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The effects of soil biota and fertilization on the success of *Sapium sebiferum*

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ABSTRACT

Soil organisms can have important impacts on the structure, diversity, and invasion potential of plant communities. In particular, the short co-evolutionary history of non-native plants with soil biota could confer unusual benefits or costs to hosts in the introduced range with consequences for invasion success. We used parallel pot and field studies to examine how soil biota (active or sterilized soil) and fungicide (addition or control) affected the growth and survival of the invasive, non-native tree, *Sapium sebiferum*, and three co-occurring native tree species *Liquidambar styraciflua*, *Nyssa sylvatica*, and *Quercus nigra* in the Big Thicket National Preserve in east Texas, USA. In addition, because plant interactions with soil microbes vary with abiotic factors, we also included a fertilization treatment.

The aboveground and belowground biomass of all species were higher in the presence of an active soil biotic community than in sterilized soil. *Sapium* alone showed more than additive growth increases under the combination of both field active and fertilized soils. It was also the only species to maintain high aboveground biomass and high aboveground nitrogen levels under those conditions. The high levels of arbuscular mycorrhizal colonization found on *Sapium* compared to natives suggest that arbuscular mycorrhizae may be involved in this phenomenon.

These results indicate that belowground soil organisms provide unusual positive benefits to the invader that are not experienced by native species. This benefit may increase *Sapium* growth rates compared to natives in the forest understory and therefore facilitate *Sapium* invasion into mesic temperate forests currently experiencing high levels of anthropogenic nutrient inputs.

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1. Introduction

Soil communities can have important effects on the structure of plant communities because of their involvement in multiple processes critical for plant survival and performance (Packer and Clay, 2000; Bever, 2002; Wardle, 2002; Callaway et al., 2004a). Saprophytic soil organisms influence nutrient availability in the soil. Pathogenic soil organisms impact plant

survival and mutualistic soil organisms influence a plant's ability to acquire nutrients and protect itself from pathogens (van der Heijden and Sanders, 2002; Wardle, 2002; Callaway et al., 2003; Nijjer et al., 2007). As a result, understanding how the soil community influences plant growth and survival is necessary for understanding the structure and function of plant communities and the invasion potential of exotic species.

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There is little understanding of how belowground soil organisms may affect invasion potential (Wolfe and Klironomos, 2005). Because of differences in the length of time of association with soil biota, natives and exotics may differ in their interactions with soil biota in the introduced range. The range of interactions between plants and microbes are shaped by natural selection. Recently introduced non-native species may interact in extreme ways with native soil communities because they have not been shaped by evolution. If an invading species begins to colonize a community where native plants have co-evolved with their soil communities for a long time, the invader may receive extremely high benefits or experience high costs from interactions with the soil biota in their introduced ranges (Marler et al., 1999; Carey et al., 2004; Meiman et al., 2006; Walling and Zabinski, 2006). For example, exotic species may have the ability to connect into existing mycorrhizal networks and parasitize neighboring competitors through their own enhanced nutrient uptake (Marler et al., 1999; Carey et al., 2004). If invasive plants encounter soil biota that more strongly facilitate non-native species establishment in the introduced range than the native range, these soil biota may promote invasion (“Enhanced Mutualisms Hypothesis” – Reinhart and Callaway, 2006).

The ecological effects of soil biota can be highly context dependent. One critical factor that can influence costs and benefits to hosts is nutrient availability. For example, changes in the mycorrhizal community with high nutrient availability may change the net costs and benefits received by plant hosts from mycorrhizal associations (Hetrick et al., 1989; Johnson, 1993; Johnson et al., 1997; Marler et al., 1999). When soil resources are abundant, nitrogen and light become less and more valuable, respectively. Thus, in high fertility soils, the nutrient benefit provided by mycorrhizal fungi may become less valuable, and the photosynthesizing capacity provided by the plant becomes more valuable.

Utilizing parallel pot and field studies in a temperate forest community in east Texas, we asked the following questions: (1) Does the belowground soil community influence the survival and growth of woody native seedlings and the exotic tree *Sapium sebiferum*? (2) What is the relative importance of mycorrhizae for the survival and growth of natives compared to the exotic *Sapium*? (3) Are the interactions between seedlings and their mycorrhizal partners altered by soil fertility?

2. Materials and methods

2.1. Study system and focal species

Big Thicket National Preserve (BTNP) is a 96,000-acre national preserve in east Texas that lies at the convergence of multiple ecosystems and supports considerable biological diversity. All of the native species used in this study *Liquidambar styraciflua* (Sweetgum (L.) Hamamelidaceae), *Nyssa sylvatica* (Blackgum (Marsh.) Nyssaceae), and *Quercus nigra* (Water Oak (L.) Fagaceae), are common tree species in BTNP (Marks and Harcombe, 1981). Currently, mesic and floodplain forests of BTNP are invaded by the exotic species *Sapium sebiferum* (Chinese Tallow tree (L.) Roxb., Euphorbia-

ceae; synonyms include, *Triadica sebifera*) (Bruce et al., 1997; Siemann and Rogers, 2003). In BTNP, *Sapium* has steadily increased in abundance over the past 20 years. In some areas *Sapium* has become the most abundant understory tree and is becoming common in the overstory (Harcombe et al., 1999).

2.2. Pot experiment

2.2.1. Experimental design

In June 2004, a 20-week post germination pot study was conducted to test the effects of soil biota suppression and fertilization on the growth and survival of seedlings of three native tree species and the invader *Sapium sebiferum*. The entire experiment included 192 pots in a randomized full-factorial design with 4 species (*Liquidambar styraciflua*, *Nyssa sylvatica*, *Quercus nigra*, and *Sapium sebiferum*), 2 soil treatments (active soil or sterilized soil), 2 fertilization levels (fertilized or unfertilized), 2 fungicide levels (fungicide or no fungicide) and 6 replicates per treatment.

