INTERACTIONS BETWEEN PLANTS AND SOIL MICROBES IN FLORIDA COMMUNITIES: IMPLICATIONS FOR INVASION AND ECOSYSTEM ECOLOGY

By

SARAH RENEE BRAY

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Sarah Renee Bray

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Chair: Kaoru Kitajima
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Among ecologists there is an increasing awareness and interest in the role of soil microbes in the distribution of plants and functioning of ecosystems. This dissertation relates soil microbial community composition to plant growth, habitat type, and decomposition with particular emphasis on invasive plants.

I examined growth, physiology and competitive ability of an invasive shrub, *Ardisia crenata*, in two greenhouse experiments. When grown singly, relative growth rates (RGR) and leaf area ratio (LAR) were higher for seedlings inoculated with mycorrhizal fungi isolated from *Ardisia* roots than those inoculated with single-spore isolates and nonmycorrhizal controls. In a second experiment, *Ardisia* was grown with a conspecific or heterospecific (*Prunus caroliniana*) competitor. While neither identity of competitor nor mycorrhizal status had a great effect on *Ardisia* growth, *Prunus* growth was significantly depressed in competition with *Ardisia* in the absence of mycorrhizal fungi.

In chapter 2, I examined soil microbial communities from five different habitats prone to invasion by an invasive plant using phospholipid fatty acids (PLFA) and Biolog substrate utilization. Habitat type had the largest effect on microbial community composition. Moisture content of soils and, to a lesser extent, carbon and nitrogen contents appeared to be driving differences in biomarker PLFAs. Although the largest differences in soil microbial community composition were found among habitats, invasion altered microbial community composition within habitats.

In chapter 3, I placed litters of 20 native and non-native plant species of varying decomposability in a common site and quantified their decomposition. The composition of microbial communities on 11 of the litters was examined by PLFA at 28, 56 and 238 days. Microbial communities at the early (low moisture) sampling dates were more similar to one another than to the late (high moisture) sampling date. In addition to moisture effects, litter quality had a significant effect on microbial community composition. Both decomposition and microbial community composition were correlated with leaf chemistry. The best single predictor of decomposition rate was microbial community composition.

These results suggest that plant-microbial interactions are important in plant invasion, and explicit examination of a potential positive feedback on invasion through the microbial community should be further explored.

CHAPTER 1
INTRODUCTION

Historically, most ecologists have studied aboveground macroorganisms even though they have long recognized that processes and organisms aboveground can modify processes and species distributions belowground and vice versa (Jenny 1941, Brown 1958, Garrett 1963, Hudson 1968, Janos 1980). Much of this aboveground bias has been due to the difficulty of examining organisms and tissues in the soil matrix. Traditionally, culture-based techniques have been used to identify and isolate soil microbes. However, only a small proportion of soil microbes (<1%) are believed to be culturable (Atlas and Bartha 1998), and many researchers have treated the soil as a "black box." The advent of new techniques to study belowground microorganisms using molecular markers has led to a "renaissance" of sorts in the study of interactions of soil microorganisms with aboveground macrophytes, allowing ecologists to proceed "through a ped darkly" (Coleman 1985).

Soil microbes have direct and indirect interactions with plants (Figure 1-1). Symbiotic microbes directly affect the host plant's fitness, resulting in alteration of plant species abundance and distribution. Pathogenic microbes have negative effects on individual plant fitness. Due to pathogens' high host-specificity, these microbes may be responsible for density-dependant plant distributions predicted from the Janzen-Connell hypothesis (e.g., Packer and Clay 2000). Mutualistic bacteria and fungi (e.g., nitrogen-fixing bacteria and mycorrhizal fungi) also directly interact with their hosts receiving carbon substrates directly from their host and supplying the plant with nutrients. Free-

living saprophytic fungi and bacteria in the soil interact indirectly with plants by decomposing their senescent tissue and replenishing inorganic soil nutrients. Therefore, whereas mutualistic soil microbes may be a proximal source of nutrients, free-living microbes supply the ultimate source of nutrients.

Availability of nutrients in the soil is one of the primary controls of productivity in ecosystems, and competition for those limiting resources is a major control of community composition. Plant species that can draw down the most limiting resource below the level at which other plants can survive (i.e., the plant with the lowest R*) should be the best competitor within a given system and will displace other species (Tilman 1982). To become a superior competitor for this limiting resource, the plant must allocate a greater proportion of its biomass to the acquisition, conservation, and/or efficient use of that resource. Such biomass investment limits the ability of the plant to compete for other limiting resources. The resultant trade-off means that species with different allocation patterns or suites of functional traits related to resource acquisition will be superior competitors based upon resource availability in the habitat (Chapin 1980, Tilman 1988, Chapin and Aerts 2000).

Competition for resources by plants may be modified by interactions with free-living and symbiotic microbes. Mycorrhizal fungi increase the volume of soil a plant can access, increasing their ability to acquire phosphorus. Increasing the mycorrhizal fungal richness leads to a greater use of soil phosphorus by the plant community, greater productivity and plant species richness (van der Heijden et al. 1998). Not all species of mycorrhizal fungi benefit all plants equally, however, as carbon cost of some fungal partners exceeds the benefit in increased phosphorus nutrition (Bethlenfalvay et al. 1982,

Smith and Smith 1996, Johnson et al. 1997, Graham and Eissenstat 1998). Such differential response of plants to the species of mycorrhizal fungi present may modify the competitive hierarchy (Moora and Zobel 1996, Smith et al. 1999). If dominant, superior competitors harbor fungi parasitic to themselves, their competitive ability should be decreased. Work by Bever et al. (1996) has, in fact, shown that there are host-specific sporulation rates of mycorrhizal fungi. They found that these differential rates of sporulation maintained diversity through negative feedback on dominant plants as the fungus that preferentially sporulated with the dominant plant was also least beneficial to it (Bever 2002). Conversely, dominance may be achieved through the presence of mutualistic microbes or the absence or resistance to pathogenic microbes (Bever et al. 1997).

While studies of mycorrhizal fungi have demonstrated that interactions between fungi and plants influence the distribution and abundance of both groups, our knowledge and understanding of the interactions between plants and free-living microbes are more limited. Because of the difficulty in examining non-culturable soil microbes, ecologists have generally left this community as a "black box," examining the effect of soil communities on plant growth without identifying the agents (pathogenic, mutualistic or free-living) responsible. In these types of studies, plants are grown in a soil for an extended period of time to allow for the soil community differentiation over time due to plant inputs. Plants are then grown in their own or another species' soil. Some studies have shown that plant growth is higher in their own soil (a "home-soil" advantage) while others have shown the reverse (Bever 1994, Callaway et al. 2004). This would suggest

the possibility for negative or positive regulation of plant species density through the soil community although those agents responsible for the regulation are unknown.

There has been an increased interest in moving away from the "black box" and examining what free-living microbes are actually responding to different plant species and what effects changes in free-living microbes might be. The advent of molecular techniques for examining soil microbes, in particular the use of phospholipid fatty acids (PLFA) and nucleic acids, has resulted in a large number of studies of soil microbial community composition (Tiedje et al. 1999). Techniques based upon DNA and RNA have greater power in distinguishing microbial species than do PLFA techniques. However, due to the specificity of primers and probes used, nucleic acid based studies tend to examine a phylogentic subset of soil microbes. Additionally, as all nucleic acid techniques are dependant on PCR, only presence/absence, rather than quantitative analysis, is possible. While PLFAs are limited in their ability to identify species, and thus can never truly answer questions about diversity, they can be used to identify different functional groups of microbes and do allow for a broad, quantitative examination of the entire soil microbial community. Thus the use of both types of techniques will help to advance understanding of soil microbial community composition.

The field of soil ecology is still very much in an exploratory phase; however, some trends are beginning to emerge. Different environmental factors such as temperature, moisture, and soil nutrients, and different plant species support different microbial communities (Bossio and Scow 1998, Bossio et al. 1998, Staddon et al. 1998, Pennanen et al. 1999, Priha and Smolander 1999, Myers et al. 2001). Less research has been directed at the effects of free-living microbes on the plant community. Because microbes

decompose organic matter, releaing inorganic nutrients, microbial influence on nutrient availability may result in plant community changes. As decomposition rates and microbial communities are known to differ among plant communities, microbial community composition may be ultimately responsible for differences in decomposition rate.

These various plant-microbial interactions have for the most part been documented in systems with well-established associations of plant species. Plant community composition and diversity, however, are changing at global and local scales. Plant invasions, in effect, offer a "natural" experiment in which to examine the effect of changing species composition on plant-microbial interactions. Plant invasions also offer a chance to examine the interaction of native microbes and non-native plants that likely do not share an evolutionary history.

The goal of this dissertation is to use the natural experiment of non-native plant invasion to further our understanding of plant controls on symbiotic and free-living microbial community composition and vice versa. Such studies will also shed light on the functional reason for dominance of invasive plants. My dissertation is divided into three sections examining the interaction of mycorrhizal fungi with an invasive shrub, differences between soil microbial communities from different habitats with and without plant invasion, and the links between native and non-native plant litter, microbial community composition and decomposition.

In Chapter 2, I study the effect of native mycorrhizal fungi on the growth, physiology and competitive ability of an invasive shrub *Ardisia crenata* in two experiments. I hypothesized that *Ardisia*'s successful invasion may be in part due to its

ability to form beneficial partnerships with native mycorrhizal fungi it encounters in the areas it invades. This was tested in a greenhouse experiment in which *Ardisia* was grown in sterile soil, with one of two native single fungal species, or with a mix of mycorrhizal fungi isolated from *Ardisia* roots. I also hypothesized that *Ardisia* performs better in heterospecific competition than conspecific competition especially when mycorrhizae are present to modify resource competition. This was tested in a second greenhouse experiment in which *Ardisia* was grown with a single conspecific or a native heterospecific competitor, *Prunus carolinana*, with or without mycorrhizae.

As the results from Chapter 2 indicated that *Ardisia*'s growth was improved by the presence of mycorrhizal fungi found naturally occurring in its roots, I wanted to determine if invasion could result in the alteration of the soil microbial community. If invaders alter the composition of the soil microbial community to their advantage, there might be potential for positive feedback on the invasion. In a natural survey of five habitats prone to invasion by non-native plants, I sought to determine if plant invasion predictably alters soil microbial community composition and function as examined with phospholipid fatty acids (PLFA) and Biolog substrate utilization. Three alternative hypotheses could explain soil microbial community composition at the landscape level. One, habitat characteristics such as soil type, soil nutrients and moisture contents, temperature, and native plant community composition could have such a strong control on soil microbial community composition that even dense invasions may not result in a change in soil microbial community composition. Alternatively, dense invasions may alter the microenvironment experienced by soil microbes enough to alter the composition of their communities. There may also be an intermediate hypothesis where some, but not

all invaders alter soil microbial community composition as a result of functional characteristics of the invader.

The results of Chapter 3 suggested that while there were larger differences between habitats than within habitats due to invasion status, invasion does alter microbial community composition. The final chapter (Chapter 4), I sought to determine if the chemical composition of litter determines the microbial community present and how microbial community composition is related to decomposition. I hypothesized that more chemically recalcitrant litter (i.e., litter high in lignin, C:N, and low N:lignin) would have a higher proportion of fungi and would decompose more slowly. Litter with highly labile chemistry (i.e., litter high in non-fiber carbon fractions and nitrogen and low in lignin) would decompose quickly as a result of higher total microbial biomass and a higher proportion of bacteria. To test these hypotheses, I created a "common garden" experiment in which litter of varying litter quality from native and non-native plant species were allowed to decompose for the period of one year. The composition of microbes on the plant litter was examined for 11 of the species at 28, 57, and 238 days. Composition of microbial communities varied with time; however, microbial communities on leaf litter of similar quality were more similar to one another than to microbial communities on litter of very different quality. I also found the microbial community composition was the best predictor of decomposition rate of all factors examined.

Together these studies begin to paint a picture of how plant and microbial communities may feedback on one another's composition. This work additionally shows that plant-microbial interactions may be an important pathway for the success of invasive

plants in new ranges. Further studies should explicitly examine the potential positive feedbacks on invasion through soil microbial communities.

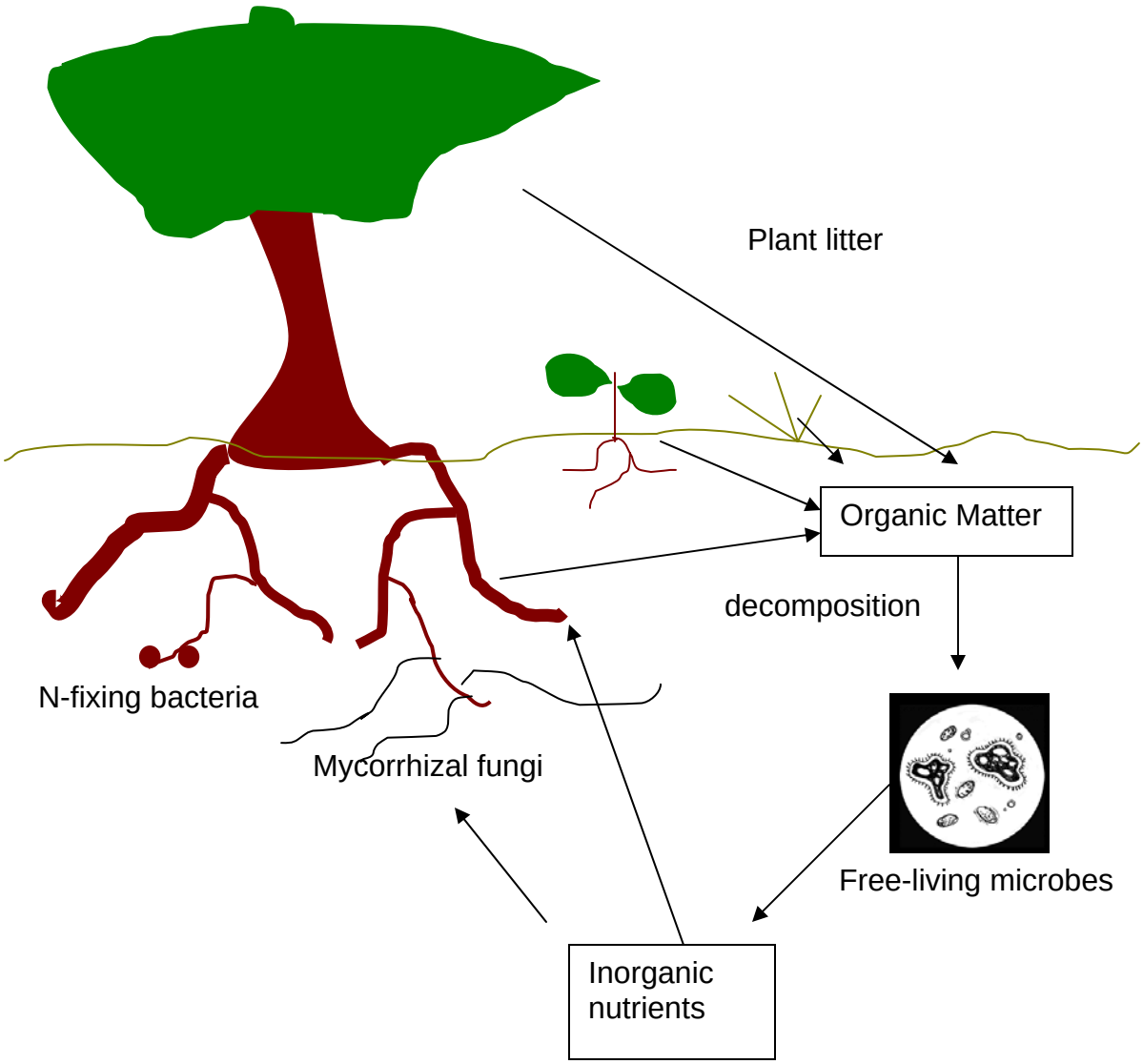


Figure 1-1: Conceptual diagram of interactions between soil microbes and plants.

CHAPTER 2
MYCORRHIZAE DIFFERENTIALLY ALTER GROWTH, PHYSIOLOGY AND COMPETITIVE ABILITY OF AN INVASIVE SHRUB

Introduction

Plants must overcome obstacles of dispersal, abiotic conditions, and competition with existing species in order to colonize and establish in a new geographical locality.[1] Arbuscular mycorrhizal fungi can aid or hinder the establishment of a new species by ameliorating or intensifying the abiotic stresses encountered in the new range. Arbuscular mycorrhizae (AM) may improve phosphorus (P) availability and enhance leaf photosynthetic rates and growth rates of the hosts (Siqueira et al. 1998, Sharma and Adholeya 2000). Due to improved P nutrition, mycorrhizal plants may allocate proportionally less to roots while increasing leaf area ratio and specific leaf area (Berta et al. 1995, Gavito et al. 2000, Lovelock et al. 1996, Son and Smith 1988). Change in allocation patterns of the host, in turn, may affect its interaction with neighboring plants for light and soil nutrients. Because the response of plants to AM is dependant on both soil-P levels and light availability (Gavito et al. 2000, Graham et al. 1997, Peng et al. 1993), effects of AM fungi on the plant invasion process must depend on these abiotic factors.

Although AM can infect a wide range of hosts from various geographical localities, the responses of host plants to mycorrhizae vary greatly depending on the combination of

[1] The information in this chapter was published in: Bray, S.R., K. Kitajima, and D.M. Sylvia. 2003. Mycorrhizae differentially alter growth, physiology, and competitive ability of an invasive shrub. Ecological Applications **13**: 565-574 and is used here with the permission of the Ecological Society of America.

plant and fungus genotypes (Johnson et al.1997, Smith and Smith 1996). Different fungal genotypes can have positive, negative or little effect on the growth of the same host species (Boerner 1990, Monzon and Azcon 1996, van der Heijden and Kuyper 2001), because AM may differ in their ability to infect a given host, efficiency of P transferred to the host, carbon demand, soil adaptation, and host compatibility (Graham et al. 1996, Johnson 1993, Johnson et al. 1997, Monzon and Azcon 1996). Thus, assessment of the effects of AM on plant invasion must consider the genotype and source of fungal isolates.

Mycorrhizal fungi may alter competitive interactions between invading and local plants. Although AM have been largely ignored as a mediator of plant invasion (Richardson et al. 2000), they have been shown to increase the growth of an invasive plant species over natives and accelerate the process of invasion in a grassland ecosystem (Marler et al. 1999). More generally, differences in competitive ability under the influence of mycorrhizae can alter community composition by favoring mycorrhizal-responsive, inferior competitors (Hartnett et al. 1993, Moora and Zobel 1996, Smith et al. 1999) or causing competitive exclusion of non-responsive dominants (Gange et al. 1999, Marler et al. 1999). Whether mycorrhizal fungi promote or inhibit the process of plant invasion must be determined by examining responses of exotic plants to multiple fungal genotypes with and without competition with native species that occupy similar ecological niches.

Here we report the results of two experiments that examined the effects of fungal isolates and abiotic environment on growth and competitive interactions of an exotic invasive shrub. Specifically, we examined the effects of various isolates of AM fungi,

soil-P, light and competition type on the growth, physiology and biomass allocation of *Ardisia crenata* Sims (Myrsinaceae, hereafter *Ardisia*). In the first greenhouse experiment, we examined the effects of light, soil-P content, and AM fungal isolates on growth, photosynthetic rates and biomass allocation patterns of *Ardisia* seedlings grown singly in pots. We hypothesized that mycorrhizal plants would exhibit higher growth rates, invest more biomass aboveground, and maximize leaf area ratio compared to nonmycorrhizal plants, and plant response to phosphorus would vary among mycorrhizal isolates. In the second greenhouse experiment, we examined the effects of AM on inter- vs. intraspecific competition between seedlings of *Ardisia* and a native shade-tolerant subcanopy tree, *Prunus caroliniana* (Mill) Aiton (Rosaceae, hereafter *Prunus*). We hypothesized that *Ardisia* would be less affected by conspecific than heterospecific competition, particularly when mycorrhizal.

Materials and Methods

Species

Ardisia is a woody evergreen shrub that was introduced as an ornamental to the southeastern United States from east Asia ca. 100 years ago (Dozier 1999). *Ardisia* is actively invading mesic forest understory in Louisiana, Texas, Hawaii, and north-central Florida (Singhurst et al. 1997). *Ardisia* forms dense monodominant patches in the understory and suppresses local species richness and diversity of native understory plant species (A. Fox and K. Kitajima, unpublished data). The architecture of the plants creates strong self- and neighbor-shading, even when plants are not in dense clumps (K. Kitajima and M. Dooley, unpublished data). Growth of *Ardisia* seedlings in the field has been shown to be positively correlated with soil-P content (Dozier 1999) and *Ardisia* roots are highly colonized by AM in the field (S. Bray, unpublished data). *Prunus* was

chosen as a heterospecific competitor in the experiment because its juveniles are abundant in forest understories where *Ardisia* typically invades (A. Fox and K. Kitajima unpublished data). These species have similar seed size, and the juveniles of both species have evergreen leaves that are common under the partially deciduous canopy of mesic hardwoods forests in north-central Florida.

Experiment 1: Effects of Soil-P, Light and Inoculum Source on Growth and Allocation

Three inocula and a sterile control were used in this experiment. The inocula were *Glomus etunicatum* (S3029), *G. fasciculatum* (S3060), and host-associated fungi. S3029 has been maintained in pot culture for 15 years; S3060 was isolated in 1997 from a tomato field in north-central Florida (Sylvia et al. 2001). These isolates have caused positive growth responses in both agronomic and native woody plants (Sylvia 1990, Sylvia et al. 1993) and will be collectively referred to as standard inocula. The host-associated inoculum (HA) was composed of a corn trap culture initiated with washed *Ardisia* roots gathered from a north-central Florida hardwood forest (29'40" N, 82'9" W). Inocula were composed soil, roots and spores produced in the corn trap cultures. Infection potential of the inocula was determined by growing corn (*Zea maize*) with 5 g of inocula for 4 wk (Sylvia 1994). Infection potential rather than most probable number was used because we were interested in comparing inocula rather than determining absolute numbers of propogules. S3060 had the highest infection potential (51.7% $\pm$ 10.2%) followed by HA (46.7% $\pm$ 7.21%) and S3029 (33.0% $\pm$ 7.00%).

Ardisia seeds were gathered from four populations in Gainesville, FL, mixed, cleaned of pulp and stored in moist sand at 4^{o}C for 24 wk. Seeds were germinated in petri dishes lined with moist filter paper at 26^{o}C. Before leaf development, seedlings

(approx. 15 d after radicle emergence) were transferred to bleach-sterilized Deepots (6.2 cm top diameter x 24.5 cm, J.M. McConkey & Co., Sumner, WA) containing a 1:1:1 steam-pasteurized mixture of soil:sand:peat moss. The soil was collected from Austin Carey Forest (29'43" N, 82' 13" W). The soil had a pH of 5.7 (2:1, H_2O:soil), 0.1% organic matter, and 1.6 mg Mehlich-I-extractable P kg^{-1}. The sand was acid washed to remove excess P, and rinsed until neutral pH was achieved. Phosphorus was added in the form of $KHPO_4$ at 0, 5, 30 or 60 mg P kg^{-1} soil. Pots were filled ¾ full with the growth medium; 5 g of inoculum were added to inoculated treatments and thoroughly mixed with the growth medium. After adding seedlings of equal mass and remaining soil, pots were randomly assigned to shading treatments with one (moderate light) or two (low light) layers of shade cloth supported by PVC frames. These treatments created mean photosynthetic photon flux densities (PPFD) of 412 µmol m^{-2} s^{-1} (moderate) and 212 µmol m^{-2} s^{-1} (low) at mid-day. The shade treatments were randomly assigned to locations within each of three blocks along a greenhouse bench. Each block contained 3 to 4 plants per treatment group. For the control, S3060 and S3029 inoculum types, there were four P levels by two light levels by 10 replicates for a total of 240 plants. The HA inoculum was used only at the 5 mg kg^{-1} P level, but had 10 replicates in each light treatment for 20 HA inoculated plants. Plants were watered when necessary and biweekly given a modified Hoagland's solution of 0.1x concentration of all nutrients, except P at 0.01x concentration (Sylvia et al. 2001).

The photosynthetic rates of the most recently fully expanded leaf were measured with a Li-6400 gas-exchange system (Li-Cor, Lincoln, NE) for three seedlings per light and inoculum treatment combination at the 5 mg kg^{-1} P level during the fourth month

after planting. All measurements were taken between 0800 and 1200. A CO_2 mixer unit maintained the CO_2 concentration of incoming reference air at 380 ppm. Temperature of the thermister block was maintained at 26°C. Flow rate was 250 mL min^{-1}. Light was supplied with a blue-red LED (LI6200-02B). Leaves were exposed to 500 μmol m^{-2} s^{-1} PPFD for 15 min for photosynthetic induction, after which quasi steady-state gas exchange rates were recorded at light levels of 800, 500, 300, 100, 60, 40, 20, 10, and 0 μmol m^{-2} s^{-1}.

Plants were harvested after 258 d and leaf area was measured immediately with a portable area meter (Li-3000, Li-Cor, Lincoln, NE). Roots of five randomly selected plants within each treatment combination were weighed for fresh mass and set aside for analysis of AM colonization. Roots of the remaining plants along with stems and leaves of all plants were dried at 60° C until constant mass was reached. The dry mass of roots used for assessment of colonization was estimated from fresh:dry mass ratios of roots. To examine biomass allocation pattern, root:shoot ratio (R/S), specific leaf area (SLA, leaf area divided by leaf mass), net assimilation ratio (NAR, net carbon assimilation on leaf area basis) and leaf area ratio (LAR, leaf area divided by total mass) were calculated. Relative growth rate (RGR) was determined using the following equation:

$$RGR\ (mg \cdot g^{-1} day^{-1}) = \frac{\ln(\textit{seedling mass at harvest}) - \ln(\textit{initial seedling mass})}{\textit{duration of study (days)}}$$

Tissue phosphorous contents were determined after grinding the dried stems and leaves with a Wiley Mill with a 20-mesh screen (Thomas Scientific, Swedesboro, NJ). Due to the small size of seedlings, two plants per block per treatment group at the 5 mg kg^{-1} P level were combined. Samples were ashed overnight at 500°C and digested with

12N HCl, followed by colorimetry methods of Murphy and Riley (1962) to determine tissue-P concentration and content per plant.

To quantify mycorrhizal colonization, *Ardisia* roots were cleared in 10% KOH at 80^{o} C for 45 min, while corn roots used for determination of inoculum potential were cleared for 15 min. A longer clearing time was necessary for *Ardisia* roots due to their high tannin content. *Ardisia* roots were then rinsed and soaked in H_2O_2 at 50^{o}C for 10 min for additional clearing. Roots were again rinsed and acidified in concentrated HCl (5 ml HCl per 200 ml^{-1} H_2O) for five minutes. The roots were then soaked overnight at room temperature in trypan blue stain, which had been used successfully to stain field-collected *Ardisia* roots (Bray, unpublished data). To estimate mycorrhizal colonization, twenty one-cm root fragments were mounted on microscope slides and examined at 100x. Roots were scored as mycorrhizal if they contained coils or arbuscules, spores, vesicles or typical AM hyphae (aseptate, large diameter, angular branching).

Experiment 2: Effects of Mycorrhizae on Seedling Competition

As a competitor of *Ardisia*, we chose *Prunus*, a shade-tolerant subcanopy tree found in north-central Florida hardwood forests. *Prunus* seedlings with four to eight leaves and 7-12 cm height grown in soil-free medium were acquired from a commercial nursery (Urban Forestry Services, Micanopy, FL). Examination of cleared and stained roots of 10 seedlings revealed no mycorrhizal colonization. Although *Prunus* spp. Have been shown to be ectomycorrhizal in some cases (Smith and Read 1997), we found no evidence of ectomycorrhizae in *Prunus caroliniana*. *Ardisia* seedlings were collected from the same invaded forest as the inoculum in experiment 1. *Ardisia* seedlings had four to eight leaves and heights of 5-10 cm. Cleared and stained *Ardisia* roots revealed a total mycorrhizal colonization level of 52% $\pm$ 2%.

Prunus and *Ardisia* were grown in hetero- and conspecific competition with (AM) or without (NM) mycorrhizal inoculum. Two plants were potted in each 3.8 L pot containing the same medium as in experiment 1 with no additional P. Plants were paired according to height and number of leaves. Twenty pots contained two *Prunus* seedlings (*Prunus* conspecific competition), twenty contained two *Ardisia* seedlings (*Ardisia* conspecific competition), and forty contained one *Ardisia* and one *Prunus* seedling (*Prunus* heterospecific competition and *Ardisia* heterospecific competition). Half of the pots were randomly assigned to the NM treatment and were drenched with 75 mg of benomyl (Benlate®) dissolved in one L of deionized water. Although benomyl may have phytotoxic effects in some species, no phytotoxic effects have been reported in either *Prunus* or *Ardisia*, and benomyl is commonly used to control fungal pathogens in horticultural nurseries growing *Prunus persica*, *Prunus dulcis*, and *Prunus serotina* (Fontanet et al.1998, Stanosz 1992). Plants in the AM treatment received 5 g of *G. fasciculatum* (S3060) inoculum to supplement indigenous AM fungi and one L of deionized water. In the AM treatments containing *Ardisia*, each pot contained S3060 and fungi already inhabiting the *Ardisia* seedling; treatments without *Ardisia* contained only S3060. Pots were then randomly placed under a shade frame (approx. 20% open-sky light) in the greenhouse. Pots were watered as needed and received the modified Hoagland's solution used in experiment 1 biweekly. The minimum and maximum temperatures averaged 18^{o}C and 34^{o}C, respectively. The average maximum PPFD in the greenhouse was 910 μmol m^{-2} s^{-1}.

At 160 d after transplanting, growth analysis was conducted with one randomly selected plant per pot to ensure statistical independence. Root:shoot, LAR, SLA, and

RGR were determined as in experiment 1. A subsample of each root system of six randomly selected individuals per treatment was used to estimate percent colonization after determining fresh mass. Fresh:dry mass ratios of remaining roots were used to estimate dry mass of the subsamples used to determine colonization. Initial mass of seedlings was estimated through an allometric relationship of leaf number (mean $\pm$ S.E.: *Ardisia* 5.1 $\pm$ 0.24; *Prunus* 5.7 $\pm$ 0.41) and height (*Ardisia* 7.2 $\pm$ 0.23 cm; *Prunus* 9.3 $\pm$ 0.35 cm) with the total dry mass for each species (*Ardisia* 1.31 $\pm$ 0.05 g; *Prunus* 0.46 $\pm$ 0.04 g). Percent mycorrhizal colonization was determined as in experiment 1. Tissue-P content was quantified only for leaves with the same method as in experiment 1.

Statistical Analyses

Allocation and growth data from both experiments were analyzed with factorial model fitting (JMP 4.0, SAS Institute). For the analysis of the first experiment, the effects of soil-P, light and inoculum type (control vs. standard inocula), and their interactions on R/S, LAR, SLA, NAR, and RGR were analyzed. Because the host-associated inoculum was given only at 5 mg kg^{-1} P level, the effects of inoculum type (control, S3029, S3069, HA), light and their interactions at the 5 mg kg^{-1} P level were then examined. The results of experiment 2 were analyzed with a model that included the effects of species (*Ardisia* or *Prunus*), competitor (conspecific or heterospecific), mycorrhizal status (AM or NM), and their interactions. When treatment and interaction terms were not significant ($P \geq 0.1$), they were dropped from the model and the results of the analysis with the reduced model were reported. Tukey HSD at alpha = 0.05 was used to compare differences between means. Effects of LAR and R/S on RGR were examined with multiple regression analysis. Differences in survivorship among treatment groups were evaluated

with logistic regression models in both experiments. Percent colonization levels were converted by square root of the arcsine to achieve normality and analyzed by ANOVA.

Results

Experiment 1: Effect of Light, Soil-P and Inoculum Type

In the first analysis, effects of the standard inocula (S3029 and S060) and nonmycorrhizal control were examined at factorial combinations of two light levels and four soil-P levels. Overall, there was no difference in RGR or biomass allocation patterns among three mycorrhizal treatments (S3029, S3060 and control) in any combination of light or soil-P. Thus, inoculum type was dropped from the ANOVA model. Neither soil-P nor light affected RGR, SLA or NAR. Leaf area ratio and R/S, however, were affected by light and soil-P (Table 2-1, 2-2). Leaf area ratio was positively correlated with RGR and explained 37% of the variance in RGR ($P < 0.0001$) while R/S was negatively correlated with RGR and explained 15% of the variance ($P < 0.0001$). Leaf area ratio and R/S together explained 39% of the variance in RGR ($P < 0.0001$) in a multiple regression. A total of twenty-seven seedlings of 260 died over the course of the experiment, but survivorship was not affected by treatments.

In the second analysis, the effects of HA inoculum on seedling growth and biomass allocation were compared to the standard inocula and control, at the 5 mg kg^{-1} P level (Figure 2-1a-d). Relative growth rates of plants with HA inoculum were twice that of the standard inocula and control ($P < 0.0001$), while LAR was 2.5-3x that of the other treatment groups ($P < 0.0001$). For pooled data across treatments, LAR was positively correlated with RGR ($P < 0.0001$) and explained 35% of the variance in RGR. Conversely, R/S of plants inoculated with HA were half that of the other inoculum treatments ($P = 0.0006$). Root:shoot ratio was negatively correlated with RGR ($P <$

0.0001) and explained 23% of the variance in RGR. Together LAR and R/S explained 39% of the variance in RGR ($P < 0.0001$). Leaf area ratio was the only morphological measure affected by light treatment, with plants in low light having higher values than those in moderate light ($P_{light} = 0.003$). Inoculum effects on SLA were nearly significant ($P = 0.06$); plants inoculated with HA had greater SLA (193.1 $\pm$ 17.49 g/cm^2 vs. 136.9 $\pm$ 14.47 g/cm^2) than controls and other inoculum sources. NAR was not affected by light or inoculum.

Both shoot-P concentration and content differed among treatments ($P = 0.005$ and $P = 0.04$, respectively). Plants inoculated with S3060 had the highest P concentration and content, while HA inoculum had the lowest P concentration and second-highest content (Figure 2-1 e, f).

Leaf gas exchange data showed similar trends to RGR in relation to inoculum type (Table 2-3). The maximum photosynthetic rate of plants inoculated with HA were approximately twice that of plants with S3060 and controls under moderate light. Due to the small size of plants and leaves inoculated with S3029 under moderate light, no gas exchange rates are available for this treatment group. Plants inoculated with HA had rates approximately twice that of controls in both light treatments. Dark respiration rates of all inocula were similar to the dark respiration rates of control plants.

Mycorrhizal colonization of plants mirrored the growth and biomass allocation differences among inocula ($P <0.0001$); plants with HA inoculum were highly colonized (mean $\pm$ S.E.: 67 $\pm$ 7%) whereas standard isolates (S3029 & S3060) had low colonization rates (17 $\pm$ 7% and 7 $\pm$ 7%, respectively). Controls were virtually non-colonized (0.8 $\pm$ 0.8%). Septate hyphae, presumably saprophytic, were observed externally with many

root samples, primarily concentrated in plants inoculated with S3060 and S3029. Colonization levels were not related to original inoculum potential as determined by the corn assay.

Experiment 2: Effects of Mycorrhizae on Seedling Competition

Mycorrhizal colonization was significantly lower in plants treated with benomyl (NM treatment) than AM plants of both species ($P < 0.0001$). Benomyl was more effective in reducing colonization in *Prunus* (AM = 73 $\pm$ 7%, NM= 9 $\pm$ 10%) than *Ardisia* (AM = 59 $\pm$ 1%, NM = 38 $\pm$ 6%; Table 2-4). Competition type had no effect on colonization levels.

Nine of 80 seedlings died over the course of the experiment. Mortality was significantly higher for NM *Prunus* seedlings (7 of 9 dead seedlings, log-likelihood $\chi^2 = 6.57$, $P = 0.038$, $P_{myc} = 0.037$) than AM *Prunus* seedlings.

Relative growth rates differed significantly between treatments ($P < 0.0001$) with *Prunus* having greater RGR than *Ardisia* ($P < 0.0001$, Figure 2-2a). *Prunus* and *Ardisia* also responded differently to the competition treatment (Table 2-4, significant species x competition interaction). While *Ardisia* had the highest RGR in heterospecifc competition, the RGR of *Prunus* decreased approximately by half when grown in heterospecific competition without mycorrhizae (Figure 2-2a).

Leaf area ratio also differed among treatment groups as *Prunus* and *Ardisia* responded differently to mycorrhizal status ($P_{sp*myc} = 0.015$, Table 2-4). Unlike in experiment 1, *Ardisia* tended to have higher LAR when nonmycorrhizal in heterospecific competition, whereas *Prunus* had higher LAR with mycorrhizae in both competition types (Figure 2-2b). *Ardisia* had a higher R/S than *Prunus* ($P < 0.0001$, Figure 2-2c); however, R/S of *Ardisia* decreased under conspecific competition when mycorrhizal

($P_{comp*myc}$ = 0.04). Specific leaf area did not vary significantly among treatments and species (P = 0.18, Figure 2-2d).

Leaf-P concentration and content differed among species and treatments (Table 5; Fig. 2-2e, f). *Ardisia* had lower P concentration than *Prunus*, and was not affected by competition or mycorrhizal treatment (Figure 2-2e). *Prunus* had its highest P concentration in the NM, heterospecific competition treatment, while the other three treatments did not differ significantly from one another (Figure 2-2e). *Ardisia* in the NM, conspecific treatment had a significantly lower total P content than the other three treatment groups (Figure 2-2f). *Prunus* had lower P content in NM than AM treatments with no effect from competition (Figure 2-2f).

Discussion

Effect of Inoculum Source on *Ardisia*

Although all three types of inocula colonized *Ardisia* roots, they had strikingly different colonization levels and effects on biomass allocation and growth of the host. In experiment 1, only the HA inoculum isolated from field-collected *Ardisia* roots, but not the standard inocula, improved seedling RGR over the nonmycorrhizal control. As *Ardisia* had no response to soil-P nor was higher RGR accompanied by an increase in P content or concentration, it appears that the benefit of the HA mycorrhizae was mediated through changes in allocation and physiology rather than improved P nutrition. Plants inoculated with HA inoculum had less relative investment in roots, greater leaf area and higher A_{max} than the control. HA inoculum phenotypically altered LAR of *Ardisia* seedlings from a low value typical for shade tolerant species to a higher value. LAR is generally thought to be the primary determinant of RGR both across and within species

(Poorter and Remkes 1990) and in this study accounted for 35-37% of the variation in RGR. In a study by Lovelock et al. (1996), shade-tolerant seedlings of *Beilschmiedia pendula* also increased their RGR through an increase in LAR when mycorrhizal and its morphology became more similar to that of more light-demanding plants.

The lack of positive effects on morphology and growth by the standard inocula despite their positive effects on tissue phosphorus concentration was surprising, especially given that they have been shown to increase the biomass of several species, including woody, native plants (Sylvia 1990, Sylvia et al. 1993, Sylvia et al. 2001). Possibly the costs of the two *Glomus* species were greater than their benefits at the light levels used in this experiment. Mycorrhizal fungi can demand up to 20% of the total C budget of a plant in extreme cases (Peng et al. 1993), and carbon costs can vary widely among fungal genotypes (Graham et al. 1996). The differences between inoculum types may also be mediated by fungal diversity. Isolates S3029 and S3060 represent single-spore cultures, while the HA inoculum was likely composed of multiple fungal species and strains. At least one of these may have been more effective than the two *Glomus* species. Greater numbers of fungal species have been shown to increase the productivity of grass macrocosms

In contrast to the strong effect of HA inoculum in experiment 1, suppression of mycorrhizal fungi in experiment 2 had no effect on RGR of *Ardisia* seedlings, even though colonization rates were reduced from 59% to 38%. These seedlings were collected from a dense *Ardisia* population in the field and were presumably colonized with mycorrhizae similar to HA. The magnitude of reduction of mycorrhizal colonization (33% reduction) in this study was comparable to the reductions in many

other studies, (Kahiluoto et al. 2000, Moora and Zobel 1996, Smith et al. 1999). However, it has been suggested that the relationship between mycorrhizal colonization and plant benefit is curvilinear with benefit to the plant eventually reaching a plateau at some colonization level (Gange and Ayers 1999). *Ardisia* may have reached its maximal benefit at or before a colonization rate of 38%, as found in the benomyl treatments.

That *Ardisia* has a differential response to different inoculum types may have important implications for its invasive ability. In heavily invaded areas, *Ardisia* is already associating with effective mycorrhizal fungi that alter its morphology and physiology to that of faster-growing plants. The main mode of resource competition by *Ardisia* is through casting dense shade to its neighbors. Increased LAR, enabled by the reduction in R/S due to mycorrhizae, must enhance *Ardisia*'s competitiveness for light in the forest understory.

Competitive Interactions

Ardisia seedlings grew better in heterospecific competition with *Prunus* seedlings than in conspecific competition. Conversely, *Prunus* seedlings had lower survival and growth with *Ardisia* seedlings than with conspecific seedlings, especially in nonmycorrhizal treatment (Fig. 2-2a). The architecture of *Ardisia* results in a higher amount of self- and neighbor-shading than that of *Prunus* (K. Kitajima, unpublished data). Hence, each *Ardisia* seedling is more shaded by a conspecific neighbor than a heterospecific neighbor. In conspecific competition, *Ardisia* responded with greater phenotypic plasticity of increasing LAR than in competition with less shade-casting *Prunus*.

Prunus seedling growth and survival was reduced to a greater extent by heterospecific competition in the nonmycorrhizal than in the mycorrhizal treatment. The

presence of HA and S3060 inocula reduced the negative effect of interspecific competition on *Prunus*, as is often observed in more mycorrhiza-dependant species (Hartnett et al. 1993, Moora and Zobel 1996, Smith et al. 1999). More mycorrhiza-dependant species often have a low total investment in roots (Jakobsen 1991). *Prunus* had overall higher RGR than *Ardisia*, and this difference was associated with inherently higher LAR and lower R/S of *Prunus* (Fig. 2-2 a-c). Mycorrhizae apparently allowed *Prunus* seedlings to invest less in roots and more to leaf area, and enabled them to compete more effectively with *Ardisia* seedlings for light. This finding of an apparent greater AM dependency by a faster growing species in a competitive regime is interesting because often the opposite trend has been found in the absence of heterospecific competitors (e.g. Janos 1980, Zangaro et al. 2000).

Different plant species in the same community can support different mycorrhizal communities in their rhizosphere (Bever 1994) and cause differential rates of sporulation (Bever et al. 1996). A high percentage of *Ardisia* roots are colonized by AM fungi in the field (Bray, unpublished data), likely dominated by preferred mycorrhizal fungi. The field-collected *Ardisia* seedlings in Experiment 2 had been colonized by mycorrhizal fungi, some of which remained after the benomyl treatment. These fungi colonized *Prunus* seedlings at a low level (9%), but they did not benefit growth of *Prunus* seedlings in the heterospecific competition treatment. The community composition of mycorrhizal fungi may be highly modified in the dense clump of *Ardisia* in the invaded forest. If *Ardisia* alters the composition of AM, the competitiveness of *Ardisia* may be increased.

Implications of Effects of Mycorrhizae on Exotic Species Invasion

As a new colonist in Florida, *Ardisia* is apparently not limited by the lack of potential mutualists and, in fact, benefits from the local mycorrhizal fungi. Unlike typical

invaders, *Ardisia* is highly shade-tolerant and has low RGR. Many other exotic species that have a higher RGR than *Ardisia* have a negative or neutral response to mycorrhizae when they are grown alone (Marler et al. 1999, Philip et al. 2001, Richardson et al. 2000). This appears to be the first study to document a positive response of a slow-growing exotic to native mycorrhizae. It is likely, however, that further study will show that there is no link between life history and mycorrhizal dependency in exotic species as has been found in the mycorrhizal literature as a whole (Allsopp and Stock 1992, Janos 1980, Smith and Smith 1996, Zangaro et al. 2000). Until a greater predictive framework for mycorrhizal response is developed, invasive plant response must be examined on a species by species basis.

The results of our study suggest that it is difficult to predict how competitive interactions between exotic and native plants are modified by mycorrhizae. The exotic plant's response in isolation does not necessarily predict its response to mycorrhizae in a competitive environment. Another study of competition between an exotic forb and native grass found that neither species' biomass was altered by mycorrhizae when grown in isolation, but when grown in mixture, mycorrhizae increased the growth of the exotic plant to the detriment of the native (Marler et al. 1999).

Our results also suggest that the type of mycorrhizal inoculum must be considered when evaluating mycorrhizal effects on competitive interaction between native and exotic species. The results vary depending on whether the fungal inoculum is the one preferred by the native or the exotic. Thus, studies should incorporate evaluation of both species and the mycorrhizae of the ecosystem invaded by exotic plant species.

The role of AM in mediating plant invasions and competitive interactions needs to be examined carefully. The response of exotic plants to mycorrhizae is highly variable depending on genotype interactions both in isolation and in competitive environments. Understanding how native and exotic plants respond to the local microbial community will be important for understanding the mechanisms and impacts of community invasion. Similarly, it is also imperative that we determine how exotic species potentially alter the microbial community and its ecosystem functions.

Table 2-1: ANOVA summarizing the effects of light and soil on LAR and R/S in experiment 1 (model $P < 0.05$). Fungal isolate effects (control, S3029, and S3060) were not significant and were pooled.

	LAR		R/S	
Source	F	P	F	P
Model	4.51	0.0001	3.24	0.003
Light	9.24	0.002	0.20	0.66
P-level	3.11	0.03	3.94	0.009
Light * P-level	3.37	0.02	3.24	0.02

Notes: Fungal isolate effects (control, S3029, and S3060) were not significant and were pooled. Abbreviations are: LAR, leaf area ratio; R/S, root to shoot ratio; P, phosphorus.

Table 2-2: Means of leaf area ratio (LAR) and root:shoot ratios (R/S) from all soil-P levels in experiment 1. Different letters in the same column signify significant difference in Tukey HSD values (alpha = 0.05).

	[P]	LAR ($cm^2\ g^{-1}$)	R/S	RGR ($mg\ g^{-1}\ day^{-1}$)
Moderate Light	0	20.5 a	2.38 a	5.05
	5	17.5 a	2.24 a	5.08
	30	22.5 a	1.83 a	6.05
	60	28.9 ab	1.52 b	6.62
Low Light	0	29.1 ab	1.91 a	5.77
	5	24.9 a	2.12 a	4.75
	30	38.0 b	1.66 b	7.02
	60	24.2 ab	2.08 a	5.18

Notes: Abbreviations: RGR, relative growth rate; other abbreviations as in Table 2-1.

Table 2-3: Comparison of light saturated net photosynthesis rate (Amax) and dark respiration under moderate vs. low light treatments (means $\pm$ 1 SE) from gas exchange measurements of three individuals from experiment 1.

	A_{max}		Dark Respiration	
Inoculum	Moderate	Low	Moderate	Low
Control	2.02 ± 0.55	2.02 ± 0.53	-0.279 ± 0.062	-0.310 ± 0.045
S3029	NA	3.43 ± 1.19	NA	-0.307 ± 0.132
S3060	2.65 ± 0.38	2.19 ± 0.35	-0.382 ± 0.078	-0.253 ± 0.015
HA	4.04 ± 0.28	4.42 ± 0.78	-0.356 ± 0.046	-0.313 ± 0.065

Notes: No data were collected for the moderate light treatment of isolate S3029 due to small size of leaves in this group. Abbreviations: NA, not available; HA, host-associated.

Table 2-4: ANOVA summary of the effects of species, competition, and mycorrhizae on RGR, LAR, R/S, and colonization rates from experiment 1 (model $P < 0.05$). A dash (-) indicates that the specified effect was not significant ($P > 0.1$) and was dropped from the model.

	RGR		LAR		R/S		Colonization	
Source	F	P	F	P	F	P	F	P
Species	29.1	<0.0001	0.129	0.73	62.5	<0.0001	2.03	0.16
Myc	2.94	0.09	1.16	0.28	2.93	0.09	38.9	<0.0001
Comp	3.19	0.08	2.20	0.14	0.059	0.81	-	-
Sp*Myc	1.10	0.30	6.31	0.01	-	-	12.0	0.001
Myc*Comp	0.481	0.49	-	-	4.54	0.04	-	-
Sp*Comp	11.9	0.001	3.36	0.07	-	-	-	-
Sp*Myc*Comp	3.78	0.06	-	-	-	-	-	-

Notes: Degrees of freedom were: RGR, 1,63; LAR, 1,65; R/S, 1, 66; colonization, 1,67. NS indicates that the specified effect was not significant (P > 0.1), and was dropped from the model.

Table 2-5: ANOVA summary of the effect of species, competition, and mycorrhizae for P concentration and total P content from the competition study.

	P concentration (mg P g^{-1} tissue)		Total P content (mg P)	
Source	F	P	F	P
Species	43.6	<0.0001	19.1	<0.0001
Myc	0.086	0.70	24.9	<0.0001
Comp	9.6	0.003	2.57	0.11
Sp*Myc	3.81	0.06	-	-
Myc*Comp	4.15	0.05	4.51	0.04
Sp*Comp	7.06	0.01	5.27	0.02

Notes: Degrees of freedom were, P concentration, 1,64; total P content, 1,65. The three-way interaction was dropped due to lack of significance in both tests as was the species * mycorrhizae interaction in the P content test.

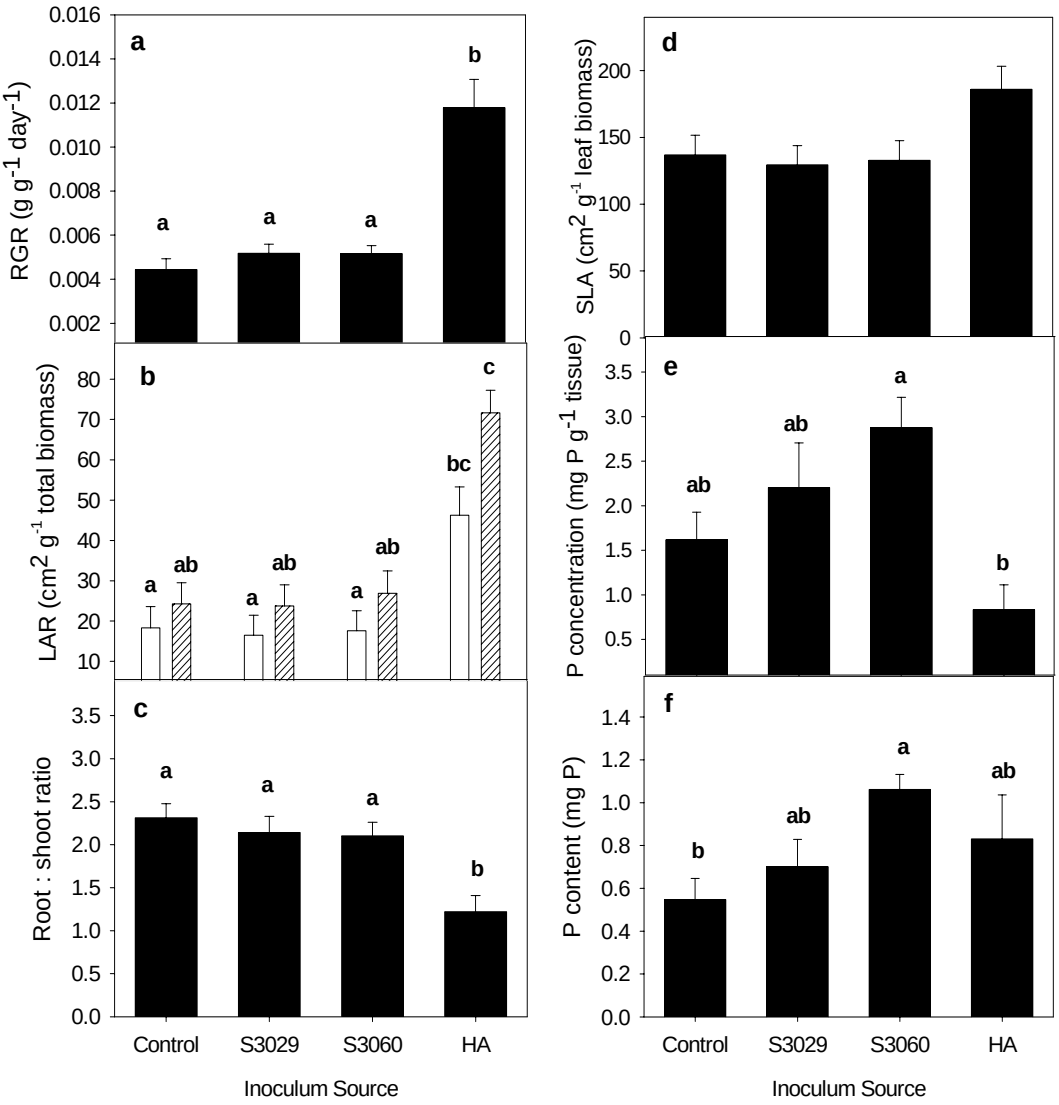


Figure 2-1: Response of *Ardisia* to light and inoculum type at 5 mg kg^{-1} P. Light treatment is distinguished only when it had a significant effect ($P < 0.05$). a) Relative growth rate in response to inoculum ($P < 0.0001$). b) Leaf area ratio in response to light ($P = 0.003$) and inoculum ($P < 0.0001$). Open bars = moderate light; hatched bars = low light. c) Root:shoot ratio in response to inoculum ($P = 0.0006$). d) Specific leaf area in response to inoculum ($P = 0.06$). e) Shoot-P concentration (mg P g^{-1} tissue) in response to inoculum ($P = 0.005$). f) Shoot-P content (mg P) in response to inoculum ($P = 0.04$). Error bars represent $\pm$ 1 SE. Letters signify difference between Tukey HSD values at alpha = 0.05.

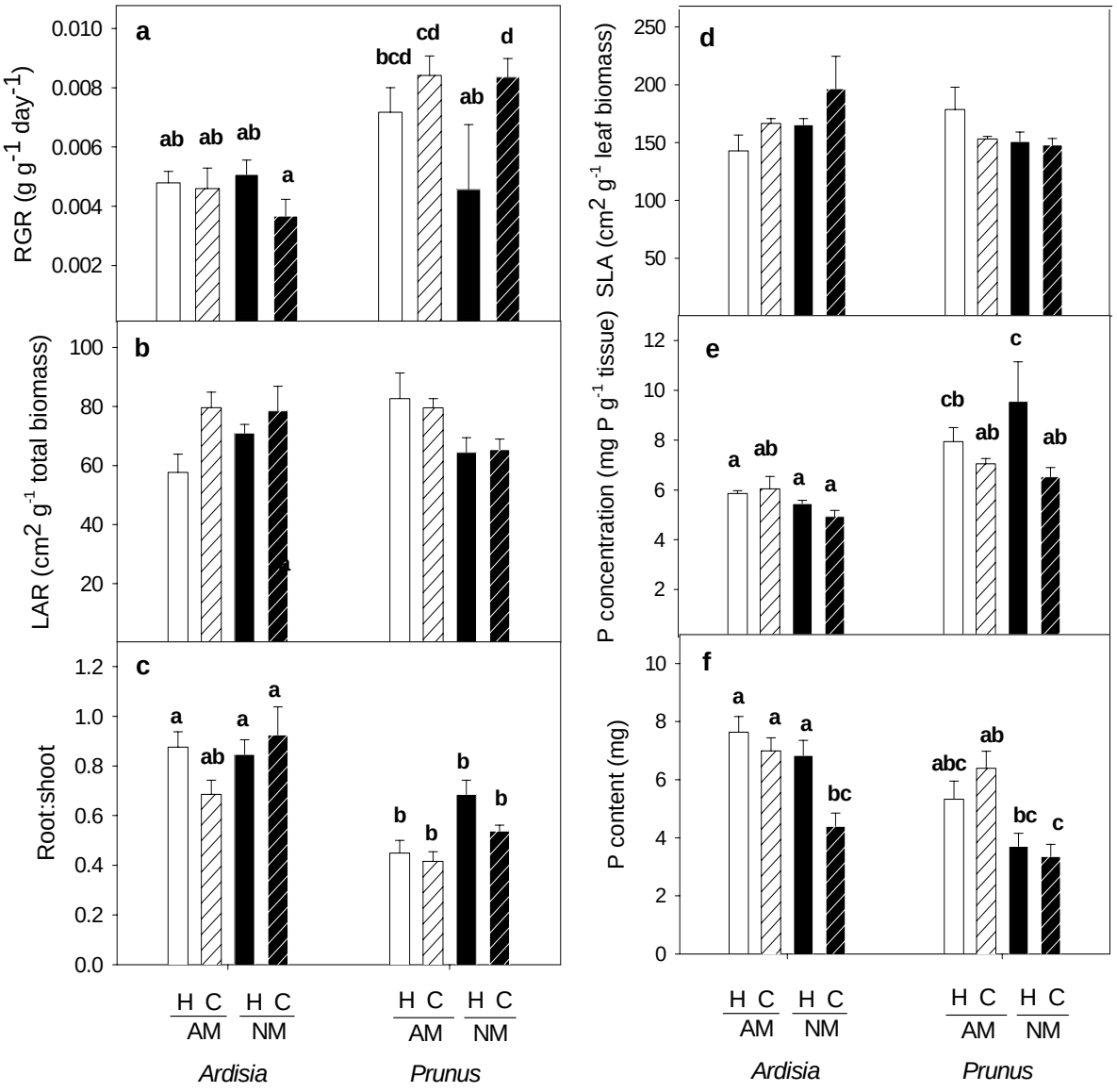


Figure 2-2: Response of *Ardisia* and *Prunus* to heterospecific or conspecific competition and mycorrhizal status. H = heterospecific competition; C = conspecific competition. a) Relative growth rate ($P < 0.0001$). b) Leaf area ratio ($P = 0.04$). c) Root to shoot ratio ($P < 0.0001$). d) Specific leaf area ($P = 0.18$). e) Leaf-P concentration (mg P g^{-1} tissue) ($P < 0.0001$). f) Leaf-P content (mg P) in response to inoculum source ($P < 0.0001$). Error bars represent $\pm$ 1 SE. Letters signify difference between Tukey HSD values at alpha = 0.05.

CHAPTER 3
SOIL MICROBIAL COMMUNITY STRUCTURE AND FUNCTION IN FLORIDA PLANT COMMUNITIES PRONE TO NON-NATIVE PLANT INVASION

Introduction

Microbes are a key component of ecosystems that function as pathogens, mutualists and decomposers. Recent studies have shown that the composition of microbial communities differs among plant communities, as influenced by biotic and abiotic factors (Waldrop et al. 2000, Myers et al. 2001, Gallo et al. 2004, Leckie et al. 2004, Waldrop and Firestone 2004). Microbial community metabolic potential and structure change across climatic gradients (Staddon et al. 1998), nitrogen levels (Pennanen et al. 1999, Gallo et al. 2004, Leckie et al. 2004), and soil moisture contents (Bossio and Scow 1998). Microbial community composition also varies under different plant species within a given community (Grayston et al. 1998, Bardgett et al. 1999, Saetre and Baath 2000, Priha et al. 2001). These differences are likely due to the differences in root exudates and turnover and the quantity and quality of aboveground litter inputs (Grayston et al. 1996).

Plant species composition is changing in many plant communities due to the introduction of non-native species. These invasions result not only in shifts in plant community composition, but can also have ecosystem-level effects such as altered nutrient levels, hydrology, and soil accumulation (Gordon 1998). Invasions, then, offer a "natural experiment" in which to examine the role of species composition in ecosystem function. It is likely that altered substrate composition due to plant composition shifts accompanied by altered abiotic soil environment will result in altered microbial

community composition. In a temperate forest, two non-native understory species supported microbial communities significantly different from a native understory species within the same forest stand (Kourtev et al. 2002). These differences extended below the root zone of the vegetation but larger scale effects were not examined. This leaves interesting questions on the potential impacts of exotic species on microbial community composition: 1) Can invasive plants alter microbial community composition beyond their crown- and root-zone of influence? 2) Do invaders have a consistent effect on microbial community composition across a landscape scale? and, 3) Does the identity of the invader and the community type it invades influence the potential effects of that invasion on microbial community composition?

The state of Florida presents many opportunities to examine a number of non-native invaders across a variety of plant communities. The Florida Exotic Pest Plant Council lists over 120 non-native species as invasive (FLEPPC 2003). These species range from grasses to trees and invade communities from freshwater marshes to upland pine savannas in freeze and freeze-free climate zones. We examined the impact of five invasive plants, ranging from trees to understory herbs, on microbial communities of plant communities in saturated south Florida everglades marsh soils to well-drained north-central Florida forest soils. We used phospholipid fatty acids (PLFA) to examine the structure of microbial communities and Biolog substrate utilization to examine the function of those communities.

Biolog and PLFA profiles have become common methods to examine microbial community composition as they are rapid and of low enough cost to allow for the analysis of the number of samples required for ecological studies. Biolog plates assay the

functional traits of microbial communities by testing their cumulative ability to metabolize 95 different carbon substrates. Because Biolog depends upon the growth of organisms within the 95 wells, it has many of the same limitations as culture-based techniques and organisms adapted to high resource availability are likely to be over-represented (Smalla et al. 1998). However, Biolog has been successfully used to differentiate microbial communities in different soil types, land management treatments and rhizospheres of different plant species (Gorlenko and Kozhevin 1994, Bossio and Scow 1995, Garland 1996). PLFA profiles are a useful technique for examining the structure of the microbial community at many levels. A common use is to examine overall "fingerprints" of microbial communities by subjecting the abundance of the various fatty acids within a sample to multivariate analysis (Frostegard et al. 1993). Major taxonomic groups of microbes, such as fungi, microeukaryotes, and Gram-negative and Gram-positive bacteria can be specifically examined by the use of biomarkers (Vestal and White 1989). In addition, functional groups such as aerobes, anaerobes, methanotrophs, and sulfate reducers can be examined by the use of fatty acid biomarkers (Harwood and Russell 1984, Dowling et al. 1986, Parkes 1987, Hill et al. 2000).

We used these two measures of microbial community structure and function to examine microbial communities within invaded and non-invaded areas within the habitat prone to invasion by each of the five invasive species. As both biotic and abiotic factors are known to influence microbial community composition, we propose two alternative hypotheses for the composition of microbial communities. Inherent characteristics (e.g. soil moisture, nutrient levels, physiognomy of vegetation) of habitats may drive

composition of microbial communities regardless of plant species. Alternatively, the invasion and dominance of non-native species may override the habitat effect, resulting in changes in the microbial community because of changes in vegtation.

Methods

Species, Sites, and Sampling

The invaders we examined included trees, *Sapium sebiferum*, *Schinus terebinthifolius*, and *Melaleuca quinquenervia*, an understory shurb, *Ardisia crenata*, and an herbaceous perennial, *Ruellia brittoniana*. In addition to being dominants in the habitats they invade, *Sapium*, *Schinus*, and *Melaleuca* have been shown to have ecosystem-level impacts on native communities such as increased soil elevation, increased litter accumulation, or altered mineralization, disturbance or hydrology (Woodall 1981, Cameron and Spencer 1989, Greenway 1994, Laroche 1994, Gordon 1998). Secondary defensive chemicals in *Schinus* are known to have allelopathic effects on other plants (Morton 1978, Mahendra K.J. et al. 1995) and the conspecific *Melaleuca alternifolia* has antibiotics that depress decomposition rates (Boon and Johnstone 1997, Bailey et al. 2003). Thus, we felt that these three species would have the greatest potential for altering microbial community composition. We chose the additional understory plants, *Ardisia crenata* and *Ruellia brittoniana* as cases in which invaders do not contribute a large percentage of the total biomass of a plant community.

The habitats that these species invade vary in their locations, physiognomy of native vegetation, moisture regimes and susceptibility to invasion by specific invaders (Table 3-1). For ease of discussion we will identify these habitats by the species to which they are most prone to invasion, i.e., Sapium-prone habitat (SA), Schinus-prone habitat (SC), Melaleuca-prone (ME), Ruellia-prone (RU), and Ardisia-prone (AR). For

consistency of presentation these habitats will appear in order of hydrological position from wettest to driest in the results figures and tables.

We selected three sites for each habitat. Most habitats included only one native plant community type (Myers and Ewel 1990) except for RU habitats in which two sites were in bottomland swamp forests and one in a cabbage palm hammock and SA habitats sampled in 2 wet prairies in Texas on clay-dominated soil and one site in Florida on sandy muck soil. The two southern-most habitats, SC and ME, were sampled in *Cladium*-dominated everglades and tropical hammock rocklands, respectively. The AR habitats were sampled exclusively in mixed hardwood forests. We chose sites that had areas of high invader density (invaded) and areas free of the invader (non-invaded). In order to minimize the chance that differences between invaded and non-invaded areas were based on site conditions prior to invasion, we chose only sites where other workers knew the history of the site. The absence of invasion in non-invaded areas was maintained either by human intervention or a clear "invasion front" was apparent. Except for the presence of the invasive species, the invaded areas appeared similar to non-invaded areas used for the study in terms of topography and soil type.

Within each site, six 5 m x 5 m plots were established—three in invaded areas and three in non-invaded areas. In most cases, each invaded plot was spatially separated from other distinct areas of invasion. When this was not possible, invaded plots were separated by at least 50 m in a large patch of the invader. Corresponding non-invaded plots were located at least 30 m from the invasion front.

Within each 5 m x 5 m plot, 20 soil cores (diameter 2 cm) were extracted systematically, approximately 80-100 cm apart, from the top 10 cm of soil, including the

litter layer. Cores within a plot were combined to create a composite sample. Samples were kept cool (~ 4 ºC) during collection in the field and frozen at the end of each day to prevent alteration of the microbial community during the remainder of field collection.

The southern most habitats, ME and SC, were collected within 3 days of each other in the July of 2002 and Florida SA and AR in August 2002. The Texas SA sites were collected in September 2002. In the summer of 2003, RU samples were collected. All samples were collected within the wet season.

Microbial Community Composition and Nutrient Analysis

Microbial community structure was examined using phospholipid fatty acid (PLFA) biomarkers. All glassware was heated at 500 °C for five hours and PTFE lined caps rinsed with hexane to remove any organic matter. A subsample weighing a total of 5 g (dry mass) from each plot was extracted in glass centrifuge tubes using single-phase phosphate-buffered methane chloroform solvent (White et al. 1979). After two hours, an additional 5 mL each of chloroform and methanol were added to break the phase. Samples were briefly shaken, vented, and allowed to separate overnight. Samples were then centrifuged and the organic layer passed through a Whatman #2 paper into a test tube where the solvent was driven off under N_2 gas. The organic phase was resuspended and separated on a silcic acid column. The phospholipid fraction was transesterified to fatty acid methyl esters (FAME) by mild alkaline methanolysis (Findlay and Dobbs 1993). For identification, samples were suspended in hexane with 19:0 fatty acid as an internal standard and analyzed with an Agilent Technologies 6890 gas chromatograph (Palo Alto, CA) with a 25m Ultra 2 phenyl methyl silicone column. The temperature program increased from 170 °C to 270C at 5 °C per minute. Peaks were identified by

MIDI peak identification software (MIDI, Inc., Newark, DE) and co-elution with standards.

Fatty acids were defined in terms of the ratio of total number of carbon atoms : number of double bonds. The position of the double bond from the methyl end of the molecule is signified by the symbol ω followed by the carbon position; cis and trans geometry are referred to by "c" and "t." The prefixes "i" and "a" signify and iso and anteiso branching; "cy" signifies a cyclopropyl fatty acid; 10Me indicates a methyl group on the tenth carbon atom from the carboxyl end of the fatty acid. The position of hydroxy groups is indicated by xOH. Fungal:bacterial ratio was calculated as (18:2ω6c)/(i15:0 + a15:0 + 15:0 + i16:0 + 16:1ω5c + i17:0 + a17:0 + 17:0 + 18:1ω7c + cy19:0) (Frostegard and Baath 1996). Gram – bacteria were represented by fatty acids 16:1ω7c, 16:1ωw7t, 17:0cy, and 18:1ω7c; Gram + bacteria by i15:0, a15:0, i16:0, a17:0, and 17:0 10 ME (O'Leary and Wilkinson 1988, Wilkinson 1988). Total PLFA (nmol g^{-1}) was used as a proxy for total biomass.

Microbial community function was examined as the ability to metabolize the 95 substrates in Biolog Gm- plates (Biolog, Inc., Haywood, CA). We inoculated one plate per plot with a 10^{-3} dilution of one-gram subsamples. Absorbance was measured at 12, 24, 48, 72, and 96 hours with a microplate reader (Bio-Rad, Hercules, CA).

A subsample of soil from each plot was dried at 60 ºC until constant weight was achieved to determine gravimetric moisture content. Subsamples were then ground with a mortar and pestle to homogenize. The carbon and nitrogen contents of soil were determined by combustion on an ECS4010 elemental combustion system (Costech Analytical Technologies, Inc, Valencia, CA).

Statistical Analyses

In statistical analyses, site was treated as the unit of replication (i.e., means of the three plots per site), yielding an n of 30. Differences in %C, %N, C:N, %moisture, total PLFA (nmol g^{-1}), and fungal:bacterial ratio among habitats and invasion-status were tested with ANOVA. Variables were log-transformed for normality and homogeneity of variances when necessary. When interaction terms of the two-way ANOVA were significant, Bonferroni-corrected t-tests were used to look for differences between invaded and non-invaded soils within each community. Correlations between soil characteristics and fungal:bacterial ratio and total PLFA were examined with simple linear and quadratic regressions.

We used principle components analysis (PCA) to summarize the multivariate data sets and create microbial community fingerprints for both PLFAs and substrate utilization from Biolog. The twenty-three most common, positively identified PLFAs comprising > 1% of the total amount of fatty acids extracted were included in the ordination as percentage of the total fatty acids per sample after arcsine-square root transformation. Absorbance values at 48 hours for 96 carbon sources for four of the five habitats examined (excluding RU) were ordinated to examine substrate utilization patterns. Average scores per site from the first two PCA axes of each ordination were subjected to MANOVA to examine the effects of habitat, invasion and habitat by invasion interaction. Finally, differences among habitats and invasion-status in the relative abundance of PLFAs that were important in structuring the PCA were determined with one- and two-way ANOVAs.

All univariate statistics were performed in JMP 4.0 (SAS Institute) and ordinations were performed in PCOrd 4.2 (MJM Software Design, Gleneden Beach, OR).

Results

Soil characteristics varied among habitats but invasion had no significant effect. ME habitats were most different from the other habitats examined with the highest moisture, carbon and nitrogen contents in the soil and lowest C:N ratio (Table 3-2). Percent C, N and moisture were highly correlated with one another (C-N $r^2 = 0.88$, C-moisture $r^2 = .61$, N-moisture $r^2 = 0.74$).

Total phospholipids and fungal:bacterial ratios differed significantly among habitat types. Only habitat type had a significant effect on total PLFA ($F_{4,20} = 22.29$, $p < 0.0001$), with RU, SA, and SC habitats having the highest total PLFA (Figure 3-1). Fungal:bacterial ratio was highest in RU and AR habitats and lowest in ME habitats ($F_{4, 20} = 69.5$, $p < 0.00001$, Figure 3-2). Although there was no main effect of invasion, there was a significant habitat by invasion interaction ($F_{4, 20} = 3.06$, $p = 0.04$). AR and ME habitats showed a trend of increased fungal : bacterial ratio with invasion, while SC, SA and RU habitats showed a trend towards decreased fungal : bacterial ratio with invasion (Figure 3-2).

Both total PLFA and fungal : bacterial ratio were correlated with the soil characteristics examined. The relationship of total PLFA with soil characteristics was unimodal (i.e., maximum biomass at intermediate values of each soil characteristic) and quadratic equations produced a better fit than linear equations. Total PLFA was best correlated with percent carbon and nitrogen (Table 3-3). Relationships between fungal:bacterial ratio and soil characteristics were monotonic and simple linear regressions produced the best fits. Moisture, nitrogen, and carbon contents were negatively correlated with fungal:bacterial ratio explaining 70%, 48%, and 26% of the

variance, respectively. Conversely, C:N was positively correlated with fungal:bacterial ratio (Table 3-3).

We examined differences in relative representation of Gram – and Gram + bacterial groups as related to habitats and invasion-status. Gram + bacteria were highest in SA and AR habitats while the inverse was true for Gram – bacteria (Figure 3-3). All effects were significant in a two-way ANOVA of Gram – PLFAs (Table 3-4). Gram – PLFAs increased with invasion in SC and RU habitats, decreased in AR habitats and had no change in ME and SA habitats. Nitrogen and carbon contents of the soil were positively correlated with Gram – bacteria and negatively correlated with Gram + bacteria. Moisture content was positively correlated only with Gram – bacteria and C:N had no significant relationship with either Gram negative or positive bacteria (Table 3-5).

To examine overall differences in microbial community composition in relation to habitat and invasion status, we ordinated the PLFA data by principle components analysis. The first two components explained 22.3% and 17.2% of the variance, respectively. Microbial communities from different habitats were primarily separated by the first axis, although the two wettest habitats were also separated from the remaining communities by the second axis (Figure 3-4). Within habitats, differences between invaded and non-invaded samples were primarily on the first axis. In MANOVA, both habitat and invasion effects were significant (Table 3-6).

The PLFA with high loadings on the first two PCA axes (loadings ≥ 0.3) included i11:0 3OH, 16:0, i16:0, 16:1ω7c, 16:1ω5c, 16:1 2OH, cy17:0, and 18:2ω6c (Table 3-7). PCA 1 was negatively correlated with 16:0 (ubiquitous fatty acid) and 16:1ω7c (Gram – PLFA), and positively correlated with i16:0 (Gram + PLFA) and 16:1 2 OH (Gram -

PLFA). PCA 2 was negatively correlated with i11:0 3OH, 16:1w5c (mycorrhizal PLFA, also often classified as Gram -), cy17:0 (Gram – PLFA, possibly an anaerobic marker), and positively correlated with 18:2ω6c (fungal PLFA). Soil characteristics were not strongly correlated with PCA 1, but were correlated with PCA 2 (Table 3-8). Moisture content, followed by %N and %C, was strongly negatively correlated with PCA 2. C:N was positively correlated with PCA 2.

We then explored the effect of habitat and invasion-status on the relative representation of these eight PLFAs with high loadings. Only habitat effect was significant for five PLFAs (i11:0 3OH, i16:0, 16:1ω5c, 16:0, and cy17:0) whereas both habitat and invasion had significant effects on the abundance of 16:1ω7c, 16:1 2OH, and 18:2ω6c. Two somewhat surprising patterns did arise. A purported mycorrhizal PLFA, 16:1ω5c, was higher in wetter communities and lower in drier communities (Figure 3-5). Similarly, a purported anaerobic marker, cy17:0, was higher in drier communities than in the wet ME and SC habitats. It is unknown what group i11:0 3OH may represent, but its concentration appeared to decrease across the moisture gradient of habitats. The remaining fatty acids, i16:0 and 16:0 were variable across the moisture gradient.

Three fatty acids, 16:1 2OH, 16:1ω7c (both Gram – PLFAs), and 18:2ω6c (a fungal PLFA) exhibited both a significant main effect of habitat and also a significant interaction effect. There was a general decrease in 16:1 2OH across the habitat moisture gradient (Figure 3-6a). At the extremes of the gradient, 16:1 2 OH tended to decrease with invasion, while the middle of the gradient showed no effect of invasion on its relative abundance. In contrast, for 16:1ω7c, there was no clear pattern across the habitat gradient as SA and AR habitats had the lowest relative concentration of 16:1ω7c (Figure

3-6b). The effect of invasion ranged from a decrease in 16:1ω7c with invasion in AR and ME habitats, an increase in RU habitats, and no discernable effect in SC and SA habitats. Finally, the fungal PLFA, 18:2ω6c, generally increased across the habitat moisture gradient (Figure 3-6c), increasing with invasion in AR and ME habitats.

The catabolic potential of microbial communities from ME, SC, SA, and AR habitats was examined as the ability of those microbes to metabolize 95 carbon substrates. Although the majority of substrates were metabolized and there was no significant difference in the number of substrates metabolized between habitats or invasion-status, rates of utilization differed. Substrate utilization patterns show lesser degree of discrimination among microbial communities than PLFAs (Figure 3-8). A MANOVA indicated only a significant effect of habitat type (Table 3-9).

Discussion

Habitat Controls of Microbial Community Composition

Our results clearly indicate that habitat was the primary control of microbial community composition across the sites we examined. Microbial communities from different habitats differed in total PLFA, fungal:bacterial ratio, relative representation of Gram – and Gram + bacteria, and several other individual PLFAs. Because plant community composition is not independent of the soil characteristics in this study, it is difficult to separate the effects of plant species composition from soil environmental variables. The major environmental gradient across the habitats examined, however, was water content of the soil and was apparently the major control of microbial community composition. Total PLFA was highest at intermediate moisture lowest at the wettest and driest habitats (ME and AR, Figure 3-1). At high water contents microbial activity is limited by oxygen availability, while at low matric potential microbial activity is limited

by water availability (Griffin 1985). Fungi are better able to withstand low matric potentials and therefore tend to dominate in drier soils and are at low levels or absent in water-logged soils (Bossio and Scow 1995, 1998, Nakamura et al. 2003). This pattern was corroborated by our results for the fungal PLFA 18:2ω6c that decreased from AR (driest) to ME (wettest) habitats (Figure 6c). Such differential responses of fungi and bacteria resulted in a decrease in fungal:bacterial ratio across the gradient from low to high water contents, with moisture explaining 51% of the variance in fungal:bacterial ratio (Figure 3-2).

Water content of the soil was also associated with higher organic matter as indicated by the high carbon and nitrogen contents in waterlogged ME habitats. Thus although carbon and nitrogen availability was highest in ME habitats, total PLFA was highest in communities with intermediate water, carbon and nitrogen contents. Heterotrophic soil microbes are generally thought to be carbon- (Alden et al. 2001, Ekbald and Nordgren 2002) or nitrogen-limited (Hart and Stark 1997). Our results indicate oxygen availability becomes a greater limitation to microbial activity and biomass than substrate availability under saturated conditions, however, when not under saturated conditions, microbial biomass (as measured by total PLFA) is positively correlated with C and N. Fungi and bacteria, however, responded in a predicted, linear fashion to increasing carbon and nitrogen availability. Fungi (as measured by 18:2ω6c) and fungal:bacterial ratio decreased with increasing C and N concentrations, and increased with C:N. Conversely, Gram – bacteria were positively correlated with carbon and nitrogen concentrations. Bacteria have higher nitrogen requirements and metabolic and growth rates than fungi (Griffin 1985). This leads to the pattern of fungi dominating

in soils with low nitrogen with bacteria dominating in soils with high nitrogen (Bardgett and McAlister 1999, Priha et al. 2001, Leckie et al. 2004). The increase in bacterial biomass with soil carbon and nitrogen concentrations in this study was primarily due to an increase in Gram – biomass. Gram – bacteria are able to quickly respond to nutrient enrichment (Griffiths et al. 1999) and have been shown to be higher in communities with higher soluble organic contents (Leckie et al. 2004). Gram + bacteria may be being out-competed at high resource availability and others studies have shown this group to decrease with increasing carbon availability (Bossio and Scow 1998).

These individual responses of particular functional groups led to an overall differentiation of microbial communities among habitats (Figures 3-4 and 3-7). There was greater separation of habitats by PLFA than by substrate utilization method. This trend has been seen in other studies (Buyer and Drinkwater 1997); however, we feel that the reduced separation discrimination of habitats by substrate utilization data could reflect an artifact of freezing soil prior to analysis. In effect, only the catabolic potential of microbes able to withstand the freezing and thawing process was examined. In examining the effect of storage on the catabolic potential of plant growth promoting bacteria, (Shishido and Chanway 1998) found that frozen soil samples were more similar to one another than to its fresh sample counterpart. Nevertheless, different habitats supported microbial communities distinct in both function and structure.

Alteration of Microbial Communities by Invasion

Overall, invasion-status had smaller, but significant, effects on soil microbial structure, as measured by PLFAs, than those of habitat (Figure 3-4, Table 3-6). Four of the five invaders (all but *Schinus*) appeared to alter microbial community structure. We predicted that invaders that dominate the community to a greater extent in terms of

biomass would have larger impacts on microbial community composition. We did not find that trend. Instead, it was only the two habitats at the extreme ends of the gradient, AR and ME, that invasion had a significant effect on PCA scores within habitats. *Melaleuca* converts wetlands dominated by *Cladium* (sawgrass, a sedge) to dense forest while *Ardisia* adds a monodominant shrub-layer without altering the overstory. Chapin and D'Antonio (Chapin et al. 1996, D'Antonio et al. 1999) predicted that invaders that change the structure of a community would have larger impacts on that system. This would seem to explain the great *Melaleuca* effects, but does not explain the effects of *Ardisia*. Kourtev et al. (2002) did find that on a local scale shrubs could alter microbial community composition. We believe that *Ardisia* may be altering microbial community composition due to its high density of carbohydrate-rich roots (S. Bray unpublished data) that may be providing a different or larger source of exudates in the top 10 cm of soil.

Our data indicate that invasion alters microbial community composition not only in the zone of influence of a single plant as previously shown (Kourtev et al. 2002) but also at a landscape level. There was a large variation in the abiotic characteristics, such as soil moisture, that we examined across sites and likely large variation in other variables not measured in this study. This variation across sites was likely responsible for the scatter in microbial community composition and the relatively low total variation (39.5%) explained by ordination of PLFA profiles.

Conclusions

Our data contribute to a growing literature base that demonstrates both soils beneath different plants support different microbial communities (Grayston et al. 1996, Westover et al. 1997, Grayston et al. 1998, Katajisto et al. 1999, Priha et al. 1999, Kourtev et al. 2002, 2003, Bardgett and Walker 2004) and microbial communities differ

among habitats with contrasting types of vegetation (Waldrop et al. 2000, Myers et al. 2001, Leckie et al. 2004). Habitat is the main control of microbial community composition, but invasion significantly modified microbial communities within a given habitat. The direction of these changes, however, was not predictable across habitat types. Changes in microbial community structure with invasion will likely be influenced by abiotic conditions in the community and the identity of the invader. As microbes are primary responsible for decomposition in most ecosystems, any alteration in microbial community structure and function with invasion may result in altered nutrient cycling and availability. The link between microbial community composition and process rates needs to be explicitly examined across plant communities.

Table 3-1: Location, mean annual temperature, mean annual rainfall, soil type and dominant vegetation of the three sites in each habitat type.

Site	Location	Mean Annual Temperature (°C)[a]	Mean Annual Rainfall (mm)[a]	Soil Order	Dominant Native Vegetation
ME Habitats					
1	26°24’ N , 80°14’ W	26.04	1560	Histosol	Sedges
2	25°55’ N, 80°26’ W	24.44	1487	Histosol	Sedges
3	26°3’ N, 80°33’ W	26.04	1560	Histosol	Sedges
SC Habitats					
1	26°8’ N, 81°3’ W	23.83	1376	Entisol	Tropical Hardwoods
2	26°2’ N, 81°17’ W	23.83	1376	Entisol	Tropical Hardwoods
3	25°56’ N, 81°18’ W	23.83	1376	Entisol	Tropical Hardwoods
RU Habitats					
1	29°37’ N, 82°19’ W	20.33	1228	Alfisol	Deciduous Soft- and Hardwoods
2	28°7’ N, 82°9’ W	22.83	1137	Mollisol	Deciduous and Evergreen Hardwoods
3	28°46’ N, 81°12’ W	22.67	1228	Alfisol	Evergreen Hardwoods and Palms
AR Habitats					
1	29°37’ N, 82°17’ W	20.33	1228	Ultisol	Deciduous and Evergreen Hardwoods
2	29°33’ N, 82°21’ W	20.33	1228	Alfisol	Deciduous and Evergreen Hardwoods
3	29°40’ N, 82°9 W	20.33	1228	Alfisol	Deciduous and Evergreen Hardwoods, Softwoods
SA Habitats					
1	29°36’ N, 82°19’ W	20.33	1228	Alfisol	Grasses, forbs,
2	29°23’N, 95°1’ W	21.78	1113	Vertisol	Grasses, forbs
3	29°22’N, 95°2’ W	21.78	1113	Vertisol	Deciduous Hardwoods

[a] Mean annual temperature and rainfall data are 30-year (1971-2000) means from the nearest weather monitoring station of the National Oceanic and Atmospheric Administration.

Table 3-2: Soil characteristics of the five habitats examined. Means ± S.D, different letters within the same column signify significant differences by Tukey HSD. Df = 1, 25.

Habitat	% Moisture	%C	%N	C:N
ME	79.29 ± 18.66 a	32.82 ± 8.81 a	2.05 ± 0.71 a	16.54 ± 1.98 c
SC	40.74 ± 8.82 b	8.81 ± 9.49 bc	0.391 ± 0.18 b	20.26 ± 10.11 bc
SA	43.28 ± 5.49 bc	9.37 ± 3.68 c	0.432 ± 0.15 b	21.91 ± 6.69 bc
RU	28.34 ± 7.68 c	12.08 ± 2.02 b	0.377 ± 0.045 b	32.61 ± 7.39 a
AR	22.20 ± 9.03 c	3.80 ± 1.42 d	0.158 ± 0.076 c	27.56 ± 10.86 ab

Table 3-3: Relationship of total PLFA and fungal:bacterial ratio with % moisture, %C, %N and C:N using quadratic fit for total PLFA and simple linear regression for fungal = biomass ratio (n = 30). All significant models had p-value ≤ 0.005 except %C vs. fungal : bacterial ratio which was significant at p = 0.03.

	Total PLFA		Fungal : Bacterial Ratio	
	R^2	Direction	r^2	Direction
% Moisture	0.32	Convex	0.70	Negative
% C	0.45	Convex	0.26	Negative
% N	0.48	Convex	0.48	Negative
C:N	NS	NS	0.52	Positive

Table 3-4: ANOVA results for the effects of habitat, invasion-status and their interaction on the relative representation of Gram – and Gram + biomarkers.

		Gram -		Gram +	
Effect	Df	F	P	F	P
Model	9, 20	26.07	<0.0001	17.96	<0.0001
Habitat	4	45.69	<0.0001	38.13	<0.0001
Invasion	1	4.23	0.0529	2.27	0.15
Hab x Inv	4	11.91	<0.00001	1.72	0.19

Table 3-5: Correlation between %N, %C, and %moisture and the relative representation of Gram – and Gram + biomarkers (n=30). All comparisons were significant at p <0.01.

	Gram -		Gram +	
	r^2	Direction	r^2	Direction
%C	0.37	Positive	0.32	Negative
%N	0.31	Positive	0.19	Negative
%moisture	0.21	Positive	NS	NS

Table 3-6: Results of MANOVA for the effects of habitat, invasion and their interaction on principle component axes 1 and 2 scores from the ordination of 23 PLFAs.

Effect	DF	F	P
Model	9, 20	28.4	<0.0001
Habitat	4	61.6	<0.0001
Invasion	1	5.74	0.03
Hab * Inv	4	0.92	0.47

Table 3-7: Loadings for the first two axes in a principle components analysis of 23 common PLFAs extracted from soil samples. Bold face indicates loadings ≥ 0.3.

Fatty Acid	PCA 1 Loading	PCA 2 Loading
Gram +		
i15:0	0.1311	0.1373
a15:0	0.1980	-0.0118
i16:0	**0.2952**	0.1035
i17:0	0.1958	-0.1475
a17:0	0.2146	-0.1363
16:0 10ME	0.2456	-0.2619
Gram -		
15:0	-0.1103	0.2806
16:1w7c	**-0.2922**	-0.2198
16:1w5c	0.0164	**-0.3532**
cy17:0	-0.2140	**-0.3141**
16:1 2 OH	**0.3478**	0.0410
17:0 10ME	0.2610	0.1541
18:1w7c	-0.2419	-0.1602
cy19:0	0.1892	0.2399
Fungi		
18:2w6c	-0.0881	**0.3527**
18:1w9c	-0.1377	0.2555
Microeukaryote		
20:4	0.0797	0.1116
Actinomycete		
18:0 10ME	0.2080	0.0591
No classification		
i11:0 3OH	0.0480	**-0.3176**
15:0 3OH	0.1190	0.0696
14:0	-0.1978	-0.0207
16:0	**-0.3237**	0.1680
18:0	-0.2192	0.2497

Table 3-8: Correlations (r) of soil characteristics with the first two axes from the ordination of 23 PLFAs.

Soil characteristic	PCA 1	PCA 2
% N	-0.135	-0.634
% C	-0.250	-0.538
% Moisture	-0.025	-0.737
C:N	-0.331	0.388

Table 3-9: The results of MANOVA for the effects of habitat, invasion and their interaction on principle component axes 1 and 2 scores derived from an ordination of metabolic activity of soil microbes on 95 substrates.

Effect	DF	F	P
Model	7, 16	2.91	0.037
Habitat	3	6.67	0.004
Invasion	1	0.32	0.57
Hab x In	3	0.007	0.99

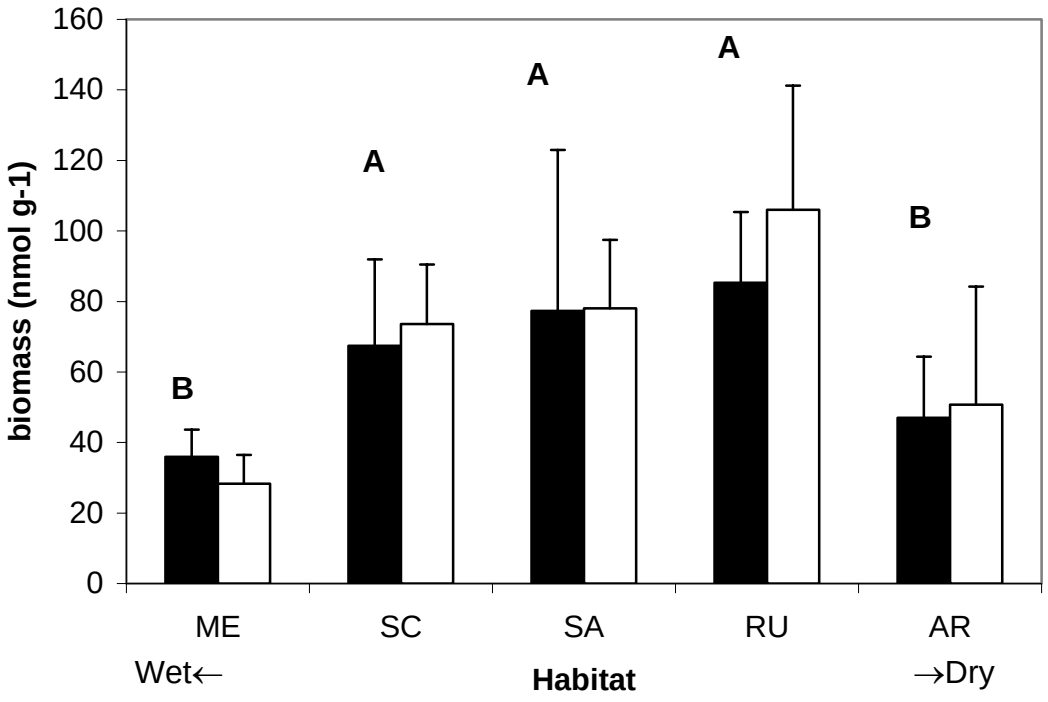


Figure 3-1: Mean soil microbial community total PLFA (nmol g^{-1} $\pm$ S.D.) for five habitats prone to invasion by five non-native species. Black bars = non-invaded; white bars = invaded. Habitat types marked by different letters are significantly different from one another by Tukey's HSD (alpha = 0.05).

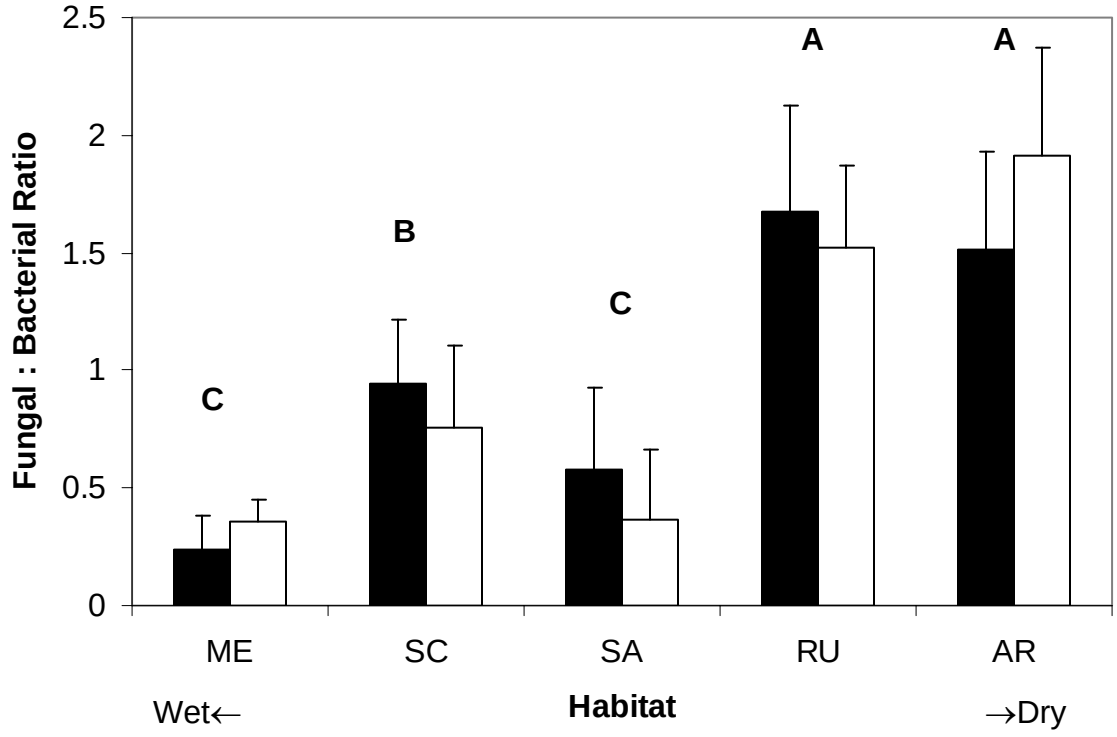


Figure 3-2: Fungal:bacterial ratios (± S.D.) for five habitats prone to invasion by five non-native species. Different letters over bars indicate significant differences between habitats as determined by Tukey's HSD (alpha = 0.05). Black bars = non-invaded; white bars = invaded.

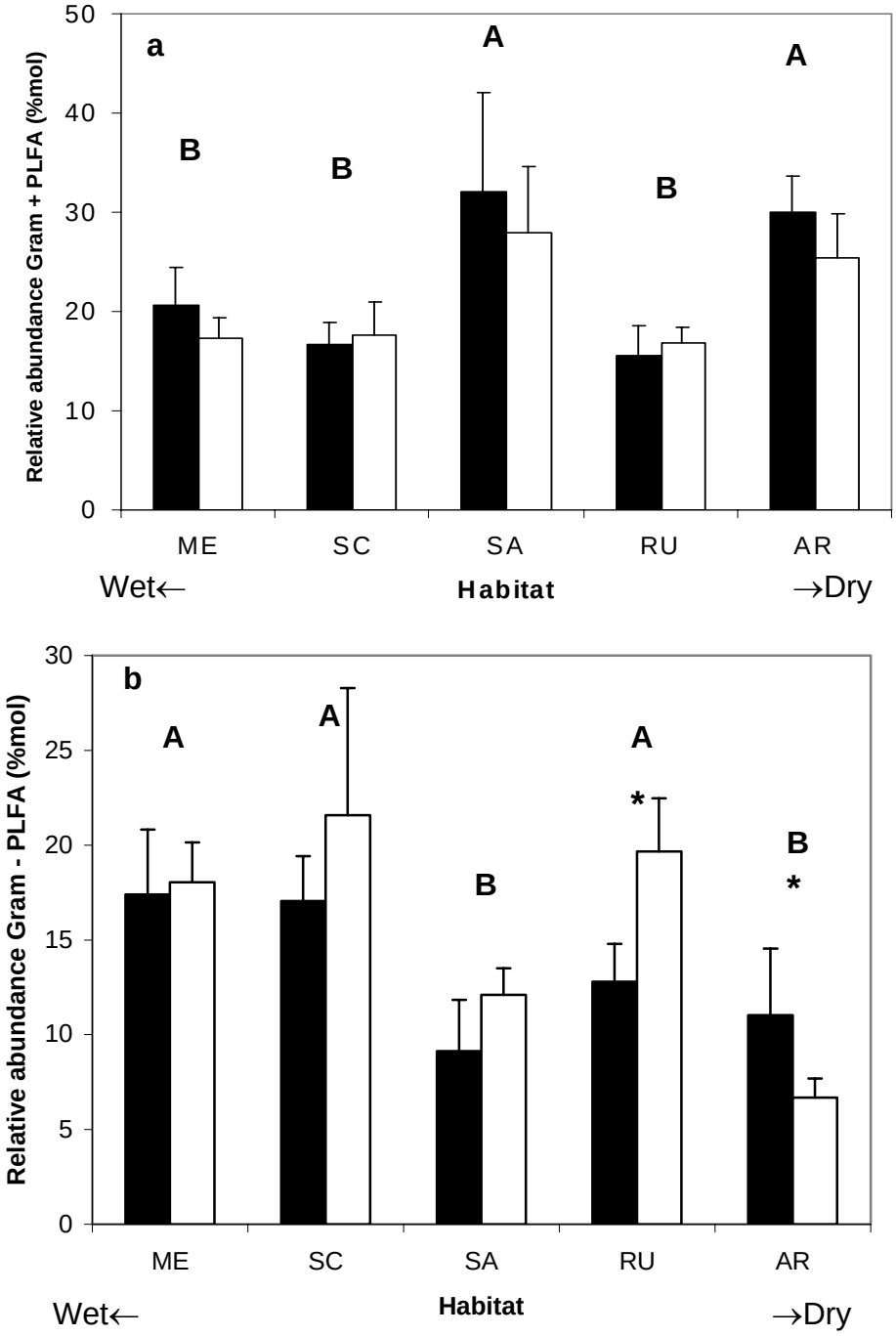


Figure 3-3: Relative representation (% of total nmoles extracted) of PLFAs across habitats and invasion-status. a) Gram + and, b) Gram – PLFA biomarkers (mean $\pm$ S.D.) Black bars = non-invaded; white bars = invaded. Different letters over bars indicate significant differences between habitats as determined by Tukey's HSD (alpha = 0.05) after pooling invaded and non-invaded areas within a habitat. Significant differences between invasion-status within a community by Bonferroni-corrected t-tests are indicated by asterisks (* = $p \leq 0.05$; ** $p \leq 0.01$).

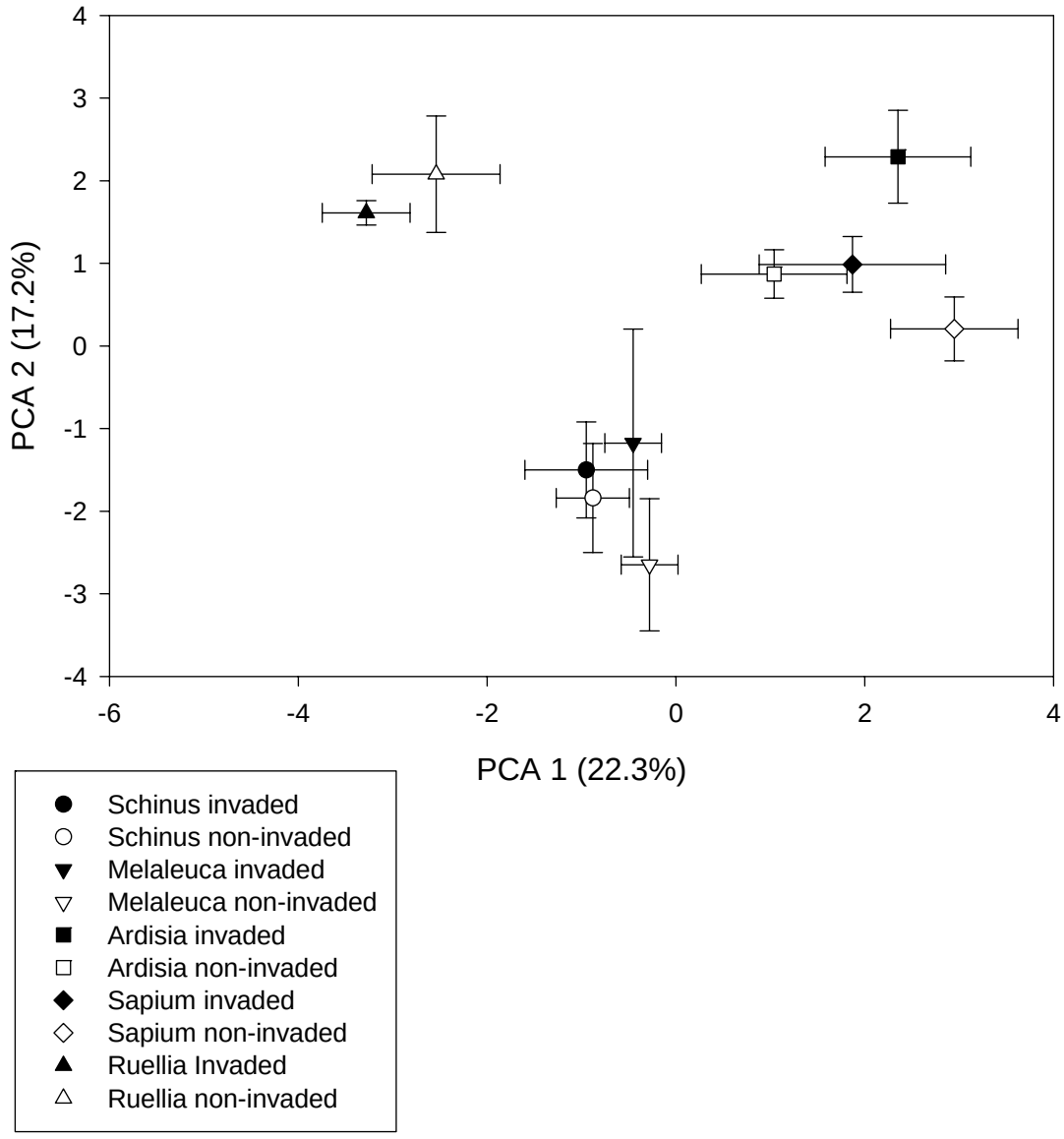


Figure 3-4: Mean (± S.D.) principle components scores by habitat and invasion-status from ordination of 23 most common microbial PLFAs found in soil samples. Downward triangles = ME, circles = SC, diamonds = SP, upward triangles = RU, squares = AR habitats. Open symbols = non-invaded, closed symbols = invaded.

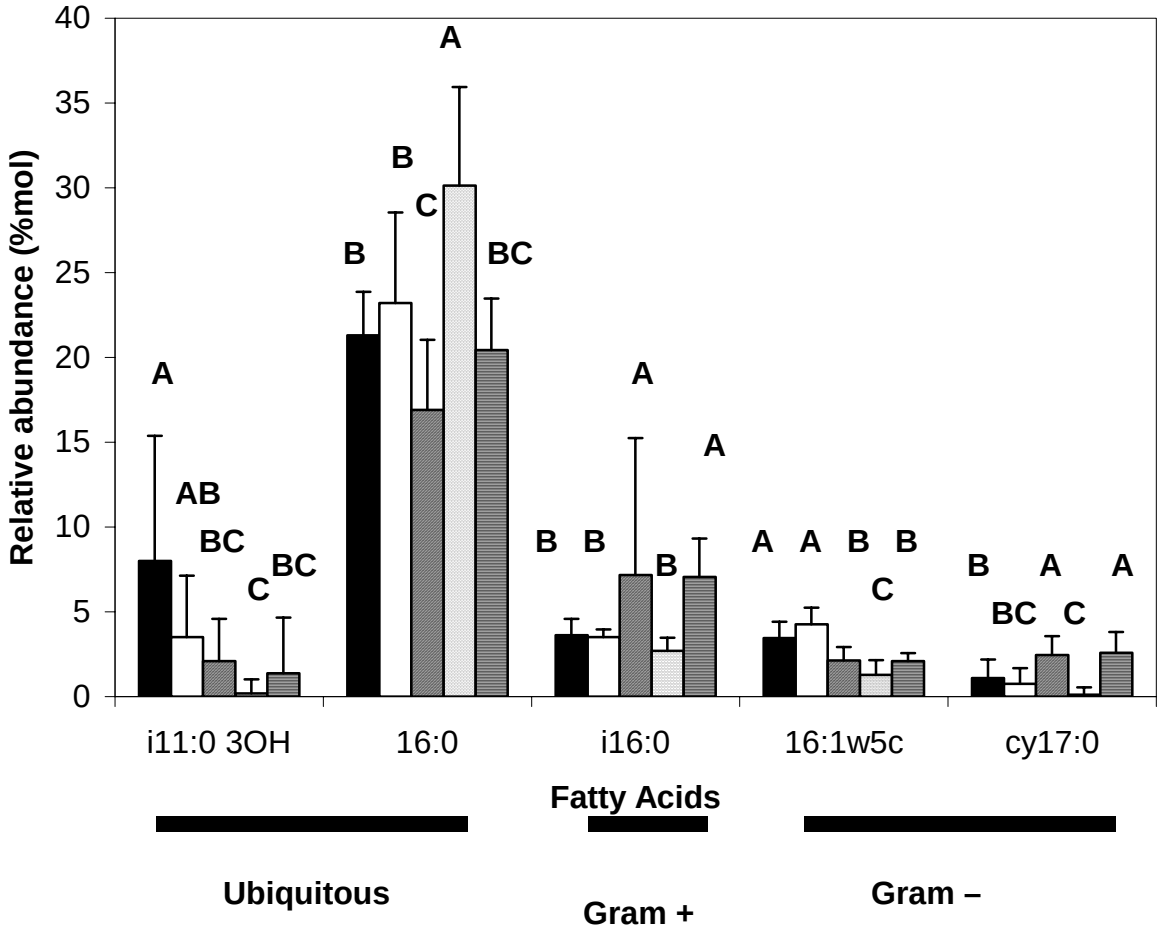


Figure 3-5: Mean (± S.D.) relative abundance of five PLFAs that had high loadings on the principle component axes that showed significant habitat effects, but no invasion effect. Solid bars = ME, open bars = SC, diagonal stripes = SA, stipled bars = RU, horizontal stripes = AR. Different letters over bars indicate significant differences between habitats as determined by Tukey's HSD (alpha = 0.05).

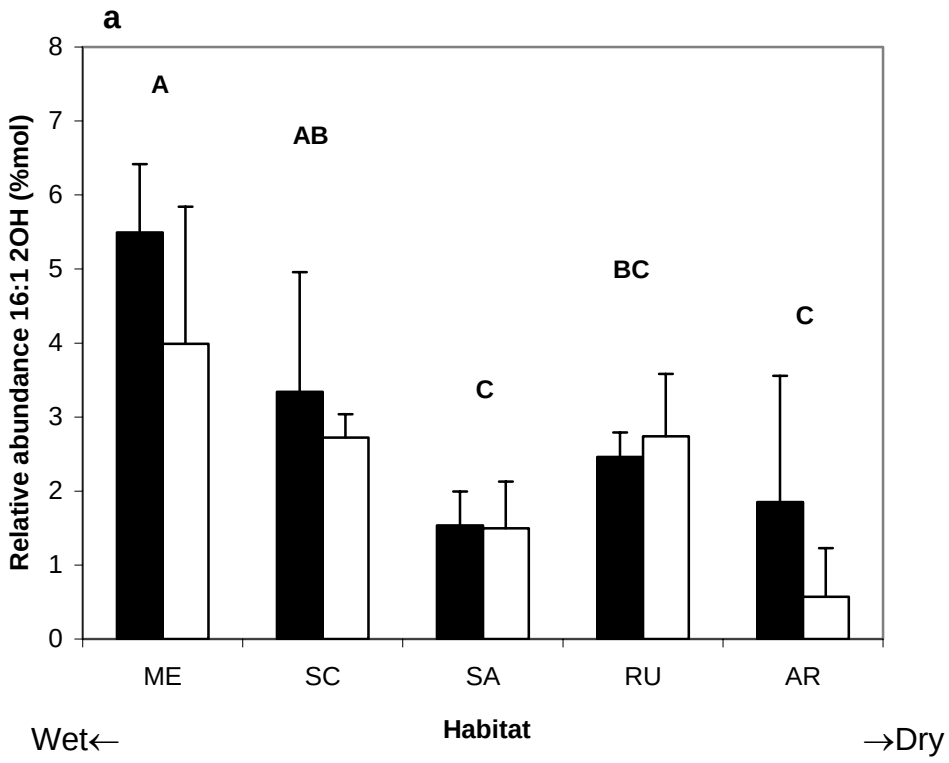
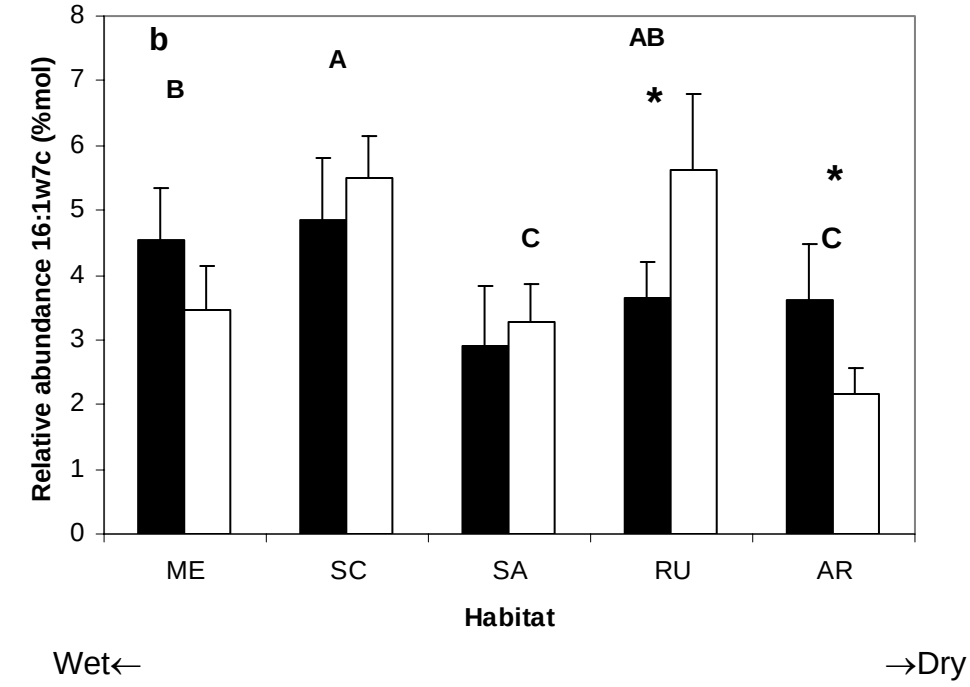


Figure 3-6: Mean (+ S.D.) relative abundance of PLFAs with high loadings (>0.3) in the PCA analysis showing significant habitat and invasion effects. a) 16:1 2OH, Gram – biomarker, b) 16:1ω7c, Gram – biomarker, and c) 18:2ω6c, fungal biomarker. Black bars = non-invaded, open bars = invaded. Different letters over bars indicate significant differences between habitats as determined by Tukey's HSD (alpha = 0.05) after pooling invaded and non-invaded areas within a habitat. Significant differences between invasion-status within a community by Bonferroni-corrected t-tests are indicated by asterisks (* = $p \leq 0.05$; ** $p \leq 0.01$).



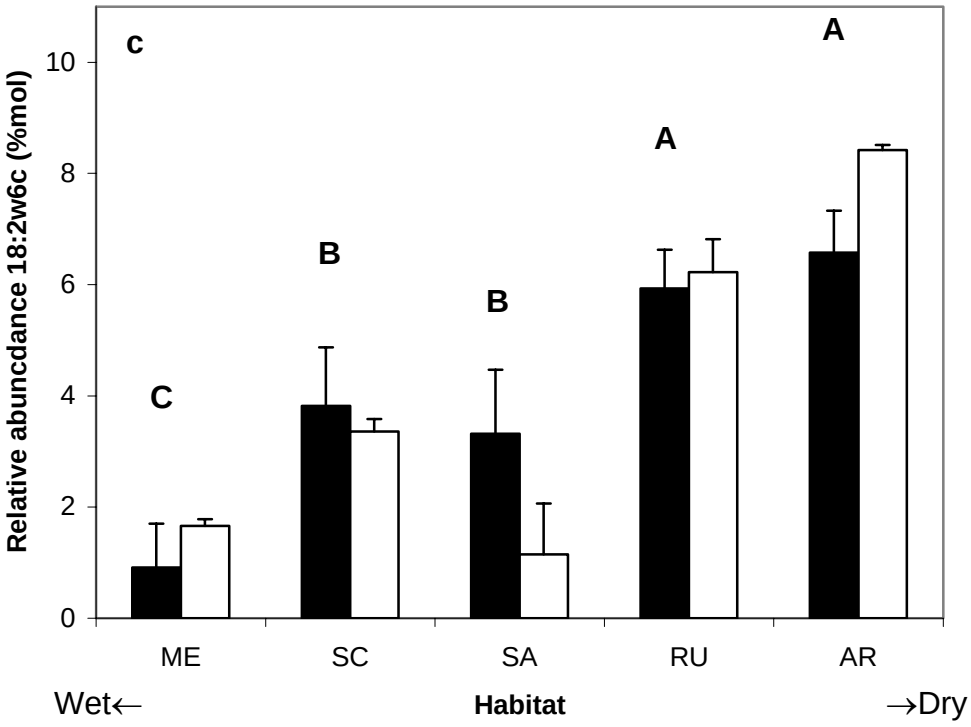


Figure 3-6. Continued

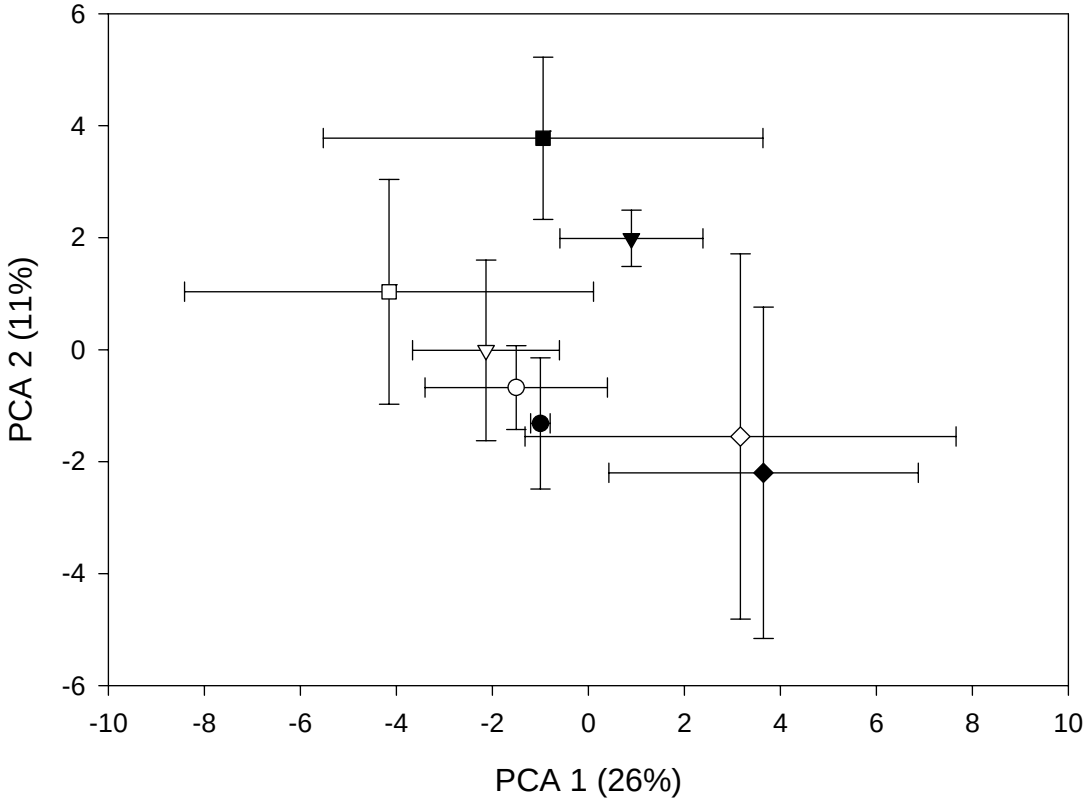


Figure 3-7: Mean (+ SD) principle components scores by habitat and invasion-status from the ordination of metabolism of 95 carbon sources by soil microbial communities. Symbols are as described in Figure 3-4.

CHAPTER 4
LINKS BETWEEN LITTER QUALITY, DECOMPOSITION AND MICROBIAL COMMUNITY COMPOSITION ON NATIVE AND NON-NATIVE PLANT LITTER

Introduction

The decomposition of plant litter is dependant on climate, substrate quality and decomposers. Within a given climate, leaf chemical composition is an excellent predictor or decomposition. The optimal measure of litter quality as a predictor of decomposition depends upon the system examined and the length of the study. Nitrogen content and carbon to nitrogen ratio (C:N) are often positively and negatively correlated with decomposition rates, respectively, in studies of short duration and in species or soils with a high percentage of more labile carbon fractions (Flanagan and van Cleve 1983, Pastor et al. 1987, Taylor et al. 1989). Other studies have shown that carbon quality, as measured by proportional representation of labile and recalcitrant substrates, to be the major factor limiting decomposition (Meentemeyer 1978, Berg and Taum 1991, Hobbie 1996). Lignin is one such recalcitrant compound degraded by only a limited number of organisms, predominately brown- and white-rot Basidiomycete fungi, can degrade it. In many cases, lignin:N is the best integrator of litter quality for the duration of its decomposition (Melillo et al. 1982).

Suites of litter quality characteristics tend to be correlated with one another as a function of habitat quality. In low-resource habitats, species tend to have low nutrient contents, high carbon-based structural defenses, and long-lived leaves (Chapin 1980). Conversely, species from high-resource habitats have higher nutrient contents, lower

levels of defense, and shorter-lived leaves (Chapin 1980, Grime et al. 1996, Wright et al. 2001). Moisture levels in soil can indirectly affect resource availability. At high soil moisture contents, oxygen becomes limiting and decreases decomposition (Haynes 1986). These lowered oxygen levels lower nutrient availability and reinforce slow decomposition rates by leading to suites of leaf traits that result in slower decomposition rates.

While the role of substrate quality in decomposition is well established, the role of the microbial community has been less studied and, in general, is treated as a 'black box.' It seems likely, however, that different communities of microbial decomposers would be found on litter of different quality and that microbes are probably responding to the same litter quality factors that control decomposition rates. As bacteria have higher nitrogen requirements and faster growth and reproduction rates than fungi, bacteria might be expected to dominate on litter with high nitrogen and low C:N. Fungi, conversely, due to their slower growth, lower nutrient requirements and ability to decompose lignin, might be expected to dominate litter with high C:N and lignin:N. Changes in litter quality over the duration of decomposition will also likely control the succession of microbes on the litter. Early studies of litter decomposers have shown that the ratio of fungi to bacteria increased with increasing C:N (Witkamp 1963, 1966).

Previous studies of decomposer microbes have primarily relied upon culture-based techniques. As only an estimated <1% of soil microbes are culturable (Torsvik et al. 1996, Atlas and Bartha 1998), culture-based techniques are limited in their ability to examine the microbial community. Phospholipid fatty acid (PLFA) analysis offers a non-culture based technique that provides a measure of the living microbial community.

PLFA analysis has the advantage of providing information on specific groups of microbes through the use of signature fatty acids as well as providing an overall "fingerprint." Thus, not only can functional or taxonomic groups be examined, but also the similarities (or dissimilarities) of different microbial communities can be compared. We applied PLFA analysis as a new approach to examine decomposer microbes on different plant litters over time.

Differences between plant species, by way of their chemical composition affecting litter quality, are known to affect decomposition rate and we hypothesize it will also determine the composition of the decomposer community. Plant community composition is changing globally with the spread of non-native species and such changes can lead to changes in ecosystem function (Vitousek and Walker 1989, Mack and D'Antonio 2003, Allison and Vitousek 2004). While a consensus predictive framework for determining which invaders will be more likely to have ecosystem-level impacts has not been developed, invaders that are qualitatively different from native species are believed to be more likely to alter ecosystem processes (Chapin et al. 1996, D'Antonio et al. 1999). Those species that have traits that overlap with native species are expected to have limited or slower impacts on ecosystem traits (Mack and D'Antonio 2003). As litter quality is a continuum in which both native and exotic species are distributed, we would expect that the potential impacts of exotic species invasion on decomposition and microbial community composition would be determined by its relative position on this continuum. The evolution of increased competitive ability (EICA) hypothesis predicts that due to the lack of herbivore pressure in their new range, invasive plants evolve to

invest less in defense (Blossey and Notzold 1995) potentially resulting in higher quality litter in invaders.

The primary goal of this study was to examine the links between decomposition and microbial community composition through litter quality. To that end, we examined the decomposition of litter of twenty species of plants, both native and exotic, from a variety of habitats varying in litter quality, in a common site. By including a variety of plant species from a variety of habitats, we attempted to sample a broad range of litter quality. We hypothesized that species identity of the litter (hereafter "litter species") significantly affects decomposition rates in relation to the litter species' typical habitats, leaf longevity, and other leaf functional traits. We hypothesized that the same litter quality factors that control decomposition in this site should also control the composition of the microbial community. Litters of higher quality should support a higher total biomass with proportionally more bacteria, especially Gram-negative bacteria, while fungi should dominate litters of lower quality. The difference in microbial community composition between plant litters should decrease as decomposition proceeds and remaining litter is dominated by more recalcitrant substrates. Finally, because we hypothesize that the same litter quality factors control both decomposition rate and microbial community composition, the correlation between them should be high.

Methods

Litter Collection and Experimental Design

We chose 20 plant species with the goals of representing 1) a broad range of litter quality as measured by leaf habit and lifespan, nitrogen content and carbon fractions and 2) non-native and native species from a variety of habitats (Table 4-1). Leaf litter of each species was collected from a minimum of five sites and a minimum of five individuals

per site between the months of September and December. Only leaves that fell freely from shaken plants or had a clear abscission zone were collected; obviously green leaves and leaves with heavy herbivore damage were excluded. Litter was pooled by species and dried at room temperature for a minimum of four weeks prior to litter bag construction.

Litter bags were constructed of 1-mm fiberglass window screen and filled with 5 g of air-dried litter. Subsamples of air-dried litter were weighed, dried at 60^{o}C and re-weighed to determine initial dry mass of litter bags. A total of 30 litter bags per species were made to allow for 5 replicates at each of 6 collection dates. We strung a total of 20 litter bags (1 per species) onto a nylon line for each harvest date. Litter bags were placed in a common hardwood-dominated forest (29°40'N, 82°9'W; mean annual rainfall 1200 mm; mean annual temperature 20.3°C) in February 2004. Six lines of litter bags, representing the six sampling dates, were placed at 5 randomly determined locations within the study site. Lines radiated out from a central flag on a litter layer dominated by *Quercus nigra*, *Quercus hemispherica*, and *Pinus taeda*.

One line from each replicate was collected at 28, 57, 112, 180, 238, and 319 days. Litter bags were transported to the lab where the exterior of the bags were brushed free of adhering soil. Roots, soil, invertebrates and frass were removed and the remaining litter weighed. A subsample of litter was immediately frozen for later phospholipid analysis; the remaining litter was weighed, dried at 60 °C and reweighed after 5 days of drying. Dried samples were ground on a Wiley Mill (Thomas Scientific) through a 40 mesh.

Carbon and nitrogen contents of initial litter and litter bags was determined by combustion on an elemental analyzer (Costech, Inc., Valencia, CA). Multiplying

nitrogen concentration by litter mass and dividing by the initial mass of nitrogen determined percent initial nitrogen remaining. Carbon fractions were extracted with increasingly acidic solutions (van Soest 1963) using an Ankom 220 Fiber Analyzer (Ankom, Macadon, NY). Neutral detergent removed non-polar extracts (fats, oils waxes) and soluble cell contents (carbohydrates, starch, non-bound proteins) and comprises the non-polar fraction (NPE). Dilute acid detergent removed hemi-cellulose and bound proteins comprising the water-soluble fraction (WS). Cellulose, the acid-soluble fraction (AS), was separated from lignin with 72% H_2SO_4. The lignin fraction was corrected for ash content by ashing samples at 500 °C after the sulfuric acid step. Carbon fractions were expressed as a percentage of total mass.

Phospholipid Fatty Acid Analysis

Prior to analysis, frozen samples were ground with a Wiley Mill to pass through a 40-mesh. All glassware was heated at 500°C for 5 hours and PTFE caps were rinsed with hexane to remove organics. Phospholipids were extracted from litter using the methods of Wilkinson et al (2002). Lipids were extracted from 250 mg litter samples in two 30-minute baths in a 37°C water bath in a single-phase phosphate buffered methane-chloroform solvent. After each extraction, the supernatant liquid was transferred to a second test tube. The phase of the second test tube was broken by the addition of 4 ml of chloroform and 4 ml of buffer. After vortexing, the phases were allowed to separate overnight. The organic phase was fractionated on silicic acid columns and the phospholipid fraction collected and transesterified to fatty acid methyl esters (FAMEs) by mild alkaline methanolysis (Findlay and Dobbs 1993). Samples were resuspended in hexane with 19:0 fatty acid as an internal standard and analyzed with an Agilent

Technologies 6890 gas chromatograph (Palo Alto, CA) with a 25m Ultra 2 phenyl methyl silicone column. The temperature program increased from 170°C to 270°C at 5°C per minute. Peaks were identified with MIDI peak identification software (MIDI, Inc., Newark, DE) and by co-elution with standards. Fatty acids were defined in terms of total number of carbon atoms : number of double bonds. The position of the double bond from the methyl end of the molecule is signified by the symbol ω followed by the carbon position; cis and trans geometry are referred to by "c" and "t." The prefixes "i" and "a" signify and iso and anteiso branching; "cy" signifies a cyclopropyl fatty acid.

Statistical Analysis

In order to compare litter decay rates among species in relation to litter quality, decomposition rate constants (k) for each litter type were determined using a negative exponential model:

$$\ln(X_t) = \ln(X_0) - kt$$

where X_t equals the amount of mass left at time = t, X_0 is the initial mass of litter, and t = time in years (Olson 1963). In order to improve normality, k's were log transformed and subjected to ANOVA examine differences among species. Tukey's HSD was used to examine differences between species. To examine difference between leaf lifespan categories and habitats, species averages were subjected to a Kruskal-Wallace rank test, as there were uneven sample sizes among groups and unequal variances. The relationship between decomposition rate and litter quality was examined in two ways. The individual effects of mean initial %N, %NPE, %WS, %AS, %lignin, C:N and lignin:N on mean decomposition constants were examined in simple regressions. As are auto-correlated and cannot be consider as independent of each other, we sought to create an integrative measure of overall litter quality. Therefore, we ordinated the average

initial values of %N, C:N, %NPE, %WS, %AS, %lignin, and lignin:N for each species in a principle components analysis. The first axis from that PCA explained 56% of the variance in litter quality data and we use this axis as a proxy for a litter quality ranking and refer to it as the "leaf chemistry axis" herein. Average species k's were then regressed against this leaf chemistry axis.

Similarity of microbial communities was examined using both constrained (canonical correspondence ordination, CCA) and unconstrained (principle components analysis, PCA) ordination. We performed these two types of ordinations because comparison of their result is informative. Constrained ordination is best used in ordinations when a set of independent environmental variables is believed to be structuring the community. As we hypothesize that microbial communities should be structured by litter quality characteristics, constrained ordination allows us to directly examine how litter quality is structuring microbial communities as the ordination axes are constrained to these independent variables. Unconstrained ordination, conversely, extracts the major variation in community data irrespective of any environmental variables. Therefore, if a PCA and CCA give similar results and explain a similar percentage of variance in the community data, it is assumed that the environmental variables examined are primarily responsible for the structuring of the community. Additionally, as CCA is subject to the same limitations as multiple regression and CCA can be sensitive to noise in the environmental matrix (McCune 1997), use of unconstrained ordination along with constrained ordination is often suggested (McGarigal et al. 2000).

We ran PCA and CCA ordinations on all samples simultaneously and on samples from each time point individually. We performed both types of analyses so that we could examine overall changes and controls of microbial community composition over decomposition and the differences and primary controls on microbial community composition at a given sampling date. Only the 17 PLFAs that comprised at least 1% of the total PLFAs extracted were included in the multivariate analyses. The PCA analysis was performed only on the main matrix of the 17 PLFA values. The CCA analysis was performed on the main matrix and a second matrix containing the 7 measures of litter quality, initial mass remaining (%IMR), and percent moisture. Individual PLFAs were log-transformed to improve normality. MANOVA was performed on PCA and CCA scores to determine the effects of time, litter species and litter species*time. The relationship between litter quality and functional groups of microbes (terminally branched Gram-positive bacterial PLFAs: i14:0 + i15:0 + a15:0 + i16:0 + a16:0 + i17:0 + a17:0; monounsaturated Gram-negative bacterial PLFAs: 16:1ω7 + 17:1ω7 + 18:1ω7; cyclopropyl Gram-negative bacterial PLFAs: cy17:0 + cy19:0; and fungi: 18:2ω6), fungal:bacterial ratio (18:2ω6) / (i15:0 + a15:0 + 15:0 + i16:0 + 16:1ω5c + i17:0 + a17:0 + cy17:0 + 17:0 + 18:1ω7c + cy19:0), and total biomass was examined in simple regressions for each time point. When necessary, PLFAs were transformed to achieve normality.

All regressions, analysis of variance, and non-parametric tests were performed in JMPIN 4.0 (SAS Institute 2000, Cary, NC) while PCA and CCAs were performed in PC-ORD 4.20 (MJM Software, Gleneden Beach, OR 1999).

Results

Decomposition of Litter

Most species showed two periods of rapid decomposition—during the initial 28 days and again between 112 and 238 days, corresponding with the wet season (Figure 4-1). Decomposition rate constants (k) ranged from < 0.4 to nearly 2.0 yr^{-1} with significant differences among species ($p < 0.0001$, $F_{19,80} = 23.47$; Table 2). Leaf lifespan class of the litter species had no effect on decomposition rate ($\chi^2 = 2.19$, df = 2, $p = 0.33$). Habitat affiliation of the litter species, however, did have a significant effect on decomposition rate ($\chi^2 = 9.72$, df= 2, $p = 0.0077$) such that decomposition rate was lowest in species from dry habitats and higher for litter species from wetter habitats. However, some individual species from "wet" habitats (e.g. *Juncus*, *Taxodium*, and *Acer*) had relatively slow decomposition rates (Table 4-1 and 4-2).

There was also a significant difference in decomposition constants between native and non-native species ($F_{1,98} = 47.09$, $p < 0.0001$); this result, however, should be interpreted with caution as the result would likely change with different representative native and exotic species. When the non-native species are compared with native dominants in the plant communities they invade, *Ruellia*, *Causurina*, *Schinus*, and *Sapium* have higher decomposition rates, *Imperata* had comparable rates with native dominants, and *Ardisia*, which can co-occur with several of the deciduous species with moderate decomposition rates, was comparable with those species and faster than *Pinus* with which it also co-occurs (Tables 4-1 and 4-2).

We also examined the relationship between initial litter quality and decomposition rates. Of the seven measures of litter quality examined, the concentration of non-polar extracts and lignin:N individually explained the greatest amount variation. Lignin and

nitrogen contents as well as the relative availability of nitrogen (C:N), were also significantly correlated with decomposition rate (Table 4-3). According to a backwards stepwise regression with %N, %NPE, %lignin, C:N and lignin:N, the variables %lignin and C:N were retained and produced the following model:

$$Ln(k) = 0.650 - 0.0275(\%lignin) - 0.00907(C:N)$$

This model explained 60% of the variation ($F_{2,17} = 12.76$, $p = 0.0003$). Multiple regression models can be problematic because of collinearity of predictor variables and the order in which these variables are added or removed in stepwise regression. As litter characteristics are known to co-vary, they were analyzed by principle components analysis. The first PCA axis from this analysis explained 55.6% of the total variance (eigenvalue = 3.89), with high loadings of C:N, %NPE, %WS, and lignin:N. Thus PCA 1 can be interpreted as a litter quality axis in which low values indicate high litter quality i.e., low C:N, lignin:N, %WS, and high %NPE) and high values indicate low litter quality (Table 4-4). Decomposition rate was negatively correlated with the leaf chemistry axis ($F_{1,18} = 17.57$, $p = 0.0005$, $r^2 = 0.49$).

Nitrogen concentration of litter increased over time (Figure 4-2a). Approximately half of the species showed no change or net increase of nitrogen relative to the initial total with time, indicating net immobilization of nitrogen. The three species with the fastest decomposition rates (*Ruellia*, *Sapium*, and *Taxodium*, Table 4-2) showed the largest nitrogen mobilization (= net nitrogen loss, Fig. 4-2b). Immobilization varied over the course of the study, with several species showing increased mobilization at time points with higher decomposition (Figure 4-1 and 4-2b).

Microbial Community Composition

We examined the structure of microbial communities of eleven species at 28, 57, and 238 days by multivariate analysis of PLFAs. We performed both PCA and CCA analysis on samples from all sample dates simultaneously and then each sample date individually. The PCA analysis of all sample dates explained a greater proportion of the total variance (52% in the first 2 PCA axes) than the CCA analysis (32.3% of variance in first 2 CCA axes, Figure 4-3, a-b). This indicates that while the leaf litter quality variables we measured were important, other, unmeasured variables were also important to the structuring of microbial communities.

In the PCA analysis, there is a large cluster of samples with poor litter quality from t = 1 and 2 samples dates and a smaller cluster composed of higher litter quality from primarily t = 2 samples and two t = 1 samples (*Ruellia* and *Causurina*). By t = 5, microbial communities were beginning to converge as indicated by the ellipse indicating 9 of the 11 litter species at t = 5. The CCA analysis allows differentiation of PLFA composition (i.e., microbial community composition) on the basis of variation in litter quality. Like the PCA, CCA axis 1 differentiates microbial communities of the dry periods (t = 1 and 2) with low CCA-1 scores, from t = 5 microbial communities from wet periods with high scores. CCA axis 2, in contrast, differentiates microbial communities in relation to litter quality, such that species with high scores on the leaf chemistry axis are associated with low CCA axis 2 scores (Fig. 4-3b), especially among samples from t = 1 and t = 2. In MANOVA of PCA and CCA axes 1 and 2 scores, time, species and time * species effects were all significant (Table 4-6).

When ordination analyses were done for each sampling period separately, the first two axes of the PCA analyses of individual sampling dates again described more of the

total variation than the first two axes of the CCA analyses (time 1: 50.5% vs. 36.6%, time 2: 51% vs. 35.9%, time 5: 64% vs. 38.2%). Constraining the axes by litter quality variables in CCA altered the distribution of litter species in species-score space (Figure 4-4, a-f).

In the PCA of t = 1, *Ruellia* was most different from other litter species, but there was no obvious pattern of microbial community composition in relation to litter quality (Figure 4-4a). The first axis was composed of high loadings for two Gram - PLFAs and the fungal PLFA, while the second axis was composed of negative Gram + PLFA loadings (Table 4-7). The simultaneous analysis of litter quality in the CCA for t = 1 microbial communities isolated the species with high litter quality, *Ruellia*, *Sapium* and *Schinus,* from the remaining samples on the basis of their higher non-polar extract and nitrogen contents which were negatively loaded on CCA axis 1(Figure 4-4b). *Pinus* and *Juncus* were very different from other litter species due to their high amount of initial mass remaining, and high lignin:N and water-soluble fiber content, respectively.

In the PCA of t = 2 microbial communities, Gram + PLFAs were again negatively loaded on the second axis while saturated fatty acids, fungi and two Gram – PLFAs were positively loaded on the first axis (Table 4-7). Inclusion of litter quality in the CCA for t = 2 samples, did not drastically change the relative position of litter species to one another (Figure 4-4, c-d). The primary difference between the t = 2 PCA and CCA was a greater separation of *Acer* and *Ardisia* microbial communities from one another and a better separation of poor litter quality species, *Aristida*, *Imperata* and *Juncus,* from the remaining litter by CCA. The separation of microbial communities in the CCA was

driven by the positive correlation of NPE on CCA axes 1 and 2, and the positive correlation of WS, AS, and initial mass remaining on CCA axis 1 (Table 4-7).

At t = 5, there was generally less separation of microbial communities on high and moderate litter quality (Figure 4-4, e, f). Fungi and some saturated PLFAs were negatively loaded on PCA 1 and two Gram – PLFAs and one Gram + PLFA were negatively loaded on PCA 2 (Table 4-7). Inclusion of litter quality in the CCA better separated low quality litters *Pinus*, *Aristida* and *Juncus* from one another and the remaining litter species on the basis of initial mass remaining and C:N (Figure 4-4f).

We explored the effect of litter quality on microbial groups in simple regressions at each collection date. In general, the strength of relationship between litter quality variables and microbial groups increased across time (Tables 4-8 through 4-13). The fungal PLFA and total PLFA, however, had strongest correlations with litter quality at t = 2. Three litter quality factors, %N, C:N and %IMR, explained the greatest proportion of variance across all microbial groups. C:N and %IMR were negatively correlated with monosaturated and cyclopropyl Gram – bacteria, Gram + bacteria, fungi, and total PLFA (nmol g^{-1}) and positively correlated with fungal : bacterial ratio. The opposite trends were true for %N.

Some surprising trends relating to carbon fractions were identified. Lignin content was not correlated with fungal PLFAs, but was *positively* correlated with Gram – and Gram + bacteria and total PLFA at t = 5, although these correlations were weak. On the other hand, the relationship between lignin:N and microbial groups was as expected, being negatively correlated with bacterial groups and positively correlated with fungal:bactieral ratio. The relationship between water and acid soluble fractions and

different microbial groups was not consistent and changed depending upon collection date examined. For example, water and acid soluble fractions were positively correlated with Gram + bacteria at t = 2, but negatively correlated at t = 5. Similar to individual measures of litter quality, the strength of correlation between the leaf chemistry axis (Table 4-4) and microbial groups increased with time (Table 4-14).

Linking Litter Quality, Decomposition and Microbial Communities

Just as the leaf chemistry axis was correlated with decomposition rate ($r^2 = 0.49$) and microbial groups (r^2 up to 0.77), it was highly correlated with microbial community principle components axes. The strength of the correlation between leaf chemistry and microbial community composition increased with time (Table 4-15). Leaf chemistry explained 88% of the variation in the first microbial axis from the fifth collection date and 65% of the variation in the first microbial axis from all samples ordinated simultaneously. Microbial community composition also explained the greatest amount of variation in decomposition rate constants (k) of any measures explored ($r^2 = 0.76$ for t = 5 microbial communities). As with the relationship between microbial community and litter chemistry, the strength of the correlation between microbial community and decomposition increased with time (Table 4-15).

Discussion

In this study we endeavored to examine the role of plant litter quality in the structuring of microbial communities and how those communities changed over time. In our experimental design of using field common gardens, environmental variables other than litter quality were standardized across litter species, although the environment did not stay constant. In addition to change in litter quality as decomposition progressed, the background moisture availability changed as the rainy season started in the middle of the

experiment. Indeed, some of the temporal differences in microbial community composition as detected by PLFA biomarkers appear to be better explained by differences in litter moisture than by chemical differences of the litter. Hence, sampling date appeared to have the greatest effect on microbial community composition (Figure 4-3a), but there were also significant differences among litter species due, in part, to differences in litter characteristics (Figure 4-3b). While non-polar extracts, lignin and lignin:N (Table 4-3) were the best individual predictors of decomposition rate, non-polar, acid- and water-soluble carbon fractions and C:N (Figure 4-3b) were most important in structuring microbial communities. What was particularly novel about this study was the discovery that the best correlate of decomposition rate was not a measure of litter quality, but the composition of the microbial community ($r^2 = 0.76$ for correlation of PCA1 of the microbial community at t = 5, Table 4-15).

Factors Controlling Microbial Community Composition

Time, litter species and litter species * time all had a significant effect on microbial community structure. The most obvious effect is that of time with samples collected at t = 5 being most different from other sampling dates (Figure 4-3). The t = 5 samples were collected on October 5, 2005 within four weeks of hurricanes Frances and Jeanne which together produced 325 mm of rain in the area and these samples had much higher moisture contents than samples collected at t = 1 and 2. The separation of the t = 5 group is due to an increase in Gram-negative bacterial PLFAs cy17:0, cy19:0, and 18:1w7c and Gram-positive bacterial PLFAs i15:0 and i16:0 in those samples. Other studies have shown increases in Gram-positive, terminally branched, saturated fatty acids such as i15:0, a15:0, i17:0, and a17:0 in soil and litter microbial communities under flooded conditions (Bossio and Scow 1998, Nakamura et al. 2003). Cyclopropyl fatty

acids cy17:0 and cy19:0 have additionally been suggested as biomarkers for anaerobic bacteria (Guckert et al. 1985, Vestal and White 1989), but see (Parkes and Taylor 1983, Bossio and Scow 1998). In the only analysis of microbial communities of early decomposition of plant litter in upland conditions of which we are aware, Wilkinson et al (2002) found that a15:0 and cyclopropyl fatty acids were generally higher in regularly watered samples, although the effect was dependant on litter species examined. In this study, bacterial dispersal and activity were likely limited in the first two collections by low moisture, while the saturating conditions of the fifth collection resulted in increased bacterial biomarkers overall and especially putative anaerobic biomarkers.

While time had the largest effect on microbial communities in PC and CC analyses, litter species and litter species*time interaction effects were also significant (Table 4-6). Microbial communities on poor quality litter changed less from sample date to sample date than did microbial communities on litter with high initial litter quality (Figure 4-3a). Two alternative hypotheses may explain this pattern. Resource availability may be high enough in high quality litter that decomposition and microbial succession can proceed even under low moisture levels. Alternatively (but not necessarily exclusively), greater changes in resource availability in high quality litter may be responsible for shifts in microbial community composition.

Ordination of each sampling date individually shows litter species and quality effects on microbial community structure. In early decomposition under low moisture levels, high concentration of non-polar extracts and to a lesser extent nitrogen concentration separated high quality litter microbial communities from moderate to low quality litters with higher water and acid soluble fiber contents that retained a greater

proportion of the initial mass expressed as %IMR (Figure 4-4 b, d). The only litter quality characteristic that was important to structuring microbial communities at all samples dates was %IMR. Under the higher moisture levels of t = 5, moisture, C:N, and %N had become important to the structuring of the microbial communities (Figure 4-4f). Other studies of soil microbial communities have shown differentiation of microbial communities from different plant communities differing in litter or soil C:N or nitrogen availability (Eiland et al. 2001, Gallo et al. 2004, Leckie et al. 2004, Myers et al. 2001, Waldrop and Firestone 2004a, b). It would appear from this study that moisture levels and availability of labile carbon control early colonization of litter and as decomposition proceeds, nitrogen availability becomes more important.

Examination of functional groups of microbes also shows that moisture levels controlled colonization by microbes as litter quality had low discrimination of microbial community composition at t = 1 and 2 (Tables 4-8 through 13). However, at t = 5 when water was not limiting, %N, C:N and %IMR were generally good predictors for bacterial functional groups, fungal : bacterial ratio, and total biomass as inferred by total microbial PFLA. This is consistent with other studies of soil microbial communities with both Gram-negative and Gram-positive bacteria generally increasing with nitrogen availability (either through nitrogen fertilization or decrease in C:N ratio)(Eiland et al. 2001, Leckie et al. 2004, Waldrop et al. 2004). The response of fungi, however, appears to be equivocal with fungi or fungal:bacterial biomass having a negative response (Eiland et al. 2001, Leckie et al. 2004), no response (Waldrop et al. 2004) or, as seen in this study, a positive response (Gallo et al. 2004) to increased nitrogen availability. Many of these studies show that there is not always a consistent response of individual PFLA

biomarkers within a functional group to litter quality treatments (see especially Gallo et al. 2004, Waldrop et al. 2004). We found this to be true in this study and the low amount of variance explained for a given functional group by different carbon fractions is a result of differential response of indicator fatty acids within a functional group.

Factors Controlling Decomposition Rate

Early decomposition is dominated by the leaching and decomposition of soluble and low molecular weight compounds (Swift et al. 1979, Berg et al. 1982). In our study, only 182 mm of rain fell in the first 112 days of the experiment, with 56% of that rain falling before the first sampling date. Between samples 3 and 5 (129 days), encompassing hurricanes Frances and Jeanne, nearly 900 mm of rain fell. This distribution in rainfall is likely responsible for two major periods of leaching resulting in greater relative mass losses at those points (Figure 4-1). Although a single exponential model may not be the best model to explain mass loss, we chose this model to enable us to have a single variable to examine the relationship between decomposition and litter quality.

All litter quality factors examined except for acid-soluble fiber were correlated with decomposition (Table 4-3). As with the microbial communities, it is difficult to determine which measure of initial litter quality is most responsible for controlling decomposition. However, across the three methods we used to examine relationship between litter quality and decomposition (single regression, multiple regression and the ordination of a leaf chemistry axis), lignin:N, C:N and non-polar carbon fractions were important factors. This is consistent with a large body of literature that shows decomposition rate is related to relative availability of carbon and nitrogen (Coulson and Butterfield 1978, Melillo et al. 1982, Taylor et al. 1989). Recalcitrant fractions such as

lignin are not degraded in a significant way until later in decomposition when most of the more labile fractions have been reduced significantly—more than 2 years into decomposition in a study of Scots pine litter (Berg et al. 1982). As this study was conducted for less than a year, it is not surprising that the non-polar fractions, which are readily available for fast-growing microorganisms, were highly correlated with decomposition rate.

The novel information from this study is the linking of litter quality, microbial community composition and decomposition rate. Non-polar fractions and C:N, which were strongly correlated with decomposition rate, were also the litter quality measures most strongly correlated with CCA axes ordinating the microbial communities (Figure 4-3b). Additionally, the leaf chemistry axis explained a large proportion of the variation in decomposition rate, functional groups, and overall microbial community composition (Tables 4-14 and 4-15). The best predictor of decomposition rate, however, was not any measure of litter quality. Instead, microbial community composition explained the greatest amount of variation in decomposition rate (76%). These results indicate that treating microbes as a black box may limit our understanding of controls on decomposition.

Implications for Invasion

We believe that our study indicates that invaders will be more likely have a large impact on microbial community composition or nutrient release if the invader's litter quality, especially C:N, lignin:N and non-polar extracts, is significantly different from the native community as these were the best predictors of decomposition and microbial community composition. Invasive species may have a greater potential to alter microbial communities and ecosystem process if they invest less in defense. Upon introduction to a

new range, several invasive species have been shown to evolve toward less allocation to defense (EICA hypothesis) as a result of decreased herbivore pressure in their introduced range (Blossey and Notzold 1995, Siemann and Rogers 2003). Such reductions in defense allocation would result in high litter quality of invaders, higher decomposition rates and a shift from oligotrophic (i.e., able to live on low nutrient levels) to copiotrophic (i.e. requiring high levels of high quality nutrients, sensu Ohta and Hattori 1983) microbial communities. These shifts could lead to positive feedbacks on invasion by increasing nutrient availability (Ehrenfeld 2003).

Invasive species need to contribute a large proportion of the total litter inputs in order to affect nutrient cycling on an ecosystem scale. In our study, although *Ardisia* and particularly *Ruellia* have litter characteristics different from those in the plant communities they invade, they are understory shrubs without a large leaf biomass and thus will be less likely to affect nutrient cycling and microbial communities at the ecosystem scale. *Sapium*, *Causurina*, *Schinus*, and *Imperata*, conversely, can form monodominant stands and produce large amounts of litter that would enable them to affect ecosystem-level change.

Conclusions

We investigated the differences in microbial community composition and decomposition of litter among different species of litter in a common site. Even though the plant litter was subjected to the same environment, with the same land-use history and the same potential colonizing microbial community, composition of the microbial community differed among litter species. These differences in microbial community composition were a function of initial litter quality and litter quality at the time of collection. Changes in litter quality due to both decomposition and changing

environmental factors, predominately moisture content, resulted in a significant effect of time on microbial community composition. Microbial community composition was more strongly correlated with decomposition rates than any measure of litter quality. This study suggests that explicitly examining the microbial community rather than treating it as a black box may improve our understanding of the controls of early-phase decomposition.

Table 4-1: Characteristics of plant species whose litter was used in this experiment including leaf lifespan class (1 = <1 year, 2 ≅ 1 year, 3 = > 1 year), leaf type (broadleaf, needle leaf, monocot), position on moisture gradient (dry, mesic, wet), and native status, along with the chemical composition of freshly collected litter (senescent leaves that were easily abscised by shaking).

Species	Lifespan	Type	Habitat	Native?	C:N	NPE(%)	WS(%)	AS(%)	Lignin(%)
Acer rubrum	1	Broad	Wet	Yes	55.9	60.1	10.6	12.3	14.3
Ardisia crenata	3	Broad	Mesic	No	44.5	52.0	19.7	20.5	7.43
Aristida stricta	2	Monocot	Dry	Yes	128.5	12.3	40.8	22.9	23.6
Carya glabra	1	Broad	Mesic	Yes	38.8	55.5	12.2	18.5	13.6
Casuarina glauca	1	**	Mesic	No	25.1	32.7	17.2	25.6	24.2
Imperata cylindrica	2	Monocot	Dry	No	97.2	19.2	31.0	38.4	10.9
Juncus roemerianus	2	Monocot	Wet	Yes	69.2	20.1	34.9	36.0	34.9
Liquidambar styricaflua	1	Broad	Mesic	Yes	51.9	57.4	9.21	16.2	16.9
Magnolia virginiana	3	Broad	Mesic	Yes	57.5	51.8	14.1	20.3	13.1
Pinus palustris	3	Needle	Dry	Yes	58.2	34.6	14.5	27.7	22.5
Quercus chapmannii	2	Broad	Dry	Yes	59.7	50.9	11.7	19.3	17.9

Table 4-1. Continued

Species	Lifespan	Type	Habitat	Native?	C:N	NPE(%)	WS(%)	AS(%)	Lignin(%)
Quercus geminata	2	Broad	Dry	Yes	74.9	42.4	12.9	25.1	19.1
Quercus laevis	1	Broad	Dry	Yes	36.0	32.0	11.9	26.6	29.2
Quercus nigra	1	Broad	Mesic	Yes	47.7	44.2	13.6	20.7	21.3
Ruellia brittoniana	1	Broad*	Wet	No	32.4	72.2	9.29	10.2	7.92
Sabal palmetto	3	Monocot	Mesic	Yes	30.3	25.27	20.2	33.9	19.1
Sapium sebiferum	1	Broad	Wet	No	32.4	73.7	9.85	10.5	5.02
Schinus terebinthifolius	2	Broad	Mesic	No	37.8	61.1	5.05	11.9	21.6
Taxodium distichum	1	Needle	Wet	Yes	28.8	45.9	12.9	15.1	25.9
Typha latifolia	2	Monocot	Wet	Yes	54.5	21.4	23.4	17.0	37.5

* *Ruellia brittoniana* was the only non-woody broad leaf examined. **Photosynthetic tissue of *Casuarina gluaca*, treated as leaves in this study, are modified stems, needle-like in appearance.

Table 4-2: Mean and SE for decomposition rates of native and non-native litters (n=5). Different letters indicate significant differences (alpha = 0.05) between species by Tukey HSD. The leaf chemistry axis is the score from the ordination of initial plant litter chemistry. High scores indicate poor quality (high lignin:N, C:N), and low scores indicate high quality (high non-polar fraction, nitrogen). DF = 19, 80.

Species	Native?	k ($year^{-1}$)		Leaf Chemistry Axis Score
		Mean	SE	
Sapium	No	1.91^{a}	0.0852	-2.70
Ruellia	No	1.79^{a}	0.150	-2.51
Causurina	No	1.06^{b}	0.0546	-1.26
Ardisia	No	0.990^{bc}	0.159	-1.14
Carya	Yes	0.849^{bc}	0.0311	-1.45
Liquidambar	Yes	0.789^{bcd}	0.0451	-0.80
Schinus	No	0.724^{bcd}	0.0354	-0.38
Q. nigra	Yes	0.731^{bcd}	0.0673	-1.48
Magnolia	Yes	0.723^{bcd}	0.0853	-0.64
Typha	Yes	0.710^{bcd}	0.0708	2.37
Sabal	Yes	0.668^{bcd}	0.0449	-0.49
Q. geminata	Yes	0.655^{cde}	0.0333	0.56
Acer	Yes	0.647^{cde}	0.0357	-0.81
Taxodium	Yes	0.586^{cdef}	0.0684	-1.16
Imperata	No	0.531^{def}	0.0506	2.26
Q. chapmannii	Yes	0.404^{ef}	0.0167	-0.36
Pinus	Yes	0.401^{fg}	0.0165	1.51
Juncus	Yes	0.394^{fg}	0.0170	3.49
Q. laevis	Yes	0.388^{fg}	0.0242	-0.30
Aristida	No	0.387^{fg}	0.159	5.28

Table 4-3: Result of simple linear regression for log-transformed decomposition constants against individual variables of litter chemical composition. DF = 1,18. %NPE = non-polar fraction, %WS = water-soluble fraction, %AS = acid-soluble fraction.

Litter variable	Slope	r^2	*P*
%N	0.583	0.24	0.028
C:N	-0.0101	0.32	0.0088
%NPE	0.0173	0.45	0.0011
%WS	-0.0214	0.19	0.052
%AS	NS	NS	NS
%Lignin	-0.0305	0.34	0.0066
Lignin:N	-0.0193	0.47	0.0009

Table 4-4: Factor loadings for individual variables that contribute to litter quality on the leaf chemistry PCA axis 1. In general, high leaf chemistry score indicates low quality litter with low %N, high C:N, low%NPE, high %WS, high %lignin, and high lignin:N ratio.

Litter Quality Measure	Loading
%N	-0.353
C:N	0.431
%NPE	-0.425
%WS	0.442
%AS	-0.0490
%Lignin	0.282
Lignin:N	0.481

Table 4-5: Factor loadings of individual PLFAs upon the first two main axes of PCA (PC1 and PC2) and CCA (CC1 and CC2). Loadings > 0.3 in **bold**

Fatty Acid	PC 1	PC 2	CC 1	CC 2
Unclassified				
14:0	0.245	-0.100	-0.011	0.086
15:0	0.331	0.059	0.314	-0.118
16:0	0.114	-0.417	-0.153	-0.033
17:0	0.154	**-0.318**	-0.123	0.058
18:0	0.179	**-0.316**	-0.133	-0.060
i19:0	-0.033	-0.068	-0.182	0.512
20:1ω9c	0.006	-0.262	-0.267	-0.161
Gram +				
i15:0	0.348	0.191	0.536	-0.077
a15:0	0.280	-0.189	0.036	-0.124
i16:0	0.351	0.136	0.371	-0.023
Gram -				
16:1ω7c	0.300	-0.188	0.039	0.059
17:1ω8c	0.084	-0.337	-0.143	0.100
cy17:0	0.343	0.165	0.476	-0.145
18:1ω7c	**0.306**	0.211	**0.520**	0.080
cy19:0	**0.332**	0.224	**0.590**	-0.015
Fungi				
18:2ω6c	0.041	-0.422	-0.188	0.068
18:1ω9c	0.076	-0.025	0.050	0.033

Table 4-6: MANOVA summarizing the effects of time, litter species and litter species*time on PCA and CCA axes 1 and 2 scores from ordination of all samples simultaneously.

	PCA		CCA	
	F	P	F	P
Model	9.01	<0.0001	27.14	<0.0001
Time	25.89	<0.0001	194.55	<0.0001
Species	14.50	<0.0001	34.13	<0.0001
Sp*Time	4.73	<0.0001	3.97	<0.0001

Note: Model df = 29, 112; Species df = 9, 112; Time df = 2, 112; Species*time df = 18, 112.

Table 4-7: Loadings for the first two PCA axes for PCA run separately for individual sampling dates (t = 1, 2, and 5).

	T = 1		T = 2		T = 5	
Fatty Acid	PCA 1	PCA 2	PCA 1	PCA 2	PCA 1	PCA 2
Unclassified						
14:0	0.1307	**0.3481**	0.1745	0.2407	-0.1945	-0.0666
15:0	**0.3287**	0.0477	**0.3158**	-0.0603	-0.1978	**-0.3765**
16:0	**0.3605**	0.1458	**0.3440**	0.2246	-0.2842	-0.2803
17:0	0.2457	-0.2206	**0.3597**	-0.0408	-0.2059	**-0.3638**
18:0	0.2188	0.0340	**0.3091**	0.1865	-0.2990	-0.1028
i19:0	0.1531	**0.3016**	NA	NA	NA	NA
20:1ω9c	0.1751	-0.0434	0.1597	**0.3788**	-0.1396	-.0899
Gram +						
i15:0	0.1022	**-0.4039**	0.1825	**-0.4010**	**-0.3037**	0.1943
a15:0	0.2567	**-0.3499**	0.1795	**-0.4548**	-0.2692	0.2642
i16:0	0.1533	**-0.4933**	0.1843	**-0.4157**	-0.2742	0.2461
Gram -						
16:1ω7c	**0.3741**	-0.0441	**0.3399**	-0.1433	**-0.2952**	0.2216
17:1ω8c	**0.3617**	-0.0086	**0.3184**	0.0161	-0.1413	-.1089
cy17:0	0.0299	-0.0451	0.1439	0.1822	-0.2756	0.2736
18:1ω7c	0.1860	0.1305	0.1439	0.1822	-0.3068	0.1148
cy19:0	-0.0550	0.1330	-0.0326	-0.1557	-0.3001	0.1010
Fungi						
18:2ω6c	**0.3870**	0.1301	**0.3694**	-0.0044	-0.0847	**-.4984**
18:1ω9c	0.1536	**0.3576**	-0.0623	0.0443	-0.2768	-.2205

Table 4-8: Correlation between each litter quality variable and the amount of PLFA (nmol) indicating monounsaturated Gram – bacteria at times 1, 2, and 5. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$. %NPE = non-polar fraction, %WS = water-soluble fraction, %AS = acid-soluble fraction, %IMR = initial mass remaining, %moisture on a dry mass basis.

	T = 1		T = 2		T = 5	
	Direction	r^2	Direction	r^2	Direction	r^2
% N	+	0.18**	NS	–	+	0.41***
C:N	-	0.23**	NS	–	-	0.40***
%NPE	NS	–	NS	–	+	0.10*
%WS	NS	–	NS	–	-	0.09*
%AS	NS	–	NS	–	-	0.26***
%lignin	NS	–	NS	–	+	0.19**
Lignin:N	-	0.29***	NS	–	-	0.12**
%IMR	NS	–	NS	–	-	0.45***
%moisture	NS	–	NS	–	+	0.09*

Table 4-9: Correlation between each litter quality variable and the amount of PLFA (nmol) indicating branched Gram+ fatty acids at times 1, 2, and 5. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$. %NPE = non-polar fraction, %WS = water-soluble fraction, %AS = acid-soluble fraction, %IMR = initial mass remaining, %moisture on a dry mass basis.

	T = 1		T = 2		T = 5	
	Direction	r^2	Direction	r^2	Direction	r^2
% N	+	0.16**	NS	–	+	0.40***
C:N	NS	–	NS	–	-	0.41***
%NPE	NS	–	NS	–	NS	–
%WS	+	0.12**	+	0.17**	-	0.08*
%AS	NS	–	+	0.05*	-	0.18**
%lignin	NS	–	-	0.13**	+	0.19**
Lignin:N	-	0.10*	NS	–	-	0.06
%IMR	-	0.16**	NS	–	-	0.33***
%moisture	NS	–	NS	–	+	0.11**

Table 4-10: Correlation between each litter quality variable and the amount of PLFA (nmol) indicating fungal fatty acid (18:2ω6c) at times 1, 2, and 5. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$. %NPE = non-polar fraction, %WS = water-soluble fraction, %AS = acid-soluble fraction, %IMR = initial mass remaining, %moisture on a dry mass basis.

	T = 1		T = 2		T = 5	
	Direction	r^2	Direction	r^2	Direction	r^2
% N	+	0.09*	+	0.47***	NS	
C:N	-	0.10*	-	0.45***	NS	
%NPE	NS	–	+	0.31***	NS	
%WS	NS		-	0.20**	NS	
%AS	NS		-	0.32***	NS	
%lignin	NS		NS		NS	
Lignin:N	-	0.12**	-	0.25***	NS	
%IMR	-		-	0.43***	NS	
%moisture	NS	0.06*	NS		-	0.29***

Table 4-11: Correlation between each litter quality variable and ratio fungal : bacterial PLFAs at times 1, 2, and 5. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$. %NPE = non-polar fraction, %WS = water-soluble fraction, %AS = acid-soluble fraction, %IMR = initial mass remaining, %moisture on a dry mass basis

	T = 1		T = 2		T = 5	
	Direction	r^2	Direction	r^2	Direction	r^2
% N	NS		NS		-	0.41***
C:N	+	0.11*	NS		+	0.32***
%NPE	NS		+	0.15**	NS	
%WS	NS		-	0.21**	NS	
%AS	NS		-	0.11*	+	0.10*
%lignin	NS		+	0.05	NS	
Lignin:N	+	0.11*	+	0.05	+	0.19**
%IMR	+	0.07*	NS		+	0.37***
%moisture	NS		NS		-	0.27***

Table 4-12: Correlation between each litter quality variable and the total PLFA (nmol) at times 1, 2, and 5. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$. %NPE = non-polar fraction, %WS = water-soluble fraction, %AS = acid-soluble fraction, %IMR = initial mass remaining, %moisture on a dry mass basis.

	T = 1		T = 2		T = 5	
	Direction	r^2	Direction	r^2	Direction	r^2
% N	+	0.15**	+	0.41***	+	0.15**
C:N	-	0.08*	-	0.39***	-	0.14**
%NPE	NS		+	0.25***	+	0.21**
%WS	NS		-	0.26***	-	0.20**
%AS	NS		-	0.29***	-	0.17**
%lignin	NS		NS		+	0.08*
Lignin:N	NS		-	0.11**	NS	
%IMR	NS		-	0.27***	-	0.17**
%moisture	NS		NS		NS	

Table 4-13: Correlation between each litter quality variable and the amount of PLFA (nmol) indicating cyclopropyl Gram- bacteria at time 5. Only t = 5 is presented as the large number of zeros at other time points did not allow for parametric statistics. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$. %NPE = non-polar fraction, %WS = water-soluble fraction, %AS = acid-soluble fraction, %IMR = initial mass remaining, %moisture on a dry mass basis

	Direction	r^2
% N	+	0.26***
C:N	-	0.18**
%NPE	+	0.05
%WS	NS	
%AS	-	0.23**
%lignin	+	0.07*
Lignin:N	NS	
%IMR	-	0.28***
%moisture	+	0.8*

Table 4-14: Coefficient of determination (r^2) of abundance of PLFAs for microbial functional groups with leaf chemistry scores at times 1, 2, and 5. Df time 2 and 5 = 1,8; df time 1 = 1, 7. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$

	Time = 1	Time = 2	Time = 5
Total PLFA	NS	0.54**	0.62**
Fungal:Bacterial Ratio	NS	NS	0.49**
Fungi	NS	0.53**	NS
Gram+	NS	NS	0.77**
Branched Gram-	NS	NS	0.70**
Cyclopropyl Gram-	NS	NS	0.60**

Note: All significant relationships were negative correlations except for fungal:bacterial ratio which was positive.

Table 4-15: Coefficient of determination (r^2) of correlations of microbial community PCA axes (independent) vs.decomposition rate (k) (dependant), and leaf chemistry axis (from ordination of initial litter quality) (independent) vs. microbial community PCA axes (dependent). All relationships were significant at $p < 0.05$.

	T=1 PCA_1	T=2 PCA_1	T=5 PCA_1	All PCA_1	All PCA_2
K	0.53	0.62	0.76	0.76	0.58
Litter chemistry	0.44	0.52	0.88	0.65	0.53

Note: Microbial PCA vs. k df = 1,9 (except T=1 PCA, df = 1,8); litter quality vs. PCA df = 1,90 (except T=1 PCA, df = 1,8).

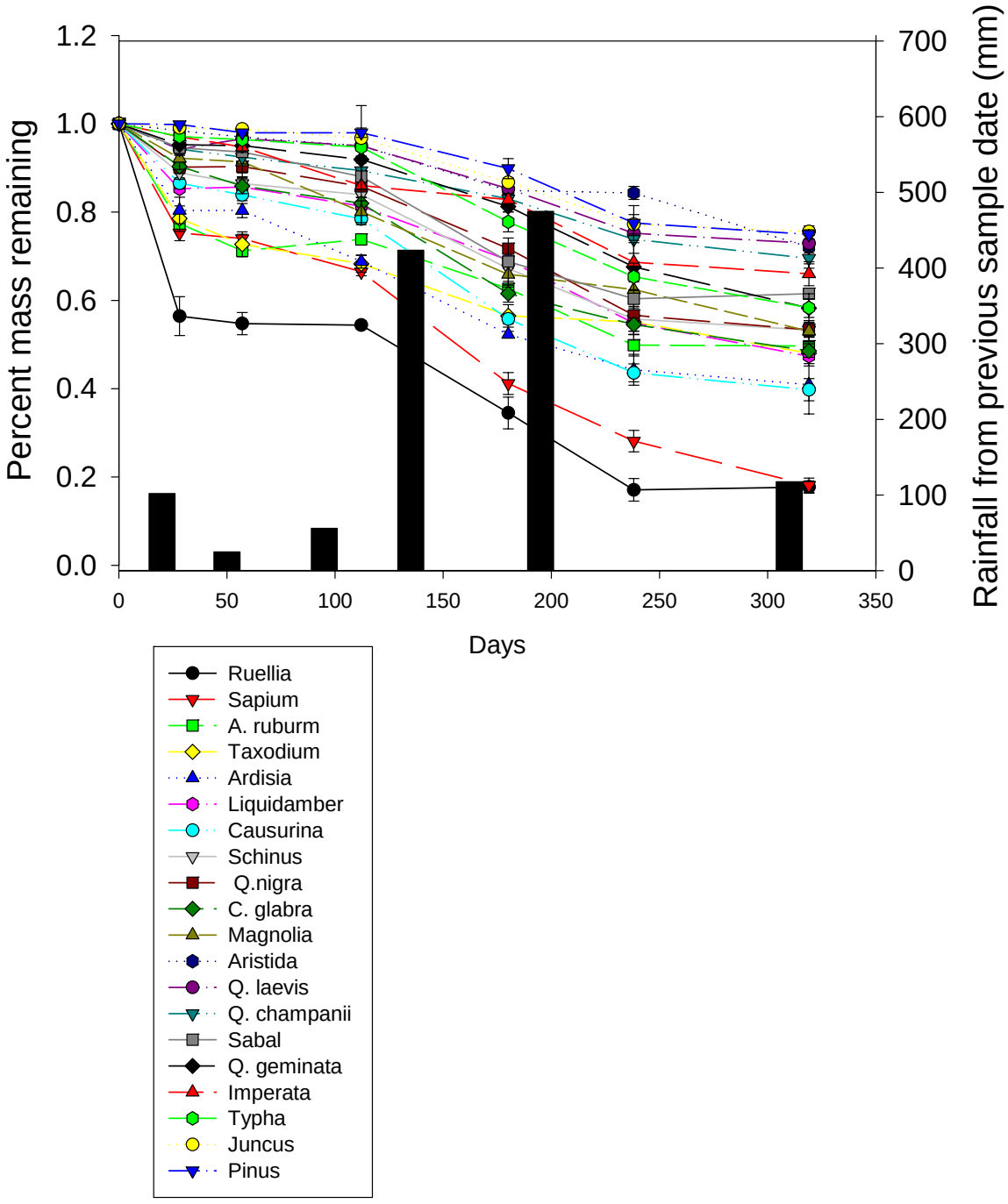


Figure 4-1: Mean ± SE of the percent mass remaining of 20 species at six collection dates. Species are shown in order of decreasing mass loss at the first collection date. Total rainfalls between sample collections are also displayed as bars.

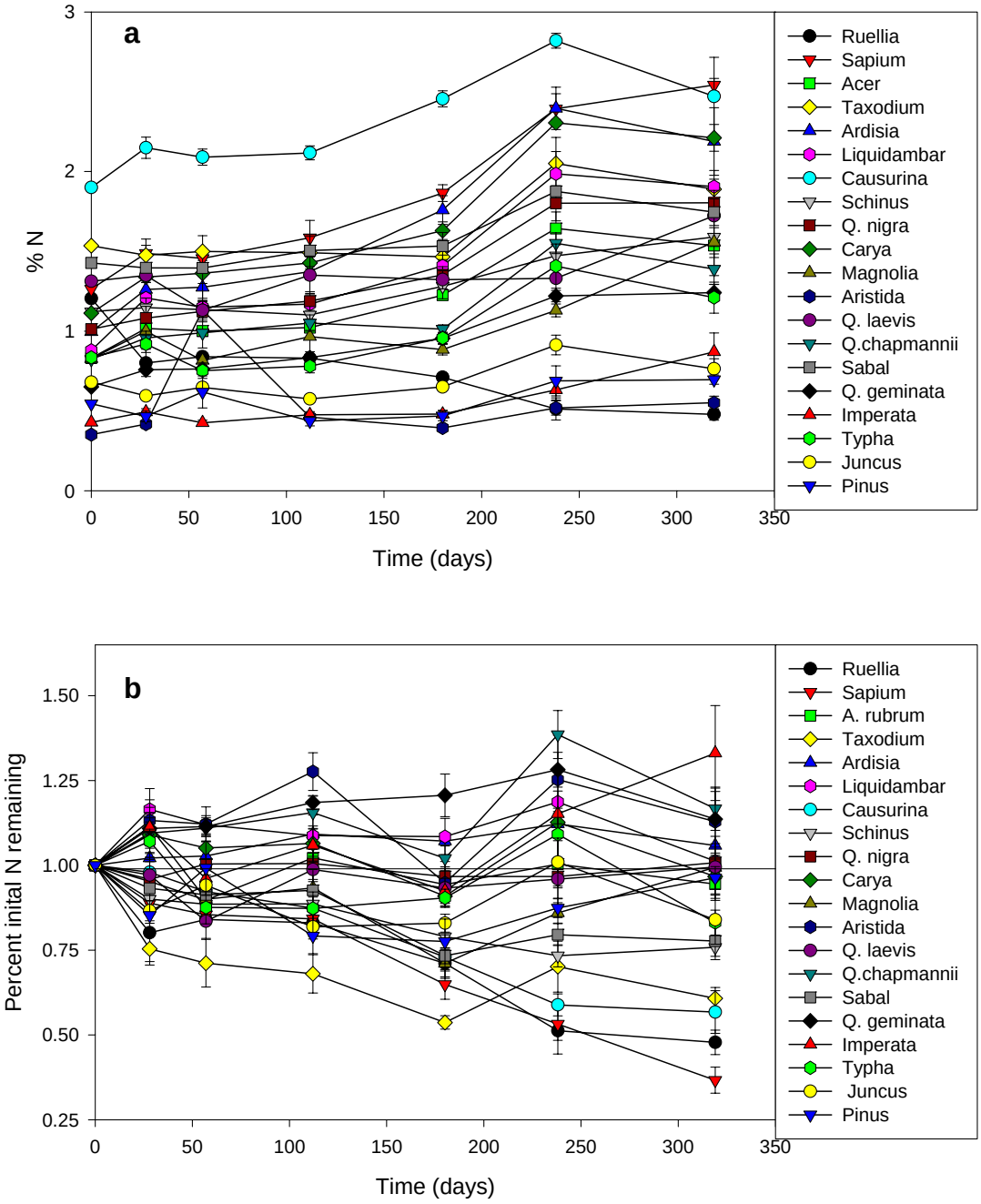


Figure 4-2: Mean ± SE for nitrogen in litter of 20 plant species at six collection dates. (a) nitrogen concentration, and (b) percent initial nitrogen remaining Species are shown in order of decreasing mass loss at the first collection date.

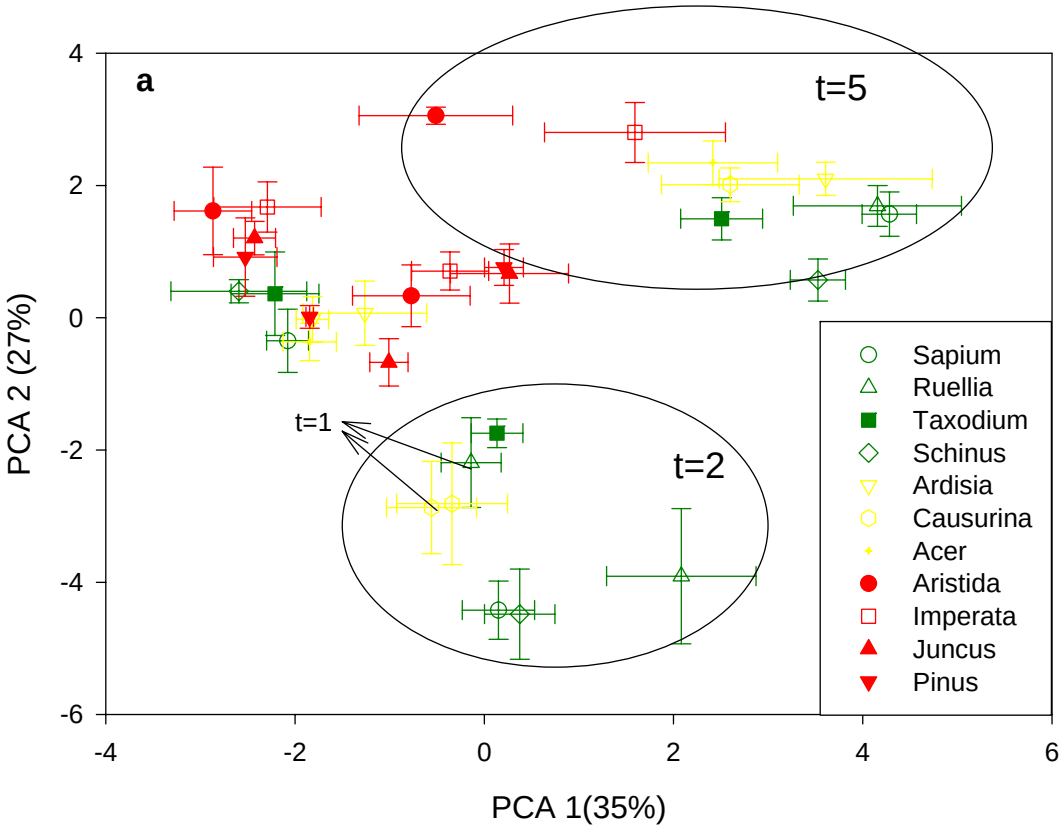


Figure 4-3 Ordinations of the 17 most common microbial PLFA found on 11 litter species from three sampling dates, showing mean values for five replicates per litter species per sampling date.: a) Principal Component Analysis (PCA). b) Canonical Correspondence Analysis including nine litter quality measures as a secondary matrix. Biplots for litter quality measures with an $r^2 \geq 0.3$ with at least one of the two canonical axes displayed. Open symbols signify non-native species, solid symbols signify native species. Litter species are presented in increasing leaf chemistry axis scores, with the 4 lowest scores representing high litter quality in green, middle litter quality in yellow, and the 4 highest scores representing low litter quality in red. Ellipses are drawn by hand to indicate groups of points from the same collection date. The large cloud of symbols not encompassed in an ellipse in (a) are predominantly litter species with low litter quality and tend to move to the right on PCA 1 over time as indicated by the arrows.

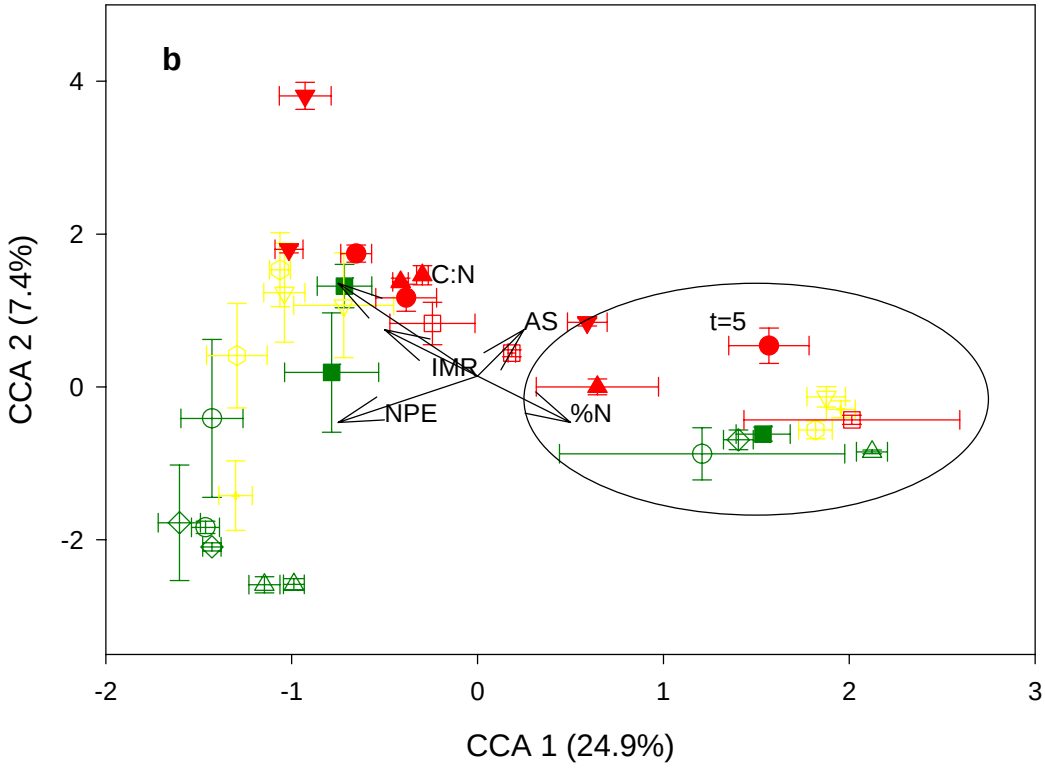


Figure 4-3. Continued.

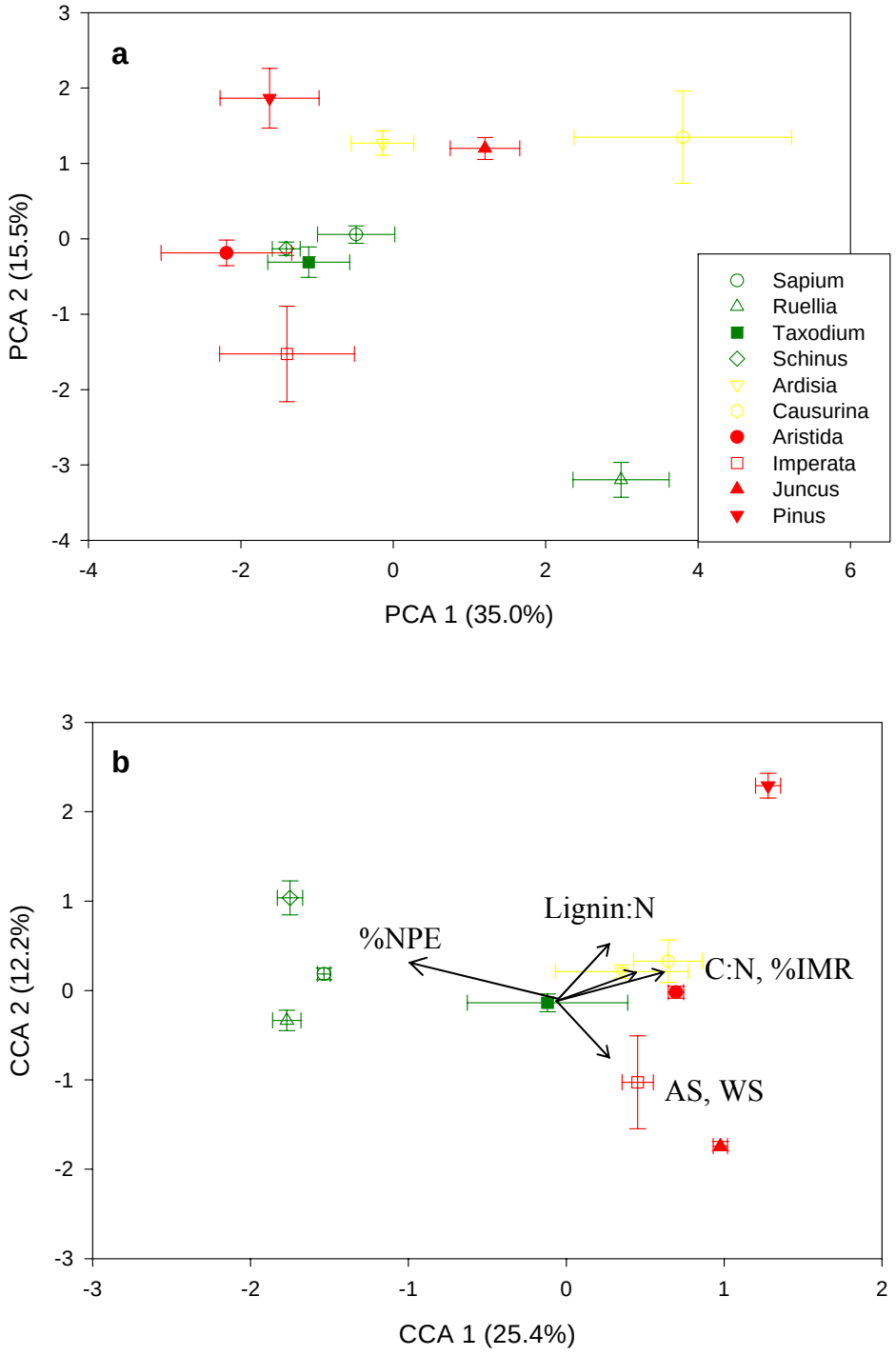


Figure 4-4: PCA and CCA analyses for 17 most common microbial PLFA and litter quality variables run separately for individual sampling dates. A) 28 day PCA, b) 28 day CCA, c) 57 day PCA, d) 57 day CCA, e) 238 day PCA, f) 238 CCA. Only litter quality measures with $r^2 \geq 0.3$ with one of the two canonical axes are displayed. See Figure 3 for symbol descriptions.

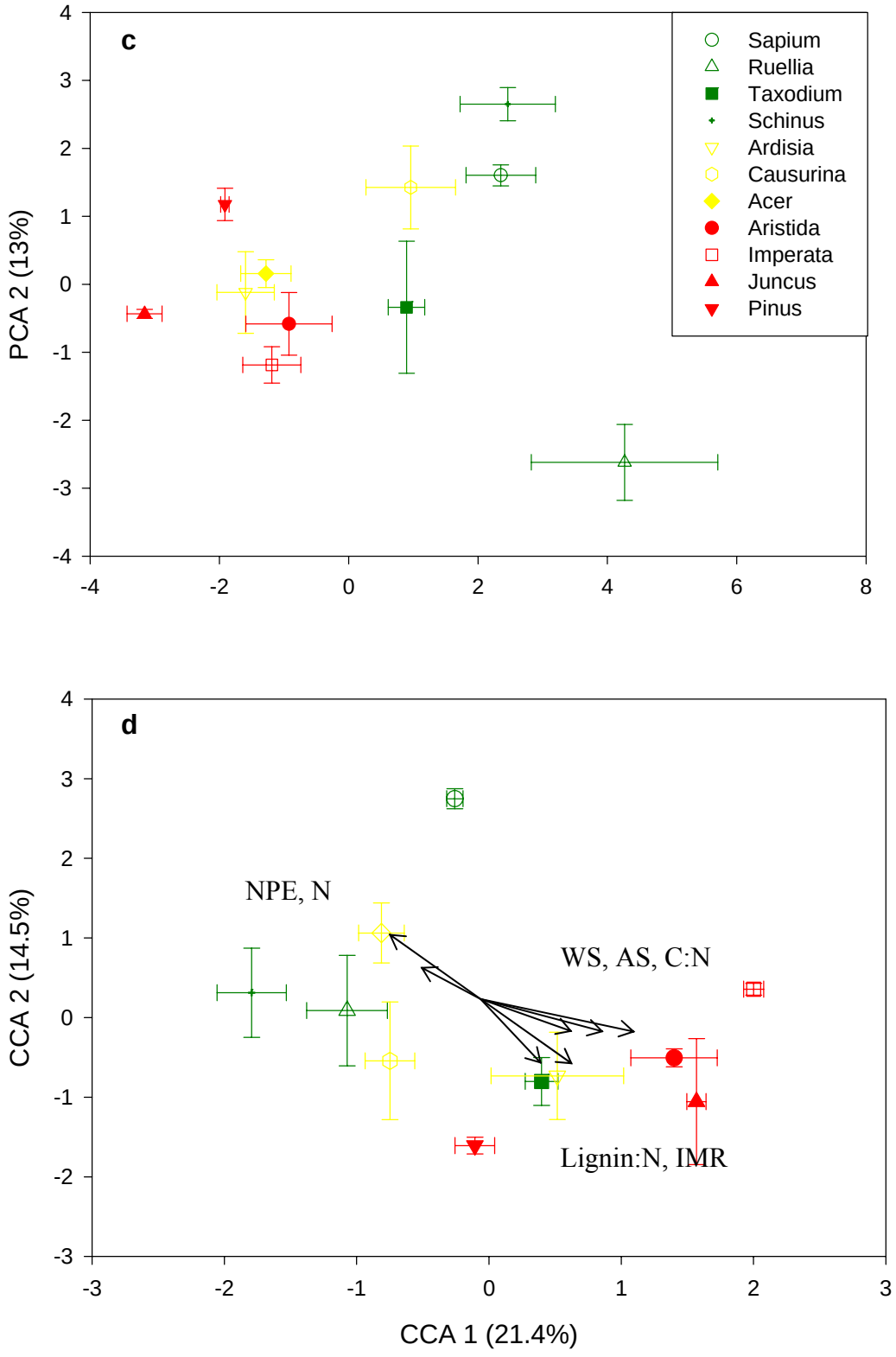


Figure 4-4. Continued.

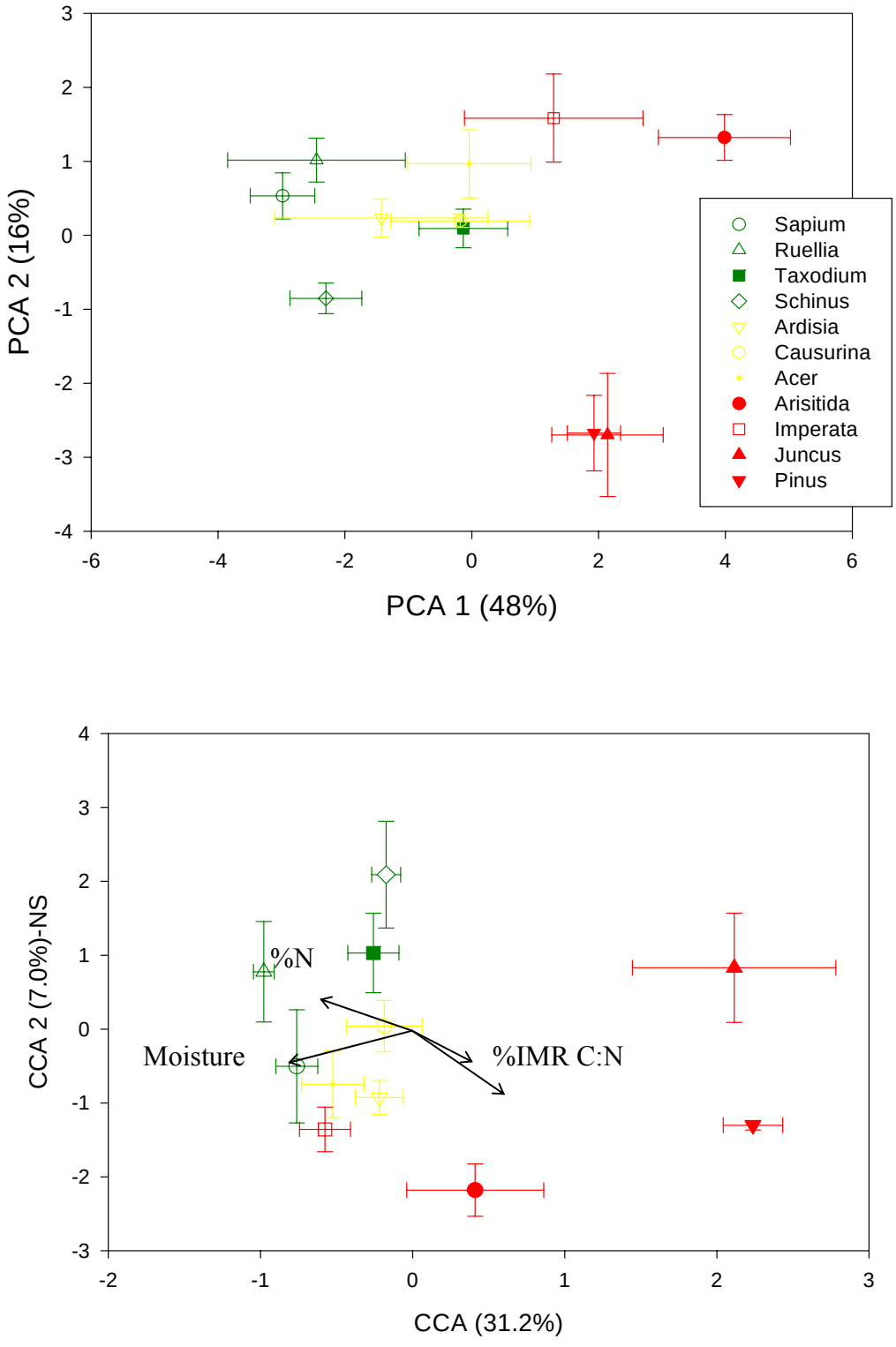


Figure 4-4. Continued.

CHAPTER 5
CONCLUSIONS

The goal of this dissertation was to evaluate the role of plant species composition in the interaction between plants and soil microbes using invasive plants as a natural experiment. I examined three primary interactions: native mycorrhizal fungi with a non-native plant, variation in free-living soil microbes in five different habitats with and without non-native plant invaders, and the links between plant litter chemical composition, microbial community composition and decomposition of plant litter.

In Chapter 2, I examined the effect of native mycorrhizae on the growth, physiology and competitive ability of an invasive shrub, *Ardisia crenta*. I hypothesized that *Ardisia* may be a successful invader because it has been able to find a beneficial fungal partner that improves its competitive ability for phosphorus. I found that *Ardisia* had the highest growth rate and photosynthetic rate when grown with the fungi with which it associates in an invaded habitat. *Ardisia*'s growth appears to be increased due to higher photosynthetic rates and a greater allocation to aboveground biomass, particularly in leaf area ratio (LAR). In a competitive environment, however, neither identity of the competitor nor mycorrhizal status had an effect on the growth rate of *Ardisia*. The absence of mycorrhizae in heterospecific competition did decrease the growth of *Prunus*. While *Ardisia* benefits from local mycorrhizal fungi, removal or disturbance of mycorrhizal fungi may be of greater detriment to natives than to *Ardisia*.

Further work with the *Ardisia* system could clarify the role of mycorrhizal fungi in mediating plant invasion. It would be useful to determine which species of fungi are

present in the host-associated inoculum and how their composition and density compare to non-invaded areas. As my results showed that the growth response of *Ardisia* is dependent upon the identity of the fungus, further growth experiments with different plant and fungal species may also be needed. For example, the results of the *Ardisia* growth and competition experiments may have been very different if fungi isolated from *Prunus*' roots were used in these experiments.

My second objective was to determine if non-native plants could alter the composition of free-living microbes (Chapter 3). The largest differences seen in microbial community structure, as measured by PLFA, and function, as measured by Biolog substrate utilization, were between habitat types. The two southern most communities, those prone to *Melaleuca* and *Schinus* invasion, were most similar to one another in comparison to the other habitats examined. Total PLFA and fungal : bacterial ratio were highly correlated with moisture content of the soil indicating that moisture content and oxygen availability may be a major control of microbial community composition. While I found that variation among microbial communities was greater between habitats than within them, there was a significant overall effect of invasion on microbial community structure. There was no effect of invasion on substrate utilization, however, suggesting that although invasion may result in a change in microbes present, due to redundancy of metabolic ability, microbial community function may not be altered by invasion.

Conclusions made from this study, however, are limited as it is only a single snapshot in time. It may be possible that at different times of year (e.g., during the dry season) similarities in soil moisture contents may cause a convergence in composition of

microbial communities across habitats. Conversely, the larger differences in soil temperature during the dry season may lead to greater differentiation of microbial communities according to latitude. Additionally, although I attribute much of the differences between microbial communities to differences in soil moisture, several environmental characteristics were not measured and may be responsible for differences in microbial community composition. A more thorough examination of environmental variables in situ or experimental alteration of these variables would lead to a better understanding of controls on microbial community composition

Finally, in Chapter 4, I explicitly examined the role of litter quality in microbial community composition. The composition of microbial communities on eleven species of native and invasive plant litter of varying quality was followed over decomposition. I also examined the relationship between the composition of the microbial communities and decomposition. I hypothesized that plant litters with similar litter quality would support similar microbial communities. This proved to be true. Whether the plant litter was native or invasive did not seem to affect the composition of the microbial community. Microbial community composition differed most among sample dates, with microbial communities of 28 and 57 days clustering together in ordination of PLFAs separate from microbial communities at 238 days. These large differences in microbial communities at early and late sample dates could be a result of microbial succession, but may also be due to moisture limitation at early sample dates.

The most novel result of this study, however, was the linking of microbial community composition to decomposition rate. I examined the correlation between seven measures of leaf chemistry, an integrated measure of leaf chemistry, microbial

community composition (the first axis from principle components ordination of PLFA data), and decomposition rate constants. Of all these relationships examined, microbial community composition was the best predictor of decomposition rate with a correlation of 76%. This result is very compelling evidence that opening the "black box" of the soil is not only of interest to microbial ecologists, but has broader implications

These results suggest many more studies. Although one of the general goals for this dissertation was to examine interactions between invasive species and microbes, this study least explicitly examined this interaction. Better pairing of native and invasive plants with respect to dominance and functional groups would place the results in better context to evaluate the impact of invasion. Additionally, many of the species used in this study are not found in hammock communities. A more realistic study would be to examine the decomposition and microbial succession of native and invasive litter pairs in invaded and non-invaded areas in which they would normally be found. Finally, the relationship found between litter quality, microbial composition and decomposition rate in this study is correlative not causal. Microcosms initiated with different starting microbial communities subjected to litter of low, moderate, and high quality might help to determine if microbes merely respond to the substrate available or are actually drivers of decomposition.

Taken together, these studies have shown that invasive plants can benefit from specific native mycorrhizal fungi, can alter microbial communities on a landscape level and may result in different decomposer microbes and decomposition rates if the litter quality of the invader is much different than native litter quality. These results suggest that plant-microbial could be an important aspect in the success of non-native plant.

Further studies should explore the potential for positive feedbacks on plant invasion through alterations of the soil microbial community by invaders.

LIST OF REFERENCES

Alden, L., F. Demoling, and E. Baath. 2001. Rapid method of determining factors limiting microbial growth in soil. Applied and Environmental Microbiology **67**:1830-1838.

Allison, S. D., and P. M. Vitousek. 2004. Rapid nutrient cycling in leaf litter from invasive plants in Hawai'i. Oecologia **141**:612-519.

Allsopp, N., and W. D. Stock. 1992. Mycorrhizas, seed size and seedling establishment in low nutrient environment. Pages 59-64 *in* D. J. Read, D. H. Lewis, A. Fitter, and I.J. Alexander, editors. Mycorrhizas in Ecosystems. CAB International, Wallingford, UK.

Atlas, R. M., and R. Bartha. 1998. Microbial Ecology: Fundamentals and Applications. Benjamin/Cummings, Redwood City, CA.

Bailey, P. C. E., S. C. Watkins, K. L. Morris, and P. I. Boon. 2003. Do Melaleuca ericifolia Sm. leaves suppress organic matter decay in freshwater wetlands? Archiv Fur Hydrobiologie **156**:225-240.

Bardgett, R. D., and E. McAlister. 1999. The measurement of soil fungal:bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands. Biology and Fertility of Soils **29**:282-290.

Bardgett, R. D., and L. R. Walker. 2004. Impact of coloniser plant species on the development of decomposer microbial communities following deglaciation. Soil Biology & Biochemistry **36**:555-559.

Bardgett, R. D., J. L. Mawdsley, S. Edwards, P. J. Hobbs, J. S. Rodwell, and W. J. Davies. 1999. Plant species and nitrogen effects of soil biological properties of temperate upland grasslands. Functional Ecology **13**:650-660.

Berg, B., and C. O. Taum. 1991. Decomposition and nutrient dynamics of litter in long-term optimum nutrition experiments. Scandinavian Journal of Forest Research **6**:305-321.

Berg, B., K. Hannus, T. Popoff, and O. Theander. 1982. Changes in organic-chemical components during decomposition. Long-term decomposition in a Scots pine forest I. Canadian Journal of Botany **60**:1310-1319.

Berta, G., A. Trotta, A. Fusconi, J. E. Hooker, M. Munro, D. Atkinson, M. Giovannetti, S. Morini, P. Fortuna, B. Tisserant, V. Gianinazzipearson, and S. Gianinazzi. 1995. Arbuscular mycorrhizal induced changes to plant growth and root-system morphology in *Prunus cerasifera*. Tree Physiology **15**:281-293.

Bethlenfalvay, G., M. Brown, and R. Pascovsky. 1982. Parasitic and mutualistic associations betwen a mycorhizal fungus and soybean: development of the host plant. Phytopathology **72**:889-893.

Bever, J. D. 1994. Feedback between plants and their soil communities in an old field community. Ecology **75**:1965-1977.

Bever, J. D. 2002. Negative feedback within a mutualism: host-specific growth of mycorrhizal fungi reduces plant benefit. Proceedings of the Royal Society of London Series B-Biological Sciences **269**:2595-2601.

Bever, J. D., J. B. Morton, J. Antonovics, and P. A. Schultz. 1996. Host-dependant sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. Journal of Ecology **84**:71-82.

Bever, J. D., K. M. Westover, and J. Antonovics. 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. Journal of Ecology **85**:561-573.

Blossey, B., and R. Notzold. 1995. Evolution of increased competitive ability in invasive nonindigenous plants–a hypothesis. Journal of Ecology **83**:887-889.

Boerner, R. E. J. 1990. Role of mycorrhizal fungus origin in growth and nutrient-uptake by *Geranium robertianum*. American Journal of Botany **77**:483-489.

Boon, P. I., and L. Johnstone. 1997. Organic matter decay in coastal wetlands: An inhibitory role for essential oil from *Melaleuca alternifolia* leaves? Archiv Fur Hydrobiologie **138**:433-449.

Bossio, D. A., and K. M. Scow. 1995. Impact of carbon and flooding on the metabolic diversity of microbial communities in soils. Applied Environmental Microbiology **61**:4043-4050.

Bossio, D. A., and K. M. Scow. 1998. Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. Microbial Ecology **35**:265-278.

Bossio, D. A., K. M. Scow, N. Gunapala, and K. J. Graham. 1998. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. Microbial Ecology **36**:1-12.

Brown, J. C. 1958. Soil fungi of some British sand dunes in relation to soil type and succession. Journal of Ecology **46**:641-664.

Buyer, J. S., and L. E. Drinkwater. 1997. Comparison of substrate utilization assay and fatty acid analysis of soil microbial communities. Journal of Microbiological Methods **30**:3-11.

Callaway, R. M., G. C. Thelen, A. Rodriguez, and W. E. Holben. 2004. Soil biota and exotic plant invasion. Nature **427**:731-733.

Cameron, G., and S. Spencer. 1989. Rapid leaf decay and nutrient release in a Chinese Tallow forest. Oecologia **80**:222-228.

Chapin, F. S. 1980. The mineral nutrition of plants. Annual Review of Ecology and Systematics **11**:233-260.

Chapin, F. S. I., and R. Aerts. 2000. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Advances in Ecological Research **30**:1-67.

Chapin, F. S. I., H. Reynolds, C. M. D'Antonio, and V. Eckhart. 1996. The functional role of species in terrestrial ecosystems. Pages 403-430 in B. Walker and W. Steffan, editors. Global Change in Terrestrial Ecosystems. Cambridge University Press, Cambridge.

Coleman, D. C. 1985. Through a ped darkly: an ecological assessment of root-soil-microbial-faunal interactions. Pages 1-21 *in* A. Fitter, D. Atkinson, D. J. Read, and M. B. Usher, editors. Ecological Interactions in Soil: Plants, Microbes and Animals. Blackwell, Oxford.

Coulson, J. C., and J. Butterfield. 1978. An investigation of the biotic factors determining the rates of plant decomposition on blanket bog. Journal of Ecology **66**:631-650.

D'Antonio, C. M., T. L. Dudley, and M. C. Mack. 1999. Disturbance and biological invasions: direct effects and feedbacks. Pages 429-468 in L. R. Walker, editor. Ecosystems of Disturbed Ground. Elsevier, Amsterdam.

Dowling, N. J. E., F. Widdle, and D. C. White. 1986. Phospholipid ester-linked fatty acids biomarkers of acetate-oxidizing sulphate-reducers and other sulphide-forming bacteria. Journal of General Microbiology **132**:1815-1825.

Dozier, H. 1999. Plant introductions and invasion: history, public awareness, and the case of *Ardisia crenata*. Ph.D. University of Florida, Gainesville.

Ehrenfeld, J. G. 2003. Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems **6**:503-523.

Eiland, F., M. Klamer, A. M. Lind, M. Leth, and E. Baath. 2001. Influence of initial C/N ratio on chemical and microbial composition during long-term composting of straw. Microbial Ecology **41**:272-280.

Ekbald, A., and A. Nordgren. 2002. Is growth of soil microorganism in boreal forest limited by carbon or nitrogen availability? Plant and Soil **242**:115-122.

Findlay, R. H., and F. C. Dobbs. 1993. Quantitative description of microbial communities using lipid analysis. Pages 777 in P. F. Kemp, editor. Handbook of Methods in Aquatic Microbial Ecology. Lewis Publishers, Boca Raton.

Flanagan, P. W., and K. van Cleve. 1983. Nutrient cycling in relation to decomposition and organic matter quality in taiga ecosystems. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **13**:795-817.

Florida Exotic Pest Plant Council. 2005. List of Florida's Invasive Species. Florida Exotic Pest Plant Council. Internet: http://www.fleppc.org/list05web.pdf. June, 2005.

Fontanet, X.,V. Estaun,A. Camprubi and C. Calvet. 1998. Fungicides added to potting substrate affect mycorrhizal symbiosis between a peach-almond rootstock and Glomus sp. Hortscience **33:** 1217-1219.

Frostegard, A., and E. Baath. 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils **22**:59-65.

Frostegard, A., E. Baath, and A. Tunlid. 1993. Shifts in the structure of soil microbial communiteis in limed forests as revealed by phospholipid fatty acid analysis. Soil Biology & Biochemistry **25**:723-730.

Gallo, M., R. Amonette, C. Lauber, R. L. Sinsabaugh, and D. R. Zak. 2004. Microbial community structure and oxidative enzyme activity in nitrogen-amended north temperate forest soils. Microbial Ecology **48**:218-229.

Gange, A. C. and R. L. Ayres. 1999. On the relation between arbuscular mycorrhizal colonization and plant "benefit." Oikos **87:** 615-621.

Gange, A., D. Lindsay, and L. Ellis. 1999. Can arbuscular mycorrhizal fungi be used to control the undesirable grass *Poa annua* on golf courses? Journal of Applied Ecology **36**:909-919.

Garland, J. L. 1996. Patterns of potential C source utilization by rhizophere communities. Soil Biology & Biochemistry **28**:223-230.

Garrett, S. D. 1963. Soil Fungi and Soil Fertility. Pergamon Press, Oxford.

Gavito, M. E., P. S. Curtis, T. N. Mikkelsen, and I. Jakobsen. 2000. Atmospheric CO2 and mycorrhiza effects on biomass allocation and nutrient uptake of nodulated pea (*Pisum sativum* L.) plants. Journal of Experimental Botany **51**:1931-1938.

Gordon, D. R. 1998. Effects of invasive, non-indigenous plant species on ecosystem processes: lessons from Florida. Ecological Applications **8**:975-989.

Gorlenko, M. V., and P. A. Kozhevin. 1994. Differentiation of soil microbial communites by multivariate testing. Microbiology **63**:158-161.

Graham, J., and D. Eissenstat. 1998. Field evidence for the carbon cost of citrus mycorrhizas. New Phytologist **140**:103-110.

Graham, J., D. Drouillard, and N. Hodge. 1996. Carbon economy of sour orange in response to different *Glomus* spp. Tree Physiology **16**:1023-1029.

Graham, J., L. Duncan, and D. Eissenstat. 1997. Carbohydrate allocation patterns in citrus genotypes as affected by phosphorus nutrition, mycorrhizal colonization and mycorrhizal dependency. New Phytologist **135**:767-776.

Grayston, S. J., D. Vaughan, and D. Jones. 1996. Rhizosphere carbon flow in trees, in comparision with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Applied Soil Ecology **5**:29-56.

Grayston, S. J., S. Wang, C. D. Campbell, and A. C. Edwards. 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biology and Biochemistry **30**:369-378.

Greenway, M. 1994. Litter accession and accumulation in Melaleuca quinquenervia (Cav.) S.T. Blake wetland in Southeastern Queensland. Australian Journal of Marine and Freshwater Research **45**.

Griffin, D. M. 1985. A comparison of the roles of bacteria and fungi. Pages 221-255 *in* E. R. Leadbetter and J. S. Poindexter, editors. Bacteria in Nature: Bacterial Activities in Perspective. Plenum Press, New York.

Griffiths, B. S., K. Ritz, N. Ebblewhite, and G. Dobson. 1999. Soil microbial community structure: effects of substrate loading rates. Soil Biology & Biochemistry **31**:145-153.

Grime, J. P., J. H. C. Cornelissen, K. Thompson, and J. G. Hodgson. 1996. Evidence of a causal connection between anti-herbivore defense and the decomposition rate of leaves. Oikos **77**:489-494.

Guckert, J. B., C. P. Antworth, P. D. Nichols, and D. C. White. 1985. Phospholipid, ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. FEMS Microbiology Ecology **31**:147-158.

Hart, S. C., and J. M. Stark. 1997. Nitrogen limitation of the microbial biomass in an old-growth forest. Ecoscience **4**:91-98.

Hartnett, D. C., B. A. D. Hetrick, G. W. T. Wilson, and D. J. Gibson. 1993. Mycorrhizal influence on intraspecific and interspecific neighbor interactions among cooccurring prairie grasses. Journal of Ecology **81**:787-795.

Harwood, D. L., and N. J. Russell. 1984. Lipids in plants and microbes. George Allen and Unwin Ltd., London.

Haynes, R. J. 1986. The decomposition process: mineralization, immobilization, humus formation, and degredation. Pages 52-126 in R. J. Haynes, editor. Mineral Nitrogen in the Plant-Soil System. Academic Press, Orlando.

Hill, G. T., N. A. Mitkowski, L. Aldrich-Wolfe, L. R. Emele, D. D. Jurkonie, A. Ficke, S. Maldonado-Ramirez, S. T. Lynch, and E. B. Nelson. 2000. Methods for assessing the composition and diversity of soil microbial communities. Applied Soil Ecology **15**:25-36.

Hobbie, S. E. 1996. Temperature and plant spcies control over litter decomposition in Alaskan tundra. Ecological Monographs **66**:503-522.

Hudson, H. J. 1968. The ecology of fungi on plant remains above the soil. New Phytologist **67**:837-874.

Jakobsen, I. 1991. Carbon metabolism in mycorrhiza. Methods in Microbiology **23**:149-180.

Janos, D. P. 1980. Vesicular-arbuscular mycorrhizae affect lowland tropical rain forest plant growth. Ecology **61**:151-162.

Jenny, H. 1941. Factors of Soil Formation. McGraw-Hill, New York.

Johnson, N. C. 1993. Can fertilization of soil select less mutualistic mycorrhizae? Ecological Applications **3:** 749-757.

Johnson, N. C., J. Graham, and F. Smith. 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phytologist 135: 575-586.

Kahiluoto, H., E. Ketoja, and M. Vestberg. 2000. Creation of a nonmycorrhizal control for a bioassay of AM effectiveness. 1. Comparison of methods. Mycorrhiza **9**:241-258.

Katajisto, J., V. Huhta, and J. Laakso. 1999. Plant effects on the soil community: A microcosm experiment. European Journal of Soil Biology **35**:17-21.

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

Kourtev, P. S., J. G. Ehrenfeld, and M. Haggblom. 2003. Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. Soil Biology & Biochemistry **35**:895-905.

Laroche, F. B. 1994. Melaleuca Management Plan for the State of Florida. Florida Exotic Pest Plant Council, West Palm Beach.

Leckie, S. E., C. E. Prescott, S. J. Grayston, J. D. Neufeld, and W. W. Mohn. 2004. Characterization of humus microbial communities in adjacent forest types that differ in nitrogen availability. Microbial Ecology **48**:29-40.

Lovelock, C. E., D. Kyllo, and K. Winter. 1996. Growth responses to vesicular-arbuscular mycorrhizae and elevated CO2 in seedlings of a tropical tree, *Beilschmiedia pendula*. Functional Ecology **10**:662-667.

Mack, M. C., and C. M. D'Antonio. 2003. Exotic grasses alter controls over soil nitrogen dynamics in a Hawaiian woodland. Ecological Applications **13**:154-166.

Mahendra K.J., Y. B., Rogers J.M., Smith A.E., Boger E.T.A., Ostrander R.L., Rheingold A.L. 1995. Specific competitive inhibitor of secreted phospholipase A2 from berries of Schinus terebinthifolius. Phytochemistry **39**:537–547.

Marler, M. J., C. A. Zabinski, and R. M. Callaway. 1999. Mycorrhizae indirectly enhance competitive effects of an invasive forb on a native bunchgrass. Ecology **80**:1180-1186.

McCune, B. 1997. Influence of noisy environmental data on canonical correspondence analysis. Ecology **78**:2617-2623.

McGarigal, K., S. Cushman, and S. Stafford. 2000. Multivariate statistics for wildlife and ecology research. Springer-Verlag, New York.

Meentemeyer, V. 1978. Macroclimate and lignin control of litter decomposition rates. Ecology **59**:465-472.

Melillo, J. M., J. D. Aber, and J. F. Muratore. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology **63**:621-626.

Monzon, A., and R. Azcon. 1996. Relevance of mycorrhizal fungal origin and host plant genotype to inducing growth and nutrient uptake in *Medicago* species. Agriculture Ecosystems & Environment **60**:9-15.

Moora, M., and M. Zobel. 1996. Effect of arbuscular mycorrhiza on inter- and intraspecific competition of two grassland species. Oecologia **108**:79-84.

Morton, J. F. 1978. Brazilian pepper, its impacts on people, animals, and the environment. Economic Botany **32**:353-359.

Murphy, J. and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chemica Acta **27:** 31-36.

Myers, R. L., and J. J. Ewel, editors. 1990. Ecosystems of Florida. University of Central Florida Press, Gainesville.

Myers, R. T., D. R. Zak, D. C. White, and A. Peacock. 2001. Landscape-level patterns of microbial community composition and substrate use in upland forest ecosystems. Soil Science Society of America Journal **65**:359-367.

Nakamura, A., C. C. Tun, S. Asakawa, and M. Kimura. 2003. Microbial community responsible for the decomposition of rice straw in a paddy field: estimation by phospholipid fatty acid analysis. Biology and Fertility of Soils **38**:288-295.

Ohta, H., and T. Hattori. 1983. *Agromonas oligotrophica* gen. nov., sp. nov, a ntirogen-fixing oligotrophic bacterium. Antonio van Leeuwenhoek **49**:429-446.

O'Leary, W. M., and D. G. Wilkinson. 1988. Gram-positive bacteria. Pages 117-202 *in* C. Ratledge and D. G. Wilkinson, editors. Microbial Lipids. Academic Press, London.

Packer, A., and K. Clay. 2000. Soil pathogens and spatial patterns of seedling mortality in a temperate tree. Nature **404**:278-281.

Parkes, R. J. 1987. Analysis of microbial communities within sediments using biomarkers. Pages 147-178 *in* M. Fletcher, T. P. G. Gray, and J. G. Jones, editors. Ecology of Microbial Communities. Cambridge University Press, London.

Parkes, R. J., and J. Taylor. 1983. The relationship between fatty acid distributions and bacterial respiratory types in contemporary marine sediments. Estuaries and Coastal Shelf Science **16**:173-189.

Pastor, J., A. Stillwell, and D. Tilman. 1987. Little bluestem litter dynamics in Minnesota old fields. Oecologia **72**:327-330.

Peng, S. B., D. M. Eissenstat, J. H. Graham, K. Williams, and N. C. Hodge. 1993. Growth depression in mycorrhizal citrus at high-phosphorus supply–analysis of carbon costs. Plant Physiology **101**:1063-1071.

Pennanen, T., J. Liski, E. Baath, V. Kitunen, J. Uotilia, C. J. Westman, and J. Fritze. 1999. Structure of the microbial communities in coniferous forest soils in relation to site fertility and stand development stage. Microbial Ecology **38**:168-179.

Philip, L. J., U. Posluszny, and J. N. Klironomos. 2001. The influence of mycorrhizal colonization on the vegetative growth and sexual reproductive potential of *Lythrum salicaria* L. Canadian Journal of Botany **79**:381-388.

Poorter, H., and C. Remkes. 1990. Leaf-area ratio and net assimilation rate of 24 wild-species differing in relative growth-rate. Oecologia **83**:553-559.

Priha, O., and A. Smolander. 1999. Nitrogen transformations in soil under *Pinus sylvestris, Picea abies* and *Betula pendula* at two forest sites. Soil Biology & Biochemistry **31**:965-977.

Priha, O., S. J. Grayston, R. Hiukka, T. Pennanen, and A. Smolander. 2001. Microbial community structure and characteristics of the organic matter in soils under *Pinus sylvestris, Picea abies* and *Betula pendula* at two forest sites. Biology and Fertility of Soils **3**:17-24.

Priha, O., S. J. Grayston, T. Pennanen, and A. Smolander. 1999. Microbial activities related to C and N cycling and microbial community structure in the rhizospheres of Pinus sylvestris, Picea albies and Betula pendula seedlings in an organic and mineral soil. FEMS Microbiology Ecology **30**:187-199.

Richardson, D. M., N. Allsopp, C. M. D'Antonio, S. J. Milton, and M. Rejmanek. 2000. Plant invasions–the role of mutualisms. Biological Reviews of the Cambridge Philosophical Society **75**:65-93.

Saetre, P., and E. Baath. 2000. Spatial variation and patterns of soil microbial community structure in a mixed spruce-birch stand. Soil Biology & Biochemistry **32**:909-917.

Sharma, M. P., and A. Adholeya. 2000. Response of *Eucalyptus tereticornis* to inoculation with indigenous AM fungi in a semiarid alfisol achieved with different concentrations of available soil-P. Microbiological Research **154**:349-354.

Shishido, M., and C. P. Chanway. 1998. Storage effects on indigenous soil microbial communities and PGPR efficacy. Soil Biology & Biochemistry **30**:939-947.

Siemann, E., and W. E. Rogers. 2003. Reduced resistance of invasive varieties of the alien tree *Sapium sebiferum* to a generalist herbivore. Oecologia **135**:451-457.

Singhurst, J. R., W. J. Ledbetter, and W. C. Holmes. 1997. *Ardisia crenata* (Myrsinaceae): New to Texas. Southwestern Naturalist **42**:503-504.

Siqueira, J. O., M. A. C. Carneiro, N. Curi, S. C. d. Silva Rosado, and A. C. Davide. 1998. Mycorrhizal colonization and mycotropic growth of native woody species as related to successional groups in Southeastern Brazil. Forest Ecology and Management **107**:241-252.

Smalla, K., U. Wachtendorf, H. Heuer, W. T. Liu, and L. Forney. 1998. Analysis of Biolog GN substrate utilization patterns by microbial communities. Applied and Environmental Microbiology **64**:1220-1225.

Smith, F. A., and S. E. Smith. 1996. Mutualism and parasitism: Diversity in function and structure in the "arbuscular" (VA) mycorrhizal symbiosis. Advances in Botanical Research **22**:1-43.

Smith, M. D., D. C. Hartnett, and G. W. T. Wilson. 1999. Interacting influence of mycorrhizal symbiosis and competition on plant diversity in tallgrass prairie. Oecologia **121**:574-582.

Smith, S. E. and D. Read. 1997. Mycorrhizal Symbiosis. Academic Press, London.

Son, C. L., and S. E. Smith. 1988. Mycorrhizal growth-responses - interactions between photon irradiance and phosphorus-nutrition. New Phytologist **108**:305-314.

Staddon, W. J., J. T. Trevors, L. C. Duchesne, and C. A. Colombo. 1998. Soil microbial diversity and community structure across a climatic gradient in western Canada. Biodiversity and Conservation **7**:1081-1092.

Stanosz, G. R. 1992. Effect of cherry leaf-spot on nursery black-cherry seedlings and potential benefits from control. Plant Disease **76:** 602-604.

Swift, M. J., O. W. Heal, and J. M. Anderson. 1979. Decomposition in Terrestrial Ecosystems. Blackwell Science Publications, Oxford, England.

Sylvia, D. M. 1990. Inoculation of native woody-plants with vesicular arbuscular mycorrhizal fungi for phosphate mine land reclamation. Agriculture Ecosystems & Environment **31**:253-261.

Sylvia, D. M. 1994. Vesicular-arbuscular mycorrhizal fungi. pp. 351-378 eds. Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties, vol. 5. Soil Science Society of America, Madison, WI.

Sylvia, D. M., A. Alagely, D. O. Chellemi, and L. W. Demchenko. 2001. Arbuscular mycorrhizal fungi influence tomatoes competition with bahia grass. Biology and Fertility of Soils **34**:448-452.

Sylvia, D. M., D. O. Wilson, J. H. Graham, J. J. Maddox, P. Millner, J. B. Morton, H. D. Skipper, S. F. Wright, and A. G. Jarstfer. 1993. Evaluation of vesicular-arbuscular mycorrhizal fungi in diverse plants and soils. Soil Biology & Biochemistry **25**:705-713.

Taylor, B. R., D. Parkinson, and W. F. J. Parsons. 1989. Nitrogen and lignin content as predictors of litter decay rates: a microcosm test. Ecology **70**:97-104.

Tiedje, J. M., S. Asuming-Brempong, K. Nusslein, T. L. Marsh, and S. J. Flynn. 1999. Opening the black box of soil microbial diversity. Applied Soil Ecology **13**:109-122.

Tilman, D. 1982. Resource Competition and Community Structure. Princeton University Press, Princeton.

Tilman, D. 1988. Plant Stategies and the Dynamics and Structure of Plant Communities. Princeton University Press, Princeton.

Torsvik, V., R. Sorheim, and J. Goksoyr. 1996. Total bacterial diversity in soil and sediment communities–a review. Applied Environmental Microbiology **56**:776-781.

van der Heijden, E. W., and T. W. Kuyper. 2001. Does origin of mycorrhizal fungus or mycorrhizal plant influence effectiveness of the mycorrhizal symbiosis? Plant and Soil **230**:161-174.

van der Heijden, M. G. A., J. N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I. R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature **396**:69-72.

Vestal, J., and D. C. White. 1989. Lipid analysis in microbial ecology: Quantitative approaches to the study of microbial communities. Bioscience **39**:535-541.

Vitousek, P. M., and L. R. Walker. 1989. Biological invasion by *Myrica faya* in Hawaii - plant demography, nitrogen-fixation, ecosystem effects. Ecological Monographs **59**:247-265.

Waldrop, M. P., and M. K. Firestone. 2004a. Altered utilization patterns of young and old soil C by microorganisms caused by temperature shifts and N additions. Biogeochemistry **67**:235-248.

Waldrop, M. P., and M. K. Firestone. 2004b. Microbial community utilization of recalcitrant and simple carbon compounds: impact of oak-woodland plant communities. Oecologia **138**:275-284.

Waldrop, M. P., D. R. Zak, and R. L. Sinsabaugh. 2004. Microbial community response to nitrogen deposition in northern forest ecosystems. Soil Biology & Biochemistry **36**:1443-1451.

Waldrop, M. P., T. C. Balser, and M. K. Firestone. 2000. Linking microbial community composition to function in a tropical soil. Soil Biology and Biochemistry **32**:1837-1846.

Westover, K. M., A. C. Kennedy, and S. E. Kelley. 1997. Patterns of rhizosphere microbial community structure associated with co-occurring plant species. Journal of Ecology **85**:863-873.

White, D. C., W. M. Davis, J. S. Nickels, J. D. King, and R. J. Bobbie. 1979. Determination of the sedimentary microbial biomass by extractible lipid phosphate. Oecologia **40**:51-62.

Wilkinson, D. G. 1988. Gram-negative bacteria. *in* C. Ratledge and D. G. Wilkinson, editors. Microbial Lipids. Academic Press, London.

Wilkinson, S. C., J. M. Anderson, S. P. Scardelis, M. Tisiafouli, A. Taylor, and V. Wolters. 2002. PLFA profiles of microbial communities in decomposing conifer litters subject to moisture stress. Soil Biology & Biochemistry **34**:189-200

Witkamp, M. 1963. Microbial populations of leaf litter in relation to environmental conditions and decomposition. Ecology **44**:370-377.

Witkamp, M. 1966. Decomposition of leaf litter in relation to environment, microflora, and microbial respiration. Ecology **47**:194-201.

Woodall, S. L. 1981. Evapotranspiration and *Melaleuca*. Pages 117-123 *in* R. K. Geiger, editor. Proceedings of *Melaleuca* Symposium. Florida Division of Forestry, Tallahassee, FL.

Wright, I. J., P. B. Reich, and M. Westoby. 2001. Strategy shifts in leaf physiology, structure and nutrient content between species of high- and low-rainfall and high- and low-nutrient habitats. Functional Ecology **15**:423-434.

Zangaro, W., V.L.R. Bononir and S.B. Truffen. 2000. Mycorrhizal dependency, inoculum potential and habitat preference of native woody species in South Brazil. Journal of Tropical Ecology **16**: 603-622.

BIOGRAPHICAL SKETCH

Sarah Renee Bray was born in Des Moines, Iowa, on February 23, 1976. Her family soon moved to Nebraska and Sarah was raised in Lincoln and Seward, Nebraska. Her love of nature sprang from family vacations spent camping and hiking in Nebraska, the Rockies and the Southwest. In junior and senior high she was inspired to study biology by Terry Loontjer and James Landon. In 1994, Sarah graduated from Seward High School and started her B.A. in biology and environmental science at Coe College in Cedar Rapids, Iowa. There she participated in a summer research program with Paula Sanchini when she discovered her interests in plant ecology. While at Coe she also participated in research programs at Kennedy Space Center and in Costa Rica. Sarah graduated magna cum laude with college honors and started her graduate career under the supervision of Dr. Kaoru Kitajima in the Department of Botany at the University of Florida. While at Florida, Sarah pursued her dual interests in teaching and research. After completing her doctorate, Sarah and her husband will move to Nebraska where she has accepted a position as assistant biology professor and curator of the arboretum at Midland Lutheran College in Fremont, Nebraska.