.0001$ and $p=0.005$) (Figure 2.4).

Fine root abundance was also affected by a significant interaction between depth class and *I. cylindrica* abundance. In the 21-40 cm depth class *I. cylindrica* absent plots yielded more fine roots than either *I. cylindrica* present or abundant plots ($p<0.0001$ and $p<0.0001$, respectively). Similarly, in the 41-60 cm depth class *I. cylindrica* absent plots yielded more fine roots than *I. cylindrica* present or abundant plots ($p=0.008$ and $p<0.0001$, respectively) (Figure 2.5).

Vegetation survey

Plant species richness and diversity differed between the *I. cylindrica* abundant and *I. cylindrica* absent plots for both Shannon-Wiener ($p=0.049$) and Fisher's alpha ($p=0.042$) indices, but not Simpson's diversity ($p=0.120$) (Table 2.3).

A correlation matrix was constructed to examine the relationship between individual species and the observed diversity indices (Table 2.4).

We determined that no species were significantly negatively correlated with measures of diversity with the exception of *I. cylindrica*. An inverse correlation was also observed in a linear regression between *I. cylindrica* percent cover and Shannon-Wiener diversity index ($y=1.8926-0.0172x$, $r=-0.9027$, $p=0.0021$, $r^2=.815$) (Figure 2.6a) as well as *I. cylindrica* and Simpson's diversity index ($y=4.6486-0.0385x$, $r=-0.7299$, $p=0.0398$, $r^2=.533$) (Figure 2.6b).

Microbial Biomass N

A significant interaction was not observed between *I. cylindrica* and month of sampling. Furthermore no significant differences were observed in either *I. cylindrica* abundance or time on microbial biomass N. There is a consensus in the literature that microbial biomass is related to soil moisture (Wardle 1992, Devi and Yadava 2006). We didn't find this relationship with microbial biomass N and soil moisture ($y=4.8233+0.1803x$; $r=0.2919$; $p=0.1050$; $r^2=0.0852$).

Radial Growth

No significant differences in radial growth were observed between *I. cylindrica* present and absent plots (Figure 2.8a and 2.8b).

No *I. cylindrica* present plots were located in stands with lower than 70 % stocking (Table 2.1). Plots in stands with lower stocking densities tended to have higher five and ten year growth rates than higher stocked stands.

DISCUSSION

Mycorrhizae are responsible for many plant functions, in particular nutrient uptake. With a significant reduction in colonization by ectomycorrhizal fungi, as observed here in *P. taeda* plots with high abundance of *I. cylindrica* during the November sampling (Fig. 2.2), the volume of soil the plant is able to exploit is reduced, potentially resulting in reduced uptake of essential nutrients. Although the magnitude of reduction in mycorrhizal colonization density (percent colonization of fine feeder roots) is not dramatic, the reduced abundance of fine feeder roots also observed on the invaded sites, especially deeper in the soil profile and in the November field sampling period (Figs. 2.4 and 2.5), likely compounds the effect of reduced mycorrhizal colonization in more heavily invaded sites, and hampers the ability of trees to uptake nutrients directly. Although significant differences in mycorrhizal colonization were only noted between *I. cylindrica* present and *I. cylindrica* abundant in November, we noted a trend towards increasing cover of *I. cylindrica* leading to decreased mycorrhizal colonization in both field seasons. In particular, the *I. cylindrica* absent and *I. cylindrica* abundant plots showed a nearly significant difference ($p=0.066$), despite very low replication ($n=3$).

We found that there was no significant difference in plant diversity indices between *I. cylindrica* present, abundant and absent plots. However, we did see a significant difference among *I. cylindrica* abundant and *I. cylindrica* absent plots. We also found that *I. cylindrica* percent cover was negatively related to vegetation diversity. It is well documented that diverse plant communities are more microbially active (Kowalchuk *et al.* 2002, Zak *et al.* 2003, Eisenhauer *et*

al. 2010). Microorganisms play an imperative role in nutrient cycling, in particular through mineralization and competition for nutrients (van der Heijden *et al.* 2008). Since dense monocultures of *I. cylindrica* reduce plant diversity associated microorganisms as well as non-associated beneficial microorganisms are likely being extirpated simultaneously, disrupting nutrient cycling. However, it is difficult to determine the exact effects of *I. cylindrica* on the resident microbial population without long-term and physiological research. Regardless of what the specific effects may be, they will doubtless have implications on resource management and conservation of biodiversity.

Microbial biomass N was highly variable in all plots and no significant differences were evident between plots; either by treatment or over time. We attributed this observation to the limited number of replications employed. A combination of observed effects including reduced plant diversity associated with *I. cylindrica*, increased bare space in *I. cylindrica* present plots and physical differences observed by other researchers (such as reduced light) may have a significant effect on the relationship between microbial biomass C:N. Microbial processes are linked with nutrient cycling, fertilization and organic matter transformation (Černý *et al.* 2003), and microbial biomass is often used as an indicator of soil quality. However, high levels of variability in microbial biomass, even among nearby areas, can make generalizations difficult (Xu *et al.* 2013). This ‘natural’ heterogeneity likely contributed to the variation observed between plots and may have been more influential as a whole than the influence of *I. cylindrica*.

Significant differences in *P. taeda* growth were not observed between *I. cylindrica* percent cover by stocking percentages. Although lower growth rates would be expected in the stands with higher stocking levels, growth of trees in the *I. cylindrica*-present plots in these stands was not significantly reduced. While competitive effects of *I. cylindrica* were expected, some invasive plants may produce a short-term fertilization effect due to increased net primary productivity, and production of litter with higher decomposition rates than native vegetation (Ehrenfeld 2003). In addition, variability in the time since *I. cylindrica* first appeared may be responsible for this discrepancy.

CONCLUSION

Abundance of mycorrhizal fungi (in November) and fine feeder roots (especially in November and deeper in the soil) were significantly reduced in plots where *I. cylindrica* was present. Vegetative communities also were less diverse in *I. cylindrica* present plots. These factors likely contribute to lower overall microbial diversity and a reduction in nutrient cycling. Although impacts on growth were not observed it is expected that over time, an impact may be seen. Furthermore, these changes may lead to communities that are more susceptible to disease and secondary invasion. Therefore, it is important to both production of *P. taeda* and conservation of biodiversity to limit area affected and invaded by *I. cylindrica* as effectively as possible by prompt and repeated treatment with effective herbicides.

REFERENCES

- Roberts K.J., Anderson R.C. 2001. Effect of garlic mustard [*Alliaria petiolata* (Beib. Cavara and Grande)] extracts on plants and arbuscular mycorrhizal (AM) fungi. *The American Midland Naturalist*, 146:146-152.
- Boufalis A., Pellissier F., Trosset L. 1994. Responses of mycorrhizal fungi to allelopathy: *Cenococcum geophilum* and *Laccaria laccata* growth with phenolic acids. *Acta Botanica Gallica* 141:547–550. doi:10.1080/12538078.1994.10515197
- Brundrett M., Bougher N., Dell B., Grove T., Malajczuk N. 1996. Working with Mycorrhizas in Forestry and Agriculture.
- Brunson B., Eckhardt L.G. 2012. Assessing the impacts of cogongrass (*Imperata cylindrica* (L.) Beauv) on root-feeding bark beetle populations associated with Southern Pine Decline. *Phytopathology* 102:1-2
- Bryson C.T., Carter R. 1993. Cogongrass, *Imperata cylindrica*, in the United States. *Weed Technology*, 1005-1009.
- Cerny J., Balik J., Pavlikova D., Zitkova M., Sykora K. 2003. The influence of organic and mineral nitrogen fertilizers on microbial biomass nitrogen and extractable organic nitrogen in long-term experiments with maize. *Plant, Soil and Environment* - UZPI (Czech Republic).
- Colwell R.K. 2013. EstimateS: Statistical estimation of species richness and shared species from samples. Version 9. User's Guide and application published at: <http://purl.oclc.org/estimates>
- Daneshgar P., Jose S. 2009. *Imperata cylindrica*, an alien invasive grass, maintains control over nitrogen availability in an establishing pine forest. *Plant Soil* 320:209–218. doi:10.1007/s11104-008-9886-8
- Daneshgar P., Jose S., Collins A., Ramsey C. 2008. Cogongrass (*Imperata cylindrica*), an alien invasive grass, reduces survival and productivity of an establishing pine forest. *Forest Science* 54:579–587.
- Devi NB, Yadava PS (2006) Seasonal dynamics of soil microbial biomass c, n and p in a mixed oak forest ecosystem of Manipur, north-east india. *Applied Soil Ecology* 31:220-227.
- Dickie, I.A., Bolstridge, N., Cooper, J.A., Peltzer, D.A. 2010. Co-invasion by *Pinus* and its mycorrhizal fungi. *New Phytologist* 187, 475–484.
- Ehrenfeld J.G. 2003. Effects of exotic plant invasions on soil nutrient cycling processes. *Ecosystems* 6:503–523.
- Eisenhauer, N., Beßler, H., Engels, C., Gleixner, G., Habekost, M., Milcu, A., Partsch, S., Sabais, A.C.W., Scherber, C., Steinbeiss, S., Weigelt, A., Weisser, W.W., Scheu, S. 2010. Plant diversity effects on soil microorganisms support the singular hypothesis. *Ecology* 91, 485–496.

Gingrich, S.F. 1967. Measuring and evaluating stocking and stand density in upland hardwood forests in the Central states. *Forest Science* 13:38-53.

Hagan D.L., Jose S., Lin C.H. 2013. Allelopathic exudates of cogongrass (*Imperata cylindrica*): Implications for the performance of native pine savanna plant species in the southeastern US. *J Chem Ecol* 39: 312–322.

Holm L.G., Plucknett D.L., Pancho J.V., Herberger J.P. 1977. The world's worst weeds. 609 pp.

Holzmueeller E.J., Jose S. 2011. Invasion success of cogongrass, an alien C4 perennial grass, in the southeastern United States: exploration of the ecological basis. *Biol Invasions* 13:435–442.

Horton T.R., Bruns T.D. 1998. Multiple-host fungi are the most frequent and abundant ectomycorrhizal types in a mixed stand of Douglas fir (*Pseudotsuga menziesii*) and bishop pine (*Pinus muricata*). *New Phytologist* 139:331–339.

Hussain F., Abidi N. 1991. Allelopathy exhibited by *Imperata cylindrica* (L.) P. Beauv. Pakistan *Journal of Botany* (Pakistan).

Jose S, Cox J, Miller DL, Shilling DG, Merritt S (2002) Alien plant invasions: The story of cogongrass in southeastern forests. *Journal of Forestry* 100:41–44.

Koger C.H., Bryson C.T. 2004. Effect of Cogongrass (*Imperata cylindrica*) Extracts on germination and seedling growth of selected grass and broadleaf species. *Weed Technology* 18:236–242.

Kowalchuk G.A., Buma D.S., Boer W., Klinkhamer P.G.L., Veen J.A. 2002. Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. *Antonie Van Leeuwenhoek* 81:509–520.

Kourtev P.S., Ehrenfeld J.G., Häggblom M. 2002. Exotic plant species alter the microbial community structure and function in the soil. *Ecology* 83:3152-3166.

Krebs C. 1989. Ecological methodology. Harper Collins Publishers.

MacDonald G.E. 2004. Cogongrass (*Imperata cylindrica*)—Biology, ecology, and management. *Critical Reviews in Plant Sciences* 23:367–380.

Magurran A.E. 2013. Measuring biological diversity. John Wiley and Sons.

Pasternak Z., Diamant A., Abelson A. 2007. Co-invasion of a Red Sea fish and its ectoparasitic monogenean, *Polylabris* cf. *mamaevi* into the Mediterranean: observations on oncomiracidium behavior and infection levels in both seas. *Parasitol Res* 100:721–727.

Pimentel D., Zuniga R., Morrison D. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics, Integrating Ecology and Economics in Control Bioinvasions* IEECB S.I. 52:273–288.

Richardson D.M., Allsopp N., D'antonio C.M., Milton S.J., Rejmánek M. 2000. Plant invasions — the role of mutualisms. *Biological Reviews* 75:65–93.

Roberts K.J., Anderson R.C. 2001. Effect of Garlic Mustard [*Alliaria petiolata* (Beib. Cavara and Grande)] extracts on plants and arbuscular mycorrhizal (AM) fungi. *The American Midland Naturalist* 146:146–152.

Simberloff D., Martin J.L., Genovesi P., Maris V., Wardle D.A., Aronson J., Courchamp F., Galil B., García-Berthou E., Pascal M., Pyšek P., Sousa R., Tabacchi E., Vilà M. 2013. Impacts of biological invasions: what's what and the way forward. *Trends in Ecology and Evolution* 28:58–66.

Soil Survey Laboratory Staff. 2012. Soil survey laboratory methods manual. Soil Surv. Invest. Rep. 42. Version 4.0. Natl. Soil Surv. Ctr., Lincoln, NE.

Taylor A.F. 2002. Fungal diversity in ectomycorrhizal communities: sampling effort and species detection. *Diversity and Integration in Mycorrhizas* 244:19-28.

Tedersoo L., Suvi T., Larsson E., Kõljalg U. 2006. Diversity and community structure of ectomycorrhizal fungi in a wooded meadow. *Mycological Research* 110:734–748.

USDA Forest Service. 1982. Service foresters handbook. SE Area, State and Priv. For., Atlanta, GA

Vance E.D., Brookes P.C., Jenkinson D.S. 1987. An extraction method for measuring soil microbial biomass C. *Soil biology and Biochemistry*, 19:703-707.

Van Der Heijden M.G.A., Bardgett R.D., Van Straalen N.M. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* 11:296–310.

Wardle DA (1992) A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. *Biological Reviews* 67:321-358.

Xu X., Thornton P.E., Post W.M. 2013. A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. *Global Ecology and Biogeography* 22:737–749.

Zak D.R., Holmes W.E., White D.C., Peacock A.D., Tilman D. 2003. Plant diversity, soil microbial communities, and ecosystem function: are there any links? *Ecology* 84:2042–2050.

Table 2.1. The site conditions and past treatments listed by plot. Plots in which no *I. cylindrica* were observed have the prefix N, plots with <50 % *I. cylindrica* have the prefix P and plots in which *I. cylindrica* was present >50 % have the prefix A. DAP was applied in 2006 at a rate of 280 kg.ha⁻¹ and Urea applied in 2010 at a rate of 224 kg.ha⁻¹. DAP applied in 2006 but at a rate of 140 kg.ha⁻¹ and Urea applied in 2007 at a rate of 224 kg.ha⁻¹.

| | <i>Imperata cylindrica</i> absent | | | <i>Imperata cylindrica</i> present (<50% cover) | | <i>Imperata cylindrica</i> abundant (>50% cover) | | |
|---|-----------------------------------|---------------------------------|------------------------------------|---|------------------------------------|--|----------------------------------|------------------------------------|
| Plot number | N1 | N2 | N3 | P1 | P2 | A1 | A2 | A3 |
| <i>Imperata cylindrica</i> cover 2011 (%) | 0 | 0 | 0 | 0 | 86 | 94 | 97 | 64 |
| Stand Age | 16 | 16 | 22 | 21 | 21 | 16 | 16 | 22 |
| Basal Area (m ² /Ha) | 20.9 | 21.6 | 24.8 | 25.3 | 25.3 | 21.6 | 20.9 | 24.8 |
| Trees Per Acre | 227 | 244 | 266 | 243 | 243 | 244 | 227 | 266 |
| Percent Stocking | 70 | | 80 | | 80 | 70 | | 80 |
| GPS | 31.1484 2, -88.482 | 31.1519 8, - 88.4829 2 | 31.1482 5, - 88.4805 2 | 31.1471 4, -88.4763 | 31.1480 4, - 88.4751 8 | 31.1489 6, - 88.4819 1 | 31.147 9, - 88.481 8 | 31.1478 5, - 88.4803 8 |
| Urea | 2010 | | 2007 | | 2010 | | 2007 | |
| Diammonium phosphate application (DAP) | 2006 | | 2006 | | 2006 | | 2006 | |
| Thinned | 2009 | | Prior to 2006 | | 2009 | | Prior to 2006 | |
| Burned | X | | Cool season, 2009 | | X | | Cool season, 2009 | |

Table 2.2. Species present indexed by site. Values represent percent cover averaged across all sub-plots. Species are divided by type.

| | <i>Imperata cylindrica</i> absent | | | <i>Imperata cylindrica</i> present | | | | |
|-----------------------------|-----------------------------------|-----------|-----------|------------------------------------|-----------|-----------|-----------|-----------|
| Species | N1 | N2 | N3 | P1 | P2 | A1 | A2 | A3 |
| Bare ground | 46 | 10 | 50 | 36 | 28 | 2 | 3 | 22 |
| Grass | | | | | | | | |
| Diacanthelium aciculare | 0 | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 |
| Imperata cylindrica | 0 | 0 | 0 | 21 | 12 | 92 | 97 | 53 |
| Muhlenbergia scheberi | 0.5 | 0.5 | 3.5 | 0 | 0 | 0 | 0 | 0 |
| Unknown sp 1 | 2 | 21 | 0 | 0 | 0 | 0 | 0 | 0 |
| Unknown sp 2 | 0 | 0 | 0.5 | 0 | 0 | 0 | 0 | 0 |
| Herbaceous | | | | | | | | |
| Chamaecrista fasciculata | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| Conya canadensis | 0.5 | 2 | 1 | 1 | 0 | 0 | 0 | 0 |
| Elephantopus tomentosus | 0 | 1.5 | 0 | 0 | 0 | 0 | 0 | 0 |
| Eupatorium capillifolium | 0 | 2.5 | 0 | 0 | 0 | 0 | 0 | 0 |
| Eupatorium rotundifolium | 28.5 | 11.5 | 33 | 8.5 | 0.5 | 1 | 0 | 5 |
| Lathyrus venosus | 0 | 14 | 0.5 | 0 | 0 | 0 | 0 | 0 |
| Oxalis stricta | 0 | 0 | 0.5 | 0 | 0 | 0 | 0 | 0 |
| Tephrosia virginiana | 0 | 0 | 0 | 0.5 | 2 | 0 | 0 | 0 |
| Tragia smallii | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 |
| Shrub | | | | | | | | |
| Callicarpa americana | 2.5 | 3 | 0 | 4 | 0 | 0 | 0 | 2.5 |
| Hypericum hypericoides | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 |
| Ilex glabra | 0 | 0 | 0 | 1 | 30 | 0 | 0 | 0 |
| Illex vomitoria | 6 | 3.5 | 3 | 6 | 8 | 3 | 0 | 0 |
| Rubus sp. | 2.5 | 21 | 0 | 1.5 | 3 | 0.5 | 0 | 0.5 |
| Vaccinium stamineum | 0.5 | 0 | 0 | 0 | 0.5 | 2 | 0 | 0 |
| Tree | | | | | | | | |
| Halesia diptera | 8 | 0.5 | 0 | 0 | 0.5 | 0 | 0 | 0 |
| Morella cerifera | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pinus taeda | 3 | 2 | 0 | 2 | 1 | 0 | 0 | 3 |
| Quercus sp. | 1 | 3 | 0 | 0 | 4 | 0 | 0 | 0 |
| Rhus copallinum | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0 |
| Vine | | | | | | | | |
| Gelsenium sempervirens | 2 | 0.5 | 12 | 18 | 3 | 0 | 0 | 14 |
| Parthenocissus quinquefolia | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| Smilax pumila | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Smilax sp. | 2.5 | 1.5 | 0.5 | 1 | 0 | 1 | 0 | 0.5 |
| Toxicodendron radicans | 0 | 0 | 0 | 1.5 | 0 | 0 | 0 | 2.5 |

Table 2.3. Percent cover of *Imperata cylindrica* and bare ground on plots, as well as species richness and measures of diversity.

| | <i>Imperata cylindrica</i> absent | | | <i>Imperata cylindrica</i> present | | <i>Imperata cylindrica</i> abundant | | |
|--------------------------------------|-----------------------------------|------|------|------------------------------------|------|-------------------------------------|------|------|
| Plot | N1 | N2 | N3 | P1 | P2 | C1 | C2 | C3 |
| <i>Imperata cylindrica</i> (% cover) | 0 | 0 | 0 | 22 | 12 | 92 | 97 | 53 |
| Bare ground (% cover) | 46 | 10 | 50 | 36 | 28 | 2 | 3 | 22 |
| Species richness | 13 | 18 | 9 | 11 | 12 | 7 | 2 | 7 |
| Shannon-Wiener | 1.79 | 2.31 | 1.21 | 1.78 | 1.84 | 0.4 | 0.03 | 1.03 |
| Simpson index | 3.62 | 7.49 | 2.36 | 4.49 | 4.44 | 1.17 | 1.01 | 1.99 |

Table 2.4. Correlation coefficients of each plant species present at the study site. Bolded values are significant at $p < .05$, $N=8$.

| Species | Diversity Index | |
|------------------------------------|-----------------|---------------|
| | Shannon-Weiner | Simpson's |
| Bare ground | 0.481 | 0.142 |
| Grass | | |
| <i>Diacantheium aciculare</i> | 0.522 | 0.779 |
| <i>Imperata cylindrica</i> | -0.903 | -0.730 |
| Unknown sp 1 | 0.551 | 0.791 |
| Unknown sp 2 | -0.046 | -0.180 |
| <i>Muhlenbergia scheberi</i> | 0.067 | -0.062 |
| Herbaceous | | |
| <i>Eupatorium rotundifolium</i> | 0.351 | 0.135 |
| <i>Elephantopus tomentosus</i> | 0.522 | 0.779 |
| <i>Tephrosia virginiana</i> | 0.343 | 0.265 |
| <i>Chamaecrista fasciculata</i> | 0.522 | 0.779 |
| <i>Conya canadensis</i> | 0.666 | 0.788 |
| <i>Oxalis stricta</i> | -0.046 | -0.180 |
| <i>Lathyrus venosus</i> | 0.523 | 0.776 |
| <i>Eupatorium capillifolium</i> | 0.522 | 0.779 |
| <i>Tragia smallii</i> | -0.655 | -0.432 |
| Shrub | | |
| <i>Rubus</i> sp. | 0.622 | 0.851 |
| <i>Callicarpa americana</i> | 0.603 | 0.578 |
| <i>Vaccinium stamineum</i> | -0.337 | -0.342 |
| <i>Ilex glabra</i> | 0.289 | 0.217 |
| <i>Hypericum hypericoides</i> | 0.280 | 0.209 |
| <i>Illex vomitoria</i> | 0.684 | 0.530 |
| Tree | | |
| <i>Halesia diptera</i> | 0.308 | 0.119 |
| <i>Rhus copallinum</i> | -0.464 | -0.402 |
| <i>Morella cerifera</i> | 0.254 | 0.056 |
| <i>Quercus</i> sp. 1 | 0.279 | 0.209 |
| <i>Quercus</i> sp. 2 | 0.602 | 0.791 |
| <i>Pinus taeda</i> | 0.629 | 0.839 |
| Vine | | |
| <i>Gelsenium sempervirens</i> | 0.174 | -0.028 |
| <i>Toxicodendron radicans</i> | 0.009 | -0.107 |
| <i>Parthenocissus quinquefolia</i> | 0.522 | 0.779 |
| <i>Smilax</i> sp. | 0.470 | 0.386 |



Figure 2.1. (A.) An example of a plot where *I. cylindrica* was abundant (>50%); (B.) An example of a plot where *I. cylindrica* was absent.

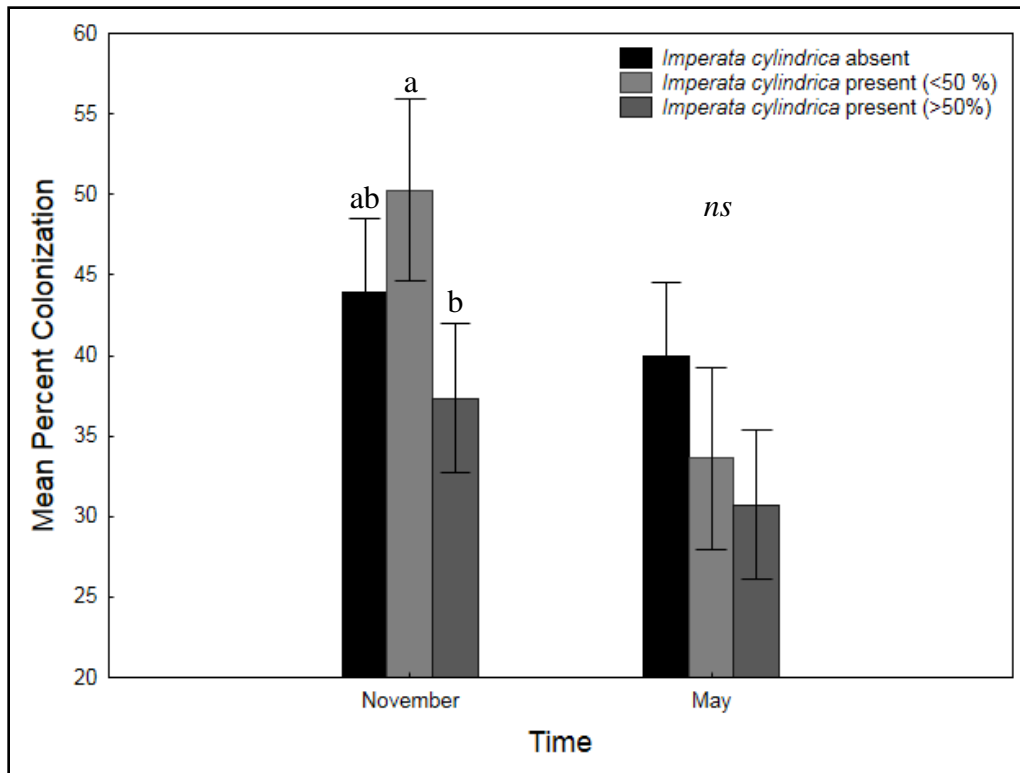


Figure 2.2. Mean values of percent colonization of mycorrhizal fungi on fine feeder roots of *P. taeda* over the course of two field seasons. Error bars represent standard error. Letters represent statistically significant different pairwise comparisons between *I. cylindrica* abundances, not between sampling seasons.

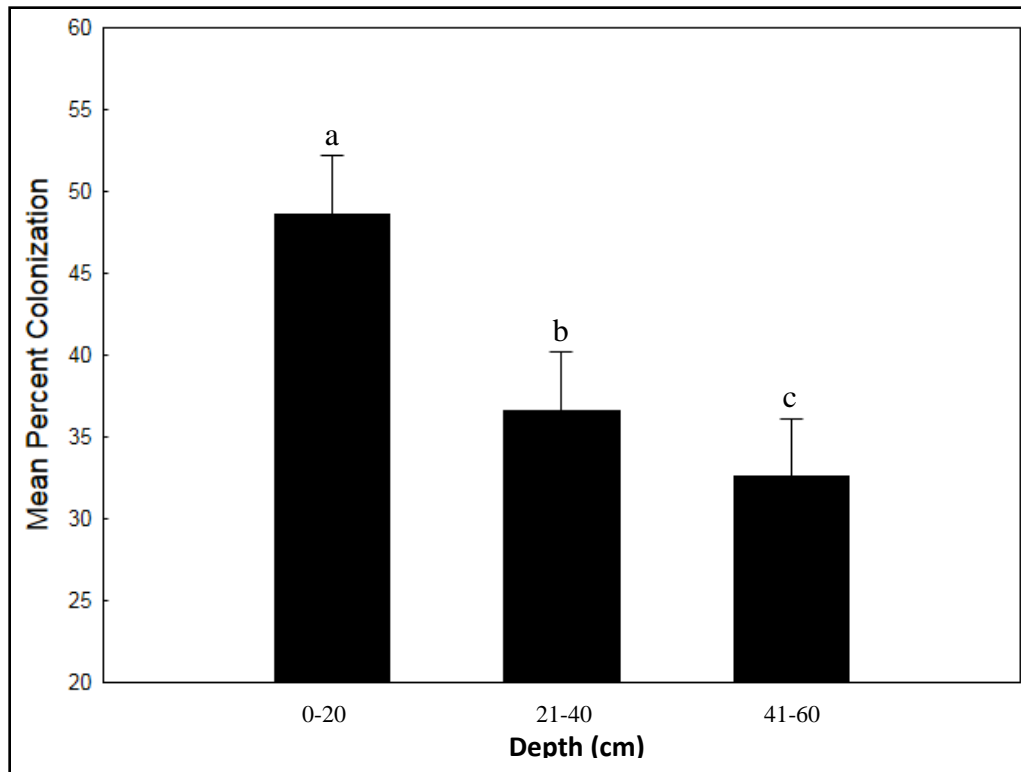


Figure 2.3. Mean values of percent colonization of mycorrhizal fungi on fine feeder roots of *P. taeda* at each of the three depth classes over the course of two field seasons. Error bars represent standard error. Letters indicate statistically significant differences.

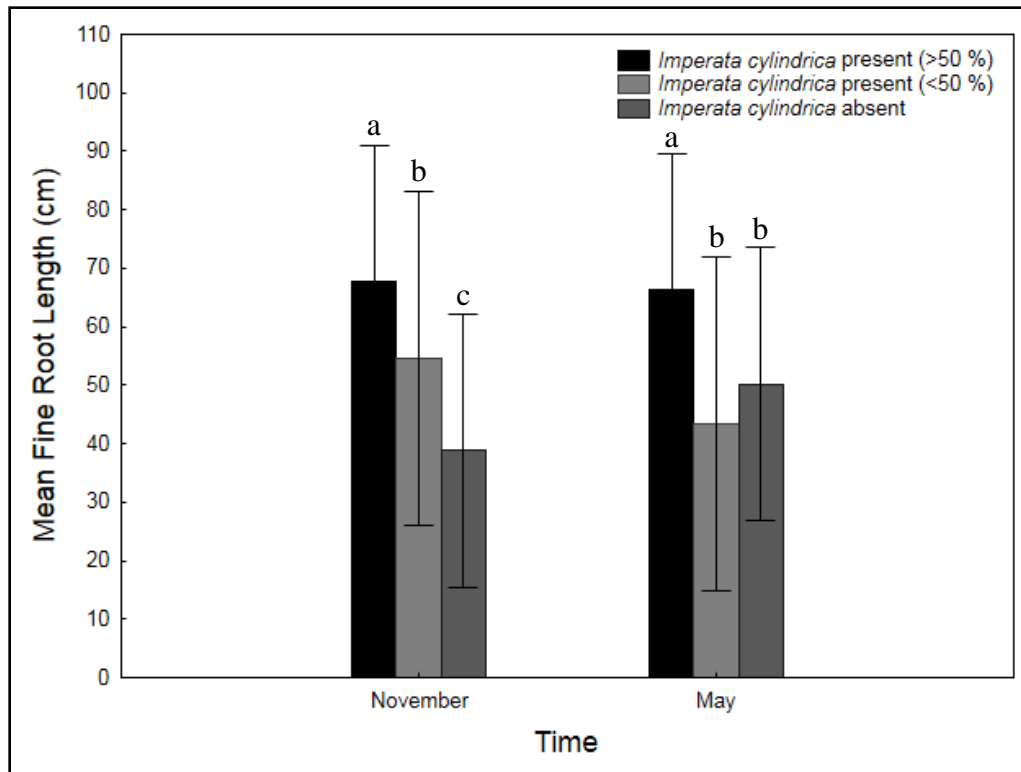


Figure 2.4. Mean values of recovered fine feeder roots of *P. taeda* over the course of two field seasons. Error bars represent standard error. Letters represent statistically significant differences between *I. cylindrica* abundances, not between sampling seasons.

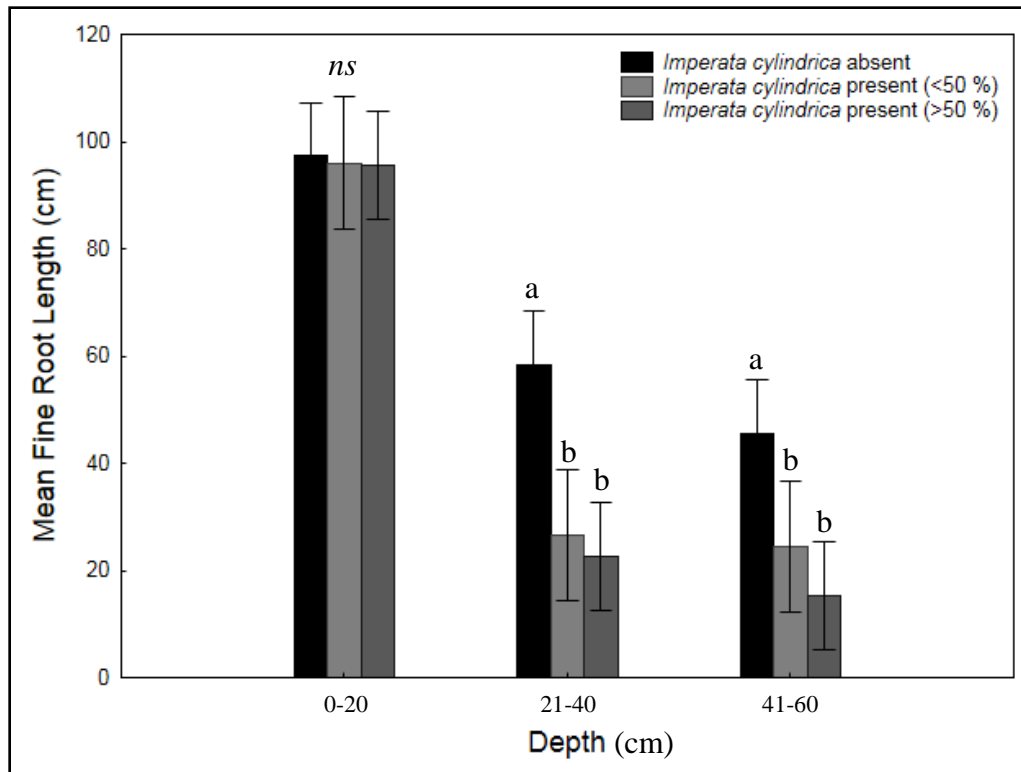


Figure 2.5. Mean values of recovered fine feeder roots of *P. taeda* over the course of two field seasons. Error bars represent 95 % confidence intervals. Letters represent statistically significant differences between depth classes, not between sampling seasons.

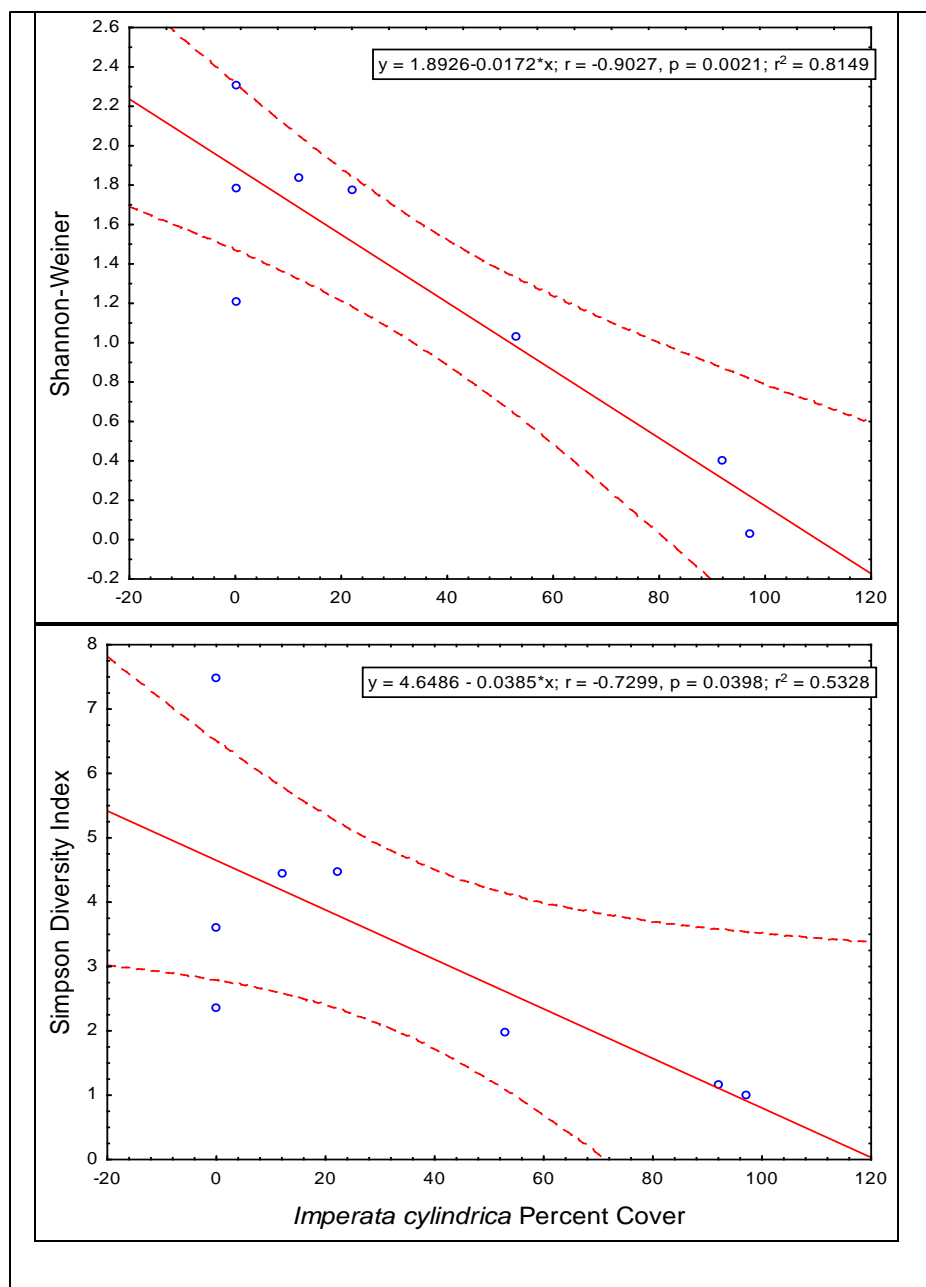


Figure 2.6. Linear regression of (A) Shannon-Weiner index and *Imperata cylindrica* percent cover (n=8); (B) Simpson diversity index and *Imperata cylindrica* percent cover (n=8).

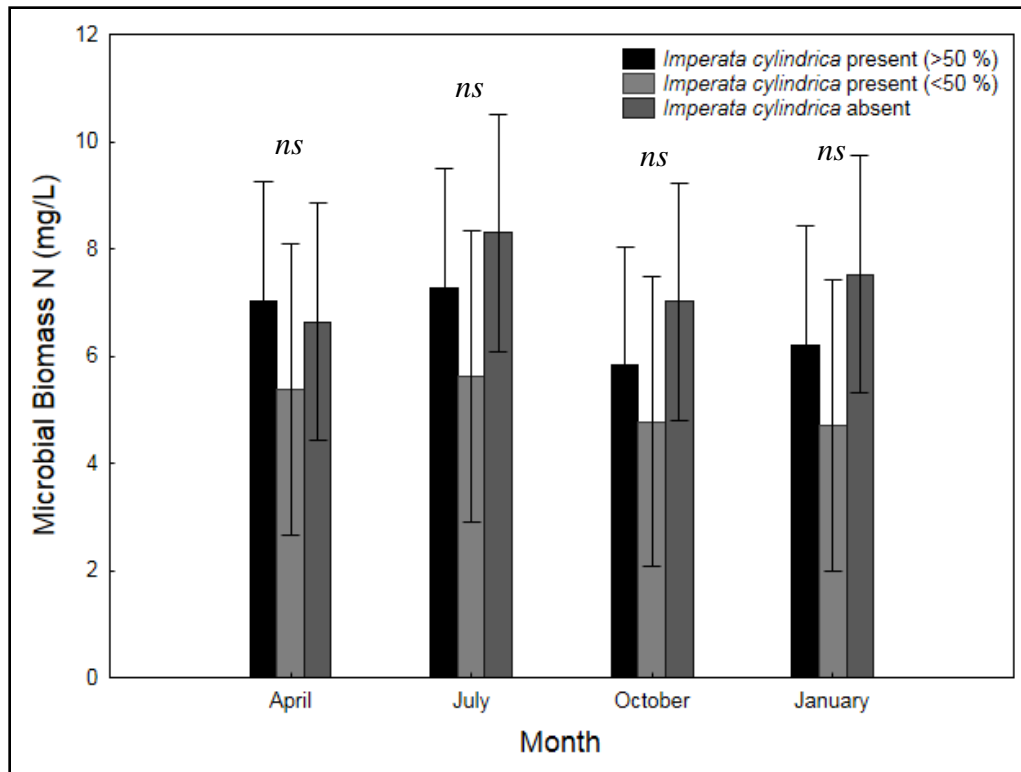


Figure 2.7. Mean values of microbial biomass N are listed for *I. cylindrica* present and absent plots by season. Error bars represent standard error, n=8.

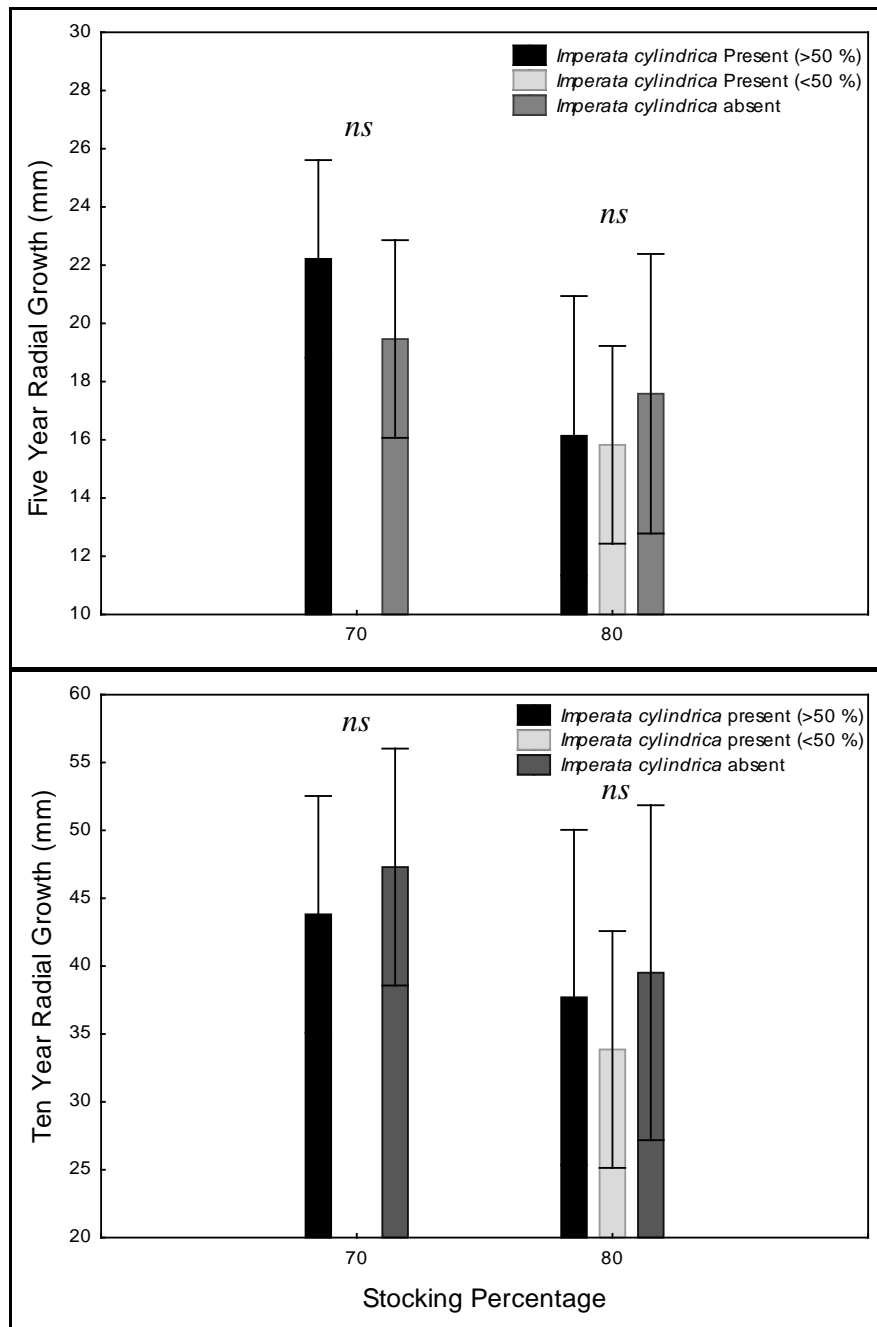


Figure 2.8. Mean values of (A) five year radial growth (B) ten year radial growth (mm) of *P. taeda* is listed by stocking percentage.