All native seeds were purchased from the Louisiana Forest Seed Company Lecompte, LA, and *Sapium* seeds were collected from trees at the University of Houston Coastal Center south of Houston, TX. All seeds were surface sterilized with a 10% bleach rinse to remove surface contaminants and germinated in topsoil sterilized twice by autoclaving at 121 °C. Flats containing seeds were placed in a greenhouse located on the Rice University campus in Houston, TX. Germinated seeds were planted into 66 ml Conetainers (Stuewe & Sons, Corvallis, Oregon) filled with sterilized topsoil and were watered as needed. These seedlings were replanted into 3.8 gal pots described below at the initiation of the experiment.

Soil used for planting germinants for the pot experiment was collected from eight sites in the Lance Rosier Unit of BTNP. Two or three holes per site were dug to a depth of 8–10 cm for soil collection at least 5 m from the base of any trees. This procedure ensured soil was not collected directly beneath specific tree species. All soil was homogenized by passing through a 1 cm screen sieve to control for nutrient differences between soils collected at different sites. It is possible however that rare soil organisms present only at a single site may cause a site specific effect to appear as a general effect. Roots were cut into 1–2 cm fragments and mixed in with the soil. All soil and roots not used as fresh inoculum were sterilized as described above. All pots were filled with an equivalent amount of sterilized field soil at the initiation of the experiment.

Two weeks after germination, seedlings of all species were transplanted into 3.8 L pots (Stuewe & Sons) filled with sterilized field soil. Seedlings were surrounded with either 30 g of fresh, homogenized field soil or sterilized field soil upon transplantation (approximately 0.01 L per pot). A 15 ml soil microbial backwash was applied to equalize the soil microbial community. The background microbial wash was manipulated by using a 38 µm sieved field solution (Johnson, 1993). We will refer to the autoclaved treatment as “sterilized” hereafter because our treatment removed mycorrhizal spores even though smaller microorganisms were present in these soils.

Rovral 4 flowable fungicide (Aventis CS, Bridgewater, NJ) was applied as a soil drench six times in the course of the 20-week experiment (three week intervals). Rovral (10% active ingredient iprodione) has been shown to reduce mycorrhizal infection and is a contact fungicide with no known systemic action (Gange et al., 1990; Ganade and Brown, 1997). Controls (no fungicide) received an equivalent amount of distilled water.

Fertilizer was applied four times during the course of the 20-week experiment. Application rates were equivalent to 4 g/m² each of N, P, and K per application. Nutrients were added as ammonium nitrate (N), superphosphate (P), and potash (K) dissolved in 40 ml of distilled water. An equivalent amount of distilled water was added to non-fertilized controls.

Initial height, diameter, leaf count, and herbivore damage measurements were taken for each seedling in the greenhouse. Successive measurements were recorded at five-week intervals over the course of the experiment. Measuring equipment was thoroughly cleaned between treatments to prevent transfer of inoculum between sources. Pots were watered as needed and periodically rotated to minimize shading and location effects. At the end of 20 weeks, we measured shoot and root dry weights and mycorrhizal infection.

2.2.2. Carbon, nitrogen, and phosphorus analysis

Aboveground biomass (leaves and stems) was ground into a fine powder and was measured for total carbon and nitrogen concentrations in an auto analyzer. Phosphorous concentrations were determined by ashing samples and using inductively coupled plasma mass spectrometry ICP-MS (Thermo Finnigan Element 2 ICP-MS).

2.2.3. Belowground mycorrhizal analysis

All tree species in this study are known to be endomycorrhizal under field conditions (Keeley, 1980; Jacobs et al., 2000; Nijjer et al., 2004). *Quercus nigra* is known to associate with both endo- and ectomycorrhizal fungi, but only endomycorrhizal fungi were measured for this study. At harvest, five randomly selected plants of each treatment were selected for endomycorrhizal analysis. Root systems were stored at 4 °C until they could be processed. Randomly selected fine root subsamples from each plant were washed on a 500- μ m sieve and stored in histocassettes in 50% ethanol until processed for endomycorrhizal staining. Roots were cleared in 10% KOH at 80 °C for 45 min and then rinsed and soaked for 1 min in 10% bleach for additional clearing (Bray et al., 2003). Roots were then rinsed and acidified in concentrated HCl (2.5%) for 5 min. Fine roots from each core were stained and dyed in trypan blue and 10 1-cm root fragments were mounted on microscope slides and measured for arbuscular mycorrhizal colonization rates using a modification of the gridline intersect method (McGonigle et al., 1990; Brundrett et al., 1994). Mounted roots were checked for the presence of arbuscules, vesicles, coils, and hyphae of arbuscular mycorrhizal fungi under a compound microscope at 400 \times magnification. Nonmycorrhizal fungi and microbes were noted and labeled as “other.” Arbuscular mycorrhizae were distinguished from non-mycorrhizal fungi by the methods of Callaway et al. (2003).

2.2.4. Data analysis

We assessed treatment effects on several plant and mycorrhizal response variables using Type III general linear models in SAS (SAS Institute, Cary, NC). We used a 4 \times 2 \times 2 \times 2 factorial general linear model for the following response variables: aboveground and belowground biomass, percent carbon, percent nitrogen, and phosphorus concentration. The model included the independent factors of species (4 species), fertilization (addition or no addition), soil treatment (active or sterilized) and fungicide (addition or no addition) and all interactions among factors. Aboveground and belowground dry weights for each plant were natural log transformed to meet the assumption of normality of residuals. Height growth for each plant was measured as ln(final ht/initial ht). Percent carbon and nitrogen were natural log transformed and phosphorus concentrations were square-root transformed to meet the assumptions of normality. All analyses met the assumption of homogeneity of variances. Following the detection of significant interactions between a treatment and the species term, we used 2 \times 2 \times 2 factorial analyses for the response of each species to identify which species were causing significant species by experimental treatment interaction terms. Within a species, we used adjusted least squares means post hoc tests to compare differences among treatment levels for significant interactions.

Mycorrhizal responses were examined using the same 4 \times 2 \times 2 \times 2 model, followed by examination of individual species when species by treatment interactions were significant. Endomycorrhizal and “other” colonization were expressed as percent root colonized. Belowground mycorrhizal and “other” colonization did not fit a normal distribution and were ranked to apply non-parametric analyses (Conover and Iman, 1981). Fisher’s Least Significant Difference post hoc tests were conducted to test for differences among treatment levels of significant main effects for variables with three or more levels. Adjusted least squares means post hoc tests were conducted to test for differences among treatment levels for significant interactions. In addition, ANCOVA with aboveground biomass as a covariate was used to test whether mycorrhizal colonization depended on soil treatment (active soil or sterilized). For species showing significant soil treatment \times biomass interactions in ANCOVA, non-parametric, Spearman rank correlations were performed (SAS Institute, Cary, NC) to test whether aboveground and/or belowground biomass was significantly correlated with belowground colonization measures.

2.3. Field experiment

2.3.1. Experimental design

In June 2004, a 2-year companion field study was initiated to test the effects of mycorrhizal suppression and fertilization on seedlings in field conditions. At the Lance Rosier unit in BTNP, ninety-six 2 m by 2 m plots with 1 m walkways were established. The entire experiment was surrounded by a 2 m buffer and a three-strand barbed wire fence to exclude feral hogs. The experiment was a full-factorial, randomized design with 4 species (*Liquidambar styraciflua*, *Nyssa sylvatica*, *Quercus nigra* and *Sapinum sebiferum*), 2 fungicide levels (fungicide or no fungicide), 2 fertilizer levels (fertilized or no

fertilizer) and 6 plot replicates. Each plot was randomly assigned to a species treatment and four conspecific seedlings were planted into the center of each plot in a 30 cm square (seedlings approximately 1 ft apart from one another).

Seedlings were germinated as described above and transported to the field site in 66 ml RLC-4 Pine Conetainers™ (Stuewe & Sons, Corvallis, Oregon). Fungicide application followed the rates and methods above. Non-fungicide controls received an equivalent amount of distilled water. Fertilizer was applied three times during the growing season as a 3:1:2 NPK granular formula at yearly application rates of 24 g/m²/yr N, 8 g/m²/yr P, and 16 g/m²/yr K and micronutrients (Vigoro Ultra Turf Fertilizer). Fertilizer was broadcast prior to forecast rain.

Seedling survival, height, diameter, leaf count, and herbivore damage were recorded four times during two growing seasons including at harvest. In August 2005, seedlings were harvested, and we measured shoot and root dry weights and mycorrhizal infection.

2.3.2. Data analysis

The second growing season (2005) experienced a heavy drought in spring and early summer followed by destruction from hurricane Rita in early fall. These two events resulted in an extremely low number of surviving replicates at the end of the experiment ($n = 52$ seedlings) precluding confidence in the results from the analysis of survival, biomass, or belowground colonization data at the end of year 2. Consequently, plant survival and change in relative height at the end of year 1 2004 were the only response variables analyzed.

Plant survival was recorded four times during the growing season and survival time of each plant was calculated as the last survey they were alive. Survival time was analyzed with a log-linear model with a polytomous, ordinal response variable (i.e., 0, 8, 13, or 20 weeks survived) to compare survival time

measurements between the different experimental treatments (SAS Institute, Cary, NC). Differences in height among species treatments were tested using a Type III General Linear Model in SAS.

Both survival and height models included the independent factors of species (four species), fertilization (addition or control), and fungicide (addition or control) and all interactions among factors. The models also included a blocking factor, plot, which was nested within the species \times fungicide \times fertilizer interaction because each plot contained a single species and received one level of each of the fungicide and fertilizer treatments. Thus, the plot factor accounted for the non-independence of seedlings within a plot. Post-hoc comparisons were conducted as described above. Individual species two-way analyses and post-hoc comparisons were conducted as described above, but also included the plot term.

3. Results

3.1. Pot experiment

3.1.1. Aboveground biomass

Aboveground biomass increased in active soil or fertilized soil for *Nyssa*, *Liquidambar*, and *Sapium*. Aboveground biomass depended on species, soil treatment, fertilizer, species \times soil treatment, and species \times fertilizer (Table 1). There was a marginally significant species \times soil treatment \times fertilizer interaction term ($P = 0.0586$). Because species was a significant factor alone and in interaction with multiple analysis terms, individual species three-way analyses were conducted to identify which species were causing the significant species by experimental treatment interaction terms.

Table 1 – Dependence of aboveground biomass, belowground biomass, root:shoot, and height growth on treatments in the pot experiment

	df	Aboveground biomass		Belowground biomass		Root:shoot		Height growth	
		F	P	F	P	F	P	F	P
Model	31	22.3	<0.0001	22.5	<0.0001	6.1	<0.0001	7.9	<0.0001
Error	153								
Sp	3	165.6	<0.0001	193.3	<0.0001	21.7	<0.0001	44.3	<0.0001
ST	1	38.3	<0.0001	21.2	<0.0001	0.1	0.7755	0.1	0.6904
Fung	1	0.8	0.3834	0.0	0.9945	0.1	0.7281	2.0	0.3460
Fert	1	101.6	<0.0001	57.5	<0.0001	11.2	0.0010	25.8	<0.0001
Sp \times ST	3	4.0	0.0088	0.6	0.5983	3.5	0.0177	3.8	0.0198
Sp \times Fung	3	2.0	0.1157	0.9	0.4218	1.1	0.3505	0.6	0.6870
Sp \times Fert	3	10.6	<0.0001	4.8	0.0031	3	0.0331	5.7	<0.0001
ST \times Fung	1	0.8	0.3736	6.2	0.0138	2	0.1640	0.9	0.7235
ST \times Fert	1	0.0	0.9735	1.0	0.3152	0	0.9004	8.7	<0.0001
Fung \times Fert	1	1.7	0.1896	1.5	0.2210	0.2	0.6256	0.7	0.8058
Sp \times ST \times Fung	3	2.3	0.0799	0.6	0.5955	1	0.3916	0.7	0.7233
Sp \times ST \times Fert	3	2.5	0.0586	0.6	0.6515	2	0.1107	9.2	<0.0001
Sp \times Fung \times Fert	3	0.2	0.9265	0.1	0.9757	1.7	0.1660	1.6	0.2073
ST \times Fung \times Fert	1	0.2	0.6753	6.2	0.0141	2.2	0.1408	0.5	0.9390
Sp \times ST \times Fung \times Fert	3	0.4	0.7274	1.2	0.3072	0.2	0.8730	2.2	0.3536

Sp, ST, Fung, and Fert refer to species, soil treatment, fungicide, and fertilization effects, respectively. Significant treatment effects ($P < 0.05$) are highlighted in bold.

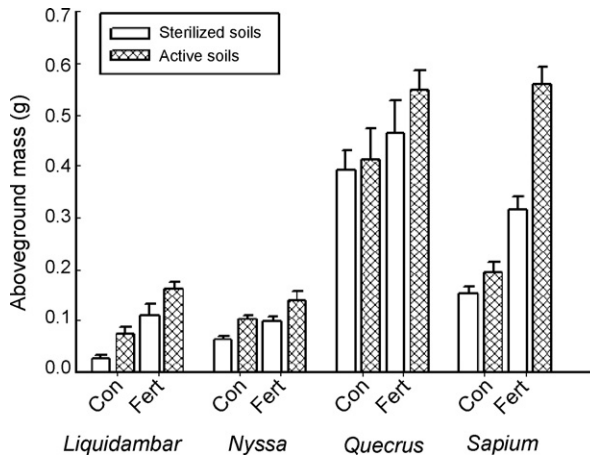


Fig. 1 – The effects of soil treatment and fertilizer on the aboveground biomass of each species in the pot experiment. Means +1 standard error are presented.

Within individual species analyses, *Nyssa*, *Liquidambar*, and *Sapium* each had significantly greater aboveground biomass in active soil compared to sterilized soil (33.6% – $P = 0.0026$, 36.3% – $P < 0.0001$, and 37.5% – $P < 0.0001$, respectively, Fig. 1) and fertilized soil (29.7% – $P = 0.0196$, 59.8% – $P < 0.0001$, and 59.9% – $P < 0.0001$, respectively, (Fig. 1) whereas *Quercus* aboveground biomass did not differ significantly between soil treatment

and fertilization treatments. These differences in response of particular species to treatments led to the significant interaction terms in the overall aboveground model.

Sapium was the only species to show synergistic (significantly more than additive) aboveground biomass increases in fertilized, active soils as indicated by a significant soil treatment × fertilization interaction effect in individual species analysis ($P = 0.0446$). Together these results suggest that *Sapium* was likely responsible for the species × soil treatment × fertilizer trend ($P = 0.0586$) in the overall aboveground biomass model.

3.1.2. Belowground biomass

Belowground biomass increased in active or fertilized soils. Belowground biomass depended on species, soil treatment, fertilizer, species × fertilizer, soil treatment × fungicide, and soil treatment × fungicide × fertilizer (Table 1). Fishers’ LSD tests showed on average that *Quercus* (a) had the highest belowground biomass followed by *Sapium* (b), *Nyssa* (c), and *Liquidambar* (d), respectively (different letters indicate significant differences). The significant soil treatment and fertilizer main effects in the overall model were driven by higher belowground biomass in active and fertilized soils compared to sterilized and non-fertilized soils, respectively (Fig. 2).

The significant soil treatment × fungicide × fertilizer interaction reflects that belowground biomass increased with fertilization and the significant soil treatment × fungicide

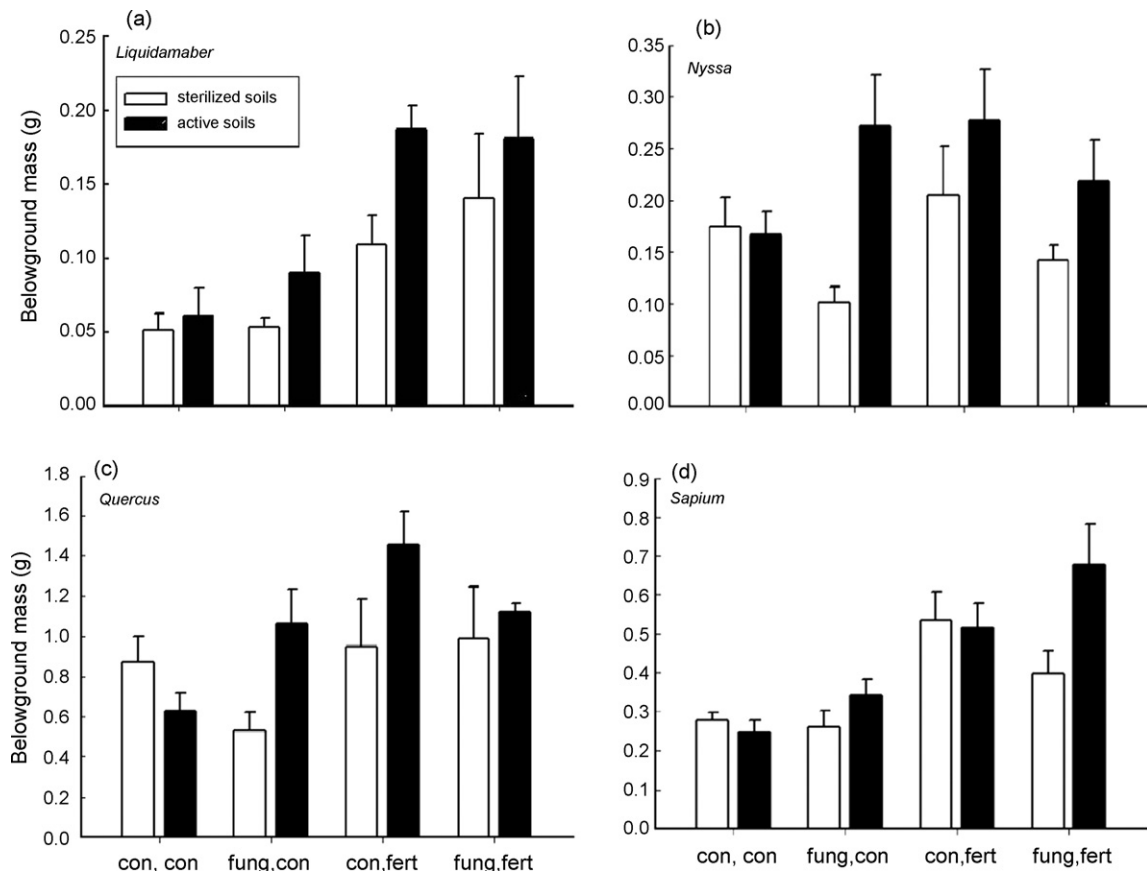


Fig. 2 – The effects of fungicide, fertilizer, and soil treatment on the belowground biomass of each (a) *Liquidambar*; (b) *Nyssa*; (c) *Quercus*; and (d) *Sapium* in the pot experiment. Means +1 standard error are displayed.

interaction reflects that plants in sterilized soils decreased biomass when treated with fungicide while plants in active soil had greater belowground biomass when treated with fungicide (Fig. 2).

Within individual species three-way analyses, *Nyssa*, *Quercus*, and *Liquidambar* all had greater belowground biomass in active compared to sterilized soils (33.4% – $P = 0.0024$, 21.9% – $P = 0.0457$, and 28.8% – $P = 0.0391$, respectively, Fig. 2). *Quercus*, *Liquidambar*, and *Sapium* had significantly higher belowground biomass with fertilizer (31.5% – $P = 0.0163$, 57.1% – $P < 0.0001$, 46.7% – $P < 0.0001$, respectively Fig. 2).

3.1.3. Height growth

Height growth was greater in fertilized conditions. Height growth depended on species, fertilizer, species \times soil treatment, species \times fertilizer, soil treatment \times fertilizer, and species \times soil treatment \times fertilizer (Table 1). In individual three-way species analyses, *Nyssa*, *Liquidambar*, and *Sapium* but not *Quercus* grew faster with fertilization ($P = 0.0028$, $P = 0.0138$, and $P < 0.0001$, respectively, Fig. 3). *Nyssa* had lower height growth in active soil relative to sterile soil as shown by a significant soil treatment main effect ($P = 0.0081$). *Sapium* had a trend for faster height growth in active soil ($P = 0.0785$). Together these suggest that *Nyssa*, *Sapium*, and *Liquidambar* are driving the significant species \times soil treatment and species \times fertilizer effects in the overall model.

Sapium was the only species to have more than additive height increases in active, fertilized soils (Fig. 3) as indicated by a significant soil treatment \times fertilization interaction effect in individual *Sapium* species analysis ($P < 0.0001$). *Sapium* likely is responsible for the significant soil treatment \times fertilizer and species \times soil treatment \times fertilizer effects in the overall model.

3.1.4. Carbon, nitrogen, and phosphorus

Carbon only varied among species (species main effect; Table 2). Fisher's LSD showed that this reflects the significantly higher carbon in the aboveground biomass of *Liquidambar* (a) and *Quercus* (a) compared to *Nyssa* (b) and *Sapium* (b) (different

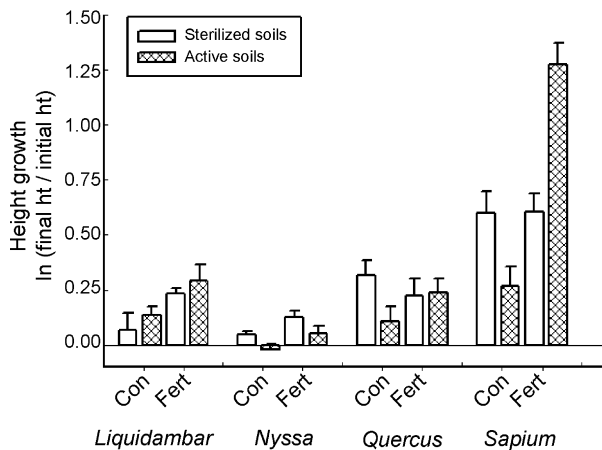


Fig. 3 – The effects of soil treatment and fertilizer on the height growth of each species. Means +1 standard error are presented.

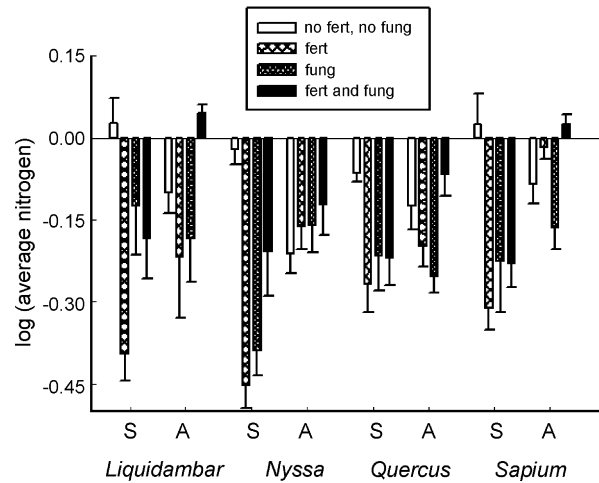


Fig. 4 – The effects of soil treatment, fertilizer, and fungicide on the log of average nitrogen found in plants. + or –1 standard errors presented. “S” and “A” stand for sterile and active soils, respectively.

letters indicate significant differences). The overall model for phosphorus was not significant ($F = 0.76$, $P = 0.8150$). The average aboveground nitrogen levels of all plant species were lower in sterilized soils that had been fertilized but not treated with fungicide (Figs. 4 and 5) and increased in active soils when plants are fertilized and treated with fungicide simultaneously (Fig. 4). Average nitrogen depended on species, soil treatment, fertilization, soil treatment \times fertilization, fungicide \times fertilization, soil treatment \times fungicide \times fertilization, and species \times soil treatment \times fungicide \times fertilization (Table 2). Phosphorus was independent of all treatments (Table 2).

3.1.5. Mycorrhizal and “other” colonization

Soil sterilization and fungicide application significantly reduced mycorrhizal colonization levels in this experiment. Mycorrhizal colonization depended on species, soil treatment, fungicide, species \times soil treatment, and soil treatment \times fungicide (Table 2). Mycorrhizal colonization was higher in *Sapium* pots compared to all other species (species main effect; Fig. 6; 92.9%, 90.8%, and 51.6% higher colonization than *Nyssa*, *Quercus*, and *Liquidambar*, respectively). The soil treatment \times fungicide interaction reflected lower overall colonization in sterilized soils and decreased colonization in active, fungicide compared to active, non-fungicide pots. The species \times soil treatment interaction in the overall model reflects that while all four species had significantly higher mycorrhizal colonization levels in active soils compared to sterile soils, *Sapium* had a much higher magnitude of colonization in field soils compared to all native species which suggests that *Sapium* is driving the species \times soil treatment interaction in the overall mycorrhizal colonization model (Fig. 6b). In addition, *Sapium* also had much higher levels of mycorrhizal colonization in fertilized soils than natives (Fig. 6b).

“Other” microbial colonization depended on species; *Nyssa* and *Sapium* had significantly lower colonization than *Quercus* or *Liquidambar* (Table 2). No species displayed significant

Table 2 – Dependence of carbon, nitrogen, phosphorus, and belowground colonization (mycorrhizal and other) on treatments in the pot experiment

	df	Carbon		Nitrogen		Phosphorus		df	Mycorrhizal colonization		Other colonization	
		F	P	F	P	F	P		F	P	F	P
Model	31	2.2	0.0008	5.5	<0.0001	0.7	0.8776	31	4.6	<0.0001	4.6	<0.0001
Error	150							125				
Sp	3	14.0	<0.0001	4.8	0.0032	0.8	0.5595	3	4.3	0.0067	40.4	<0.0001
ST	1	0.5	0.5036	17.2	<0.0001	0.6	0.4286	1	65.1	<0.0001	0.0	0.9764
Fung	1	0.0	0.9933	0.1	0.7456	<0.1	0.9205	1	6.4	0.0126	0.1	0.1803
Fert	1	0.9	0.3340	5.5	0.0200	2.3	0.1290	1	0.2	0.6811	0.8	0.3808
Sp × ST	3	0.4	0.7586	1.4	0.2570	0.5	0.6967	3	6.3	0.0005	0.3	0.8351
Sp × Fung	3	0.7	0.5514	1.5	0.2229	0.3	0.8398	3	1.0	0.4196	2.0	0.1162
Sp × Fert	3	0.7	0.5653	0.7	0.5428	0.7	0.5415	3	2.3	0.0829	2.0	0.1150
ST × Fung	1	0.5	0.4848	3.6	0.0609	0.5	0.4806	1	12.0	0.0007	0.0	0.9757
ST × Fert	1	0.1	0.7344	37.1	<0.0001	0.5	0.4806	1	0.1	0.7093	0.6	0.4277
Fung × Fert	1	1.3	0.2620	53.7	<0.0001	1.1	0.3072	1	2.6	0.1067	0.0	0.8788
Sp × ST × Fung	3	0.5	0.6913	0.1	0.9536	1.5	0.2278	3	0.2	0.8823	0.2	0.9038
Sp × ST × Fert	3	0.4	0.7646	1.1	0.3689	0.2	0.9231	3	1.8	0.1542	0.7	0.5557
Sp × Fung × Fert	3	1.9	0.1330	0.6	0.5992	0.8	0.4740	3	1.0	0.3931	1.0	0.4199
ST × Fung × Fert	1	0.4	0.5325	6.8	0.0102	1.6	0.2051	1	2.8	0.0988	<0.1	0.8363
Sp × ST × Fung × Fert	3	2.4	0.0666	4.2	0.0068	1.0	0.4039	3	0.4	0.7681	0.4	0.7400

Sp, ST, Fung, and Fert refer to species, soil treatment, fungicide, and fertilization effects, respectively. Significant treatment effects ($P < 0.05$) are highlighted in bold.

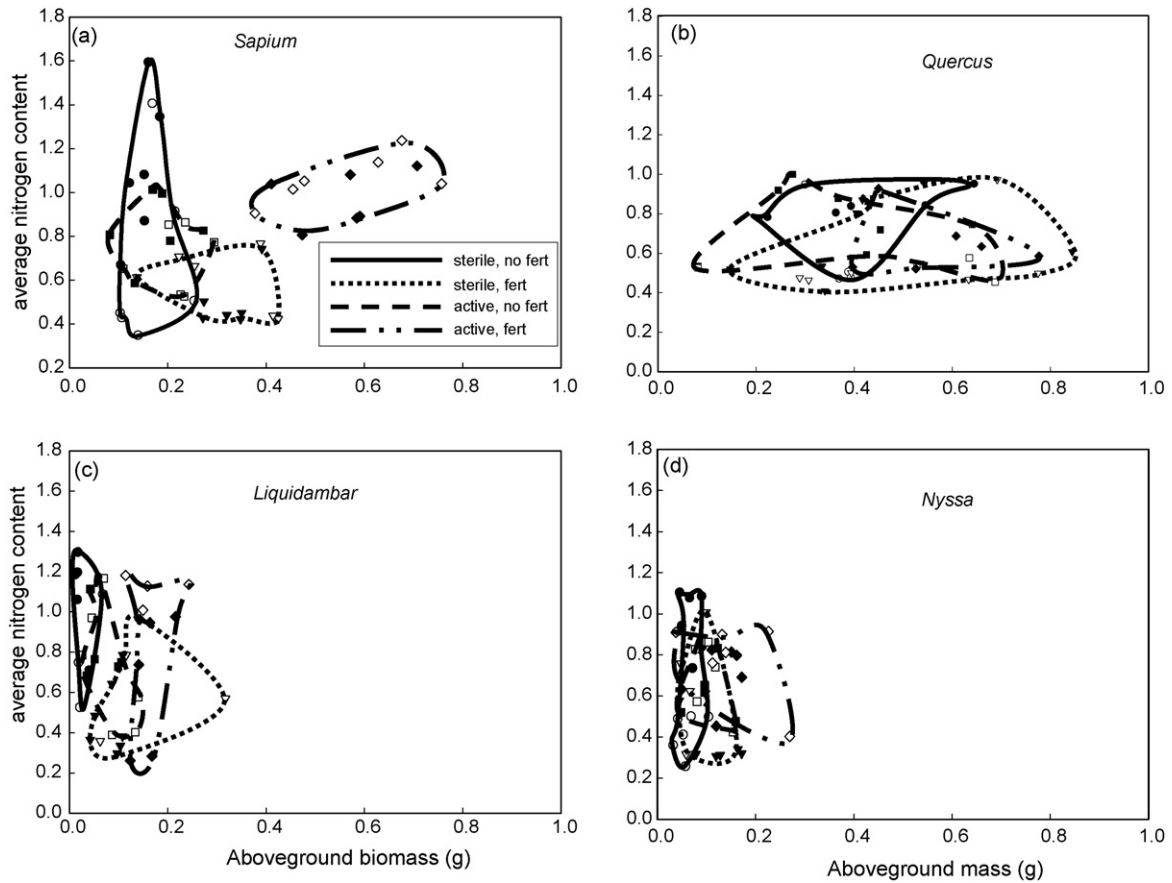


Fig. 5 – Scattergrams of average nitrogen vs. total aboveground biomass for (a) *Sapium*; (b) *Quercus*; (c) *Liquidambar*; and (d) *Nyssa*. Two way soil treatment by fertilizer interactions encircled by lines. Solid, dotted, dashed, and dotted-dashed lines represent: sterile, no fertilizer; sterile, fertilized; active, no fertilizer; active, fertilized, respectively.

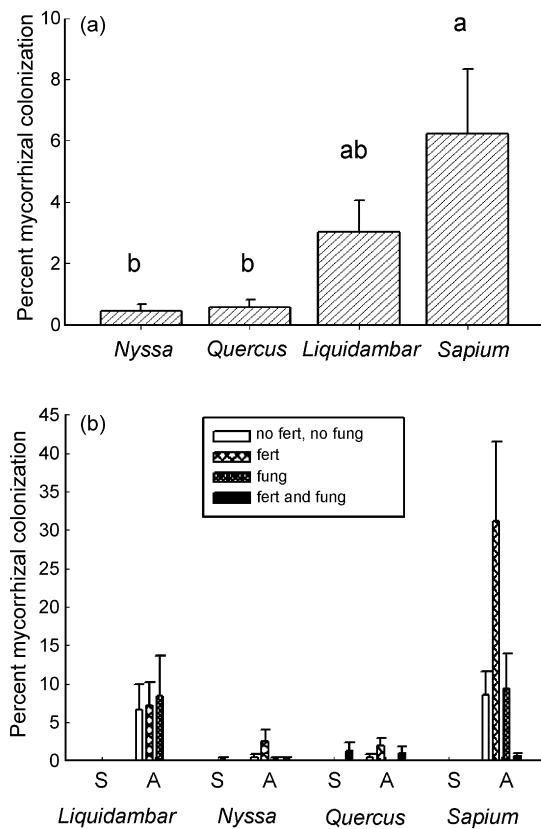


Fig. 6 – (a) The overall percent mycorrhizal colonization levels of each species. +1 Standard errors presented. Letters denote treatments that did not differ in means contrast tests. (b) The effect of soil treatment, fertilizer, and fungicide on the percent mycorrhizal colonization levels of all species. +1 Standard errors presented.

biomass \times soil treatment interactions for mycorrhizal or “other” colonization in individual plant species ANCOVA’s to warrant Spearman rank correlation analysis.

3.2. Field experiment

3.2.1. Height growth

The overall model for the relative change in height at the end of year one was not significant, nor were any of the terms in the model ($F = 0.99$, $P = 0.5211$). However, inspection of the data revealed trends for *Sapium* that paralleled those found in the aboveground biomass and height growth analyses of the pot study, with greater biomass under active, fertilized soils (Fig. 7a).

3.2.2. Survival

Survival time depended on three terms: species ($P = 0.0029$), species \times fertilizer ($P = 0.0003$), and plot (species \times fungicide \times fertilizer) ($P = 0.0079$). Individual species analyses were conducted to pinpoint the species driving the significant species \times fertilizer interaction in the overall model. *Quercus* had significant decreases in survival in fertilized conditions ($P = 0.0191$) whereas the other species did not.

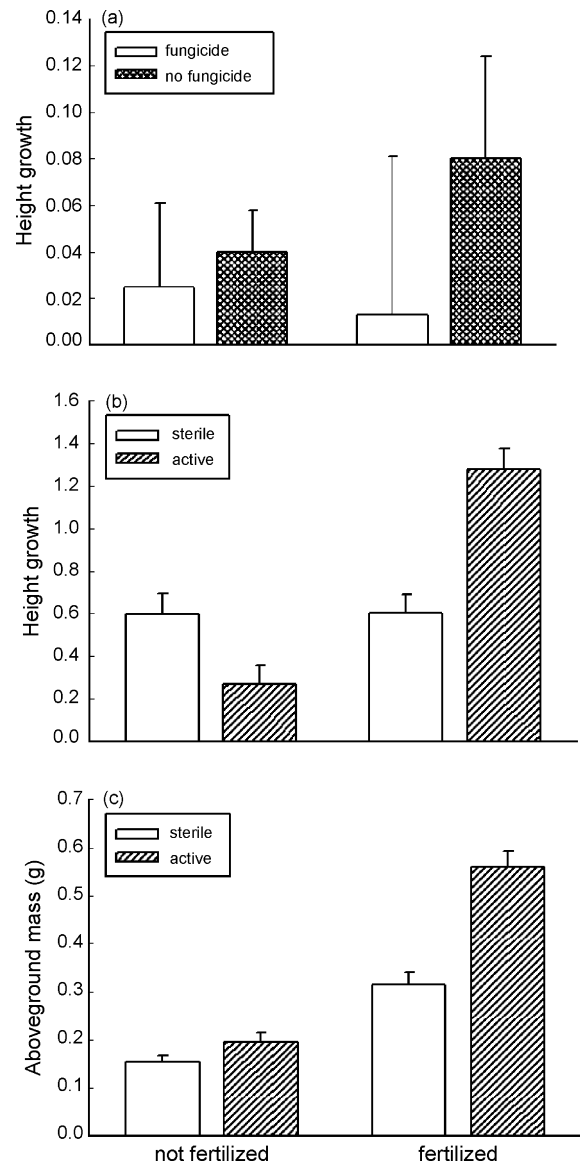


Fig. 7 – (a) The effect of fungicide and fertilizer on the height growth of *Sapium* seedlings in the field experiment. +1 Standard error presented. *Sapium*’s pattern of growth in field conditions is similar to the synergistic increases in (b) height growth and (c) aboveground biomass in active, fertilized soils in pots.

4. Discussion

The belowground soil community enhanced the growth and survival of tree seedlings in the 20-week pot experiment. Both the aboveground and belowground biomass of natives and the exotic *Sapium* decreased in sterilized soils (Figs. 1–3, Table 1). With an active soil community present, positive interactions between plants and their associated soil biota outweighed negative effects, suggesting a mutualistic interaction with the soil community was responsible for the positive aboveground growth responses seen in active field soils from east Texas. In addition, *Sapium* maintained high aboveground biomass while

hosting high levels of arbuscular mycorrhizal colonization in active soils compared to native species (Fig. 6a and b). This suggests that *Sapium* seedlings may benefit more from hosting arbuscular mycorrhizal mutualists in active soils than native species.

The prediction that fertilization would decrease the benefit of associations with mycorrhizal fungi was not supported. When mycorrhizae were present, no plant species in this study had decreased seedling height growth or biomass accumulation in fertilized conditions compared to unfertilized conditions (Figs. 1–3, Table 1). In addition, the prediction that the relative difference in seedling success with mycorrhizal associations would be more positive in unfertilized compared to fertilized soils was not supported for any species in this study (Figs. 1–3).

Results of this study did support the prediction that exotics may utilize soil biota in an unusual fashion relative to native plants in the introduced range. *Sapium* was the only species to display significant, synergistic growth increases under the combination of active soil and fertilization (Figs. 1–3 and 7), and the only species with high aboveground biomass together with high aboveground average nitrogen levels in active, fertilized soils (Fig. 5). In addition, *Sapium* showed height growth trends in the field experiment that paralleled the synergistic growth increases of *Sapium* in active, fertilized soils in the pot experiment (Fig. 6). *Sapium* also displayed significantly higher mycorrhizal colonization levels than all species and had dramatically higher levels of mycorrhizal colonization in fertilized soils than all other species. This suggests that *Sapium* experiences stronger positive interactions with the soil biota in east Texas than native species. This is one of the first studies to document the ability of an invasive species to utilize the belowground soil biota in an unusual fashion that may enhance its invasive potential. *Sapium*'s strong positive response to fertilization and unusually positive interactions with the soil biota in fertilized conditions (72.3% increase in biomass in field, fertilized soils) may aid its success as many areas where *Sapium* invades are experiencing increased anthropogenic nutrient inputs.

The “Enhanced Mutualisms Hypothesis” suggests that invasives may encounter soil biota that more strongly facilitate non-native establishment in the introduced range than in the native range (Reinhart and Callaway, 2006). Although arbuscular mycorrhizal fungi have not been thought to promote invasion because of their ubiquity and low host-specificity (generality of association), specific host-fungal pairings in the introduced range have the potential to differentially affect the growth responses elicited in plant hosts (specificity of response) (Richardson et al., 2000; Bever, 2002; Reinhart and Callaway, 2006). *Sapium*'s synergistic aboveground biomass and height growth increases in active, fertilized soils suggests that belowground soil organisms are mediating an unusual benefit to the invader not experienced by any of the native species used in this study (Figs. 1–3 and 7). The high levels of arbuscular mycorrhizal colonization found on *Sapium* compared to all other native species and similar results found in preliminary studies utilizing a mycorrhizal dip (Nijjer et al., 2004) suggest that arbuscular mycorrhizae may be involved in mediating this unusual benefit to the invader (Fig. 6a and b).

Only a few studies to our knowledge have documented clear examples of unusual mycorrhizal benefits to an invader in the introduced range; all were investigating *Centaurea maculosa* (Marler et al., 1999; Zabinski and Callaway, 2002; Carey et al., 2004). Marler et al. (1999) showed that mycorrhizae substantially increased the indirect competitive effect of *Centaurea maculosa* on *Festuca idahoensis* potentially through the mediation of a parasitic interaction. Carey et al. (2004) suggested that arbuscular mycorrhizal fungi enhance *C. maculosa* growth in its introduced range by providing a species-specific link for carbon transfer from neighboring *F. idahoensis*. However, Zabinski and Callaway (2002) found increased phosphorus uptake accessed by mycorrhizal associations in the rooting zone of neighboring plants resulted in higher aboveground biomass and luxury phosphorus concentrations in *C. maculosa* suggesting more efficient exploitation of mycorrhizal mutualists in the introduced range for phosphorus acquisition. Although the current study cannot pinpoint the exact mechanism responsible for *Sapium*'s success in active, fertilized soils, parasitism and nutrient robbing of neighbors could not have been the mechanisms responsible for *Sapium* success in this experiment because species were planted alone in pots.

Sapium is the only species that maintained a high average nitrogen content and high total aboveground biomass in active, fertilized soils (Fig. 5) which did not support the prediction of reduced benefits in fertilized conditions. In our experiment, nitrogen was added to the soil in highly available forms for uptake, and microbial transformations would not likely provide an additional benefit for plant nitrogen uptake. Arbuscular mycorrhizal fungi have been documented to aid plants in the capture and uptake of complex and inorganic forms of nitrogen from the soil and transfer large quantities to the plant (Read and Perez-Moreno, 2003; Govindarajula et al., 2005; Tu et al., 2006). Results of the current study support the idea that arbuscular mycorrhizae may translocate available inorganic forms of nitrogen in the soil to the aboveground biomass of *Sapium* plants. Arbuscular mycorrhizae could increase the efficiency of plant nitrogen uptake by increasing the area of nutrient absorption in the soil and translocating these nutrients to *Sapium*. Because arbuscular mycorrhizal fungi differ drastically in their colonization and growth capacities, specific plant-fungal interactions may have differing effects on the ability of mycorrhizae to translocate nutrients to plants and promote plant growth (Tu et al., 2006). *Sapium* may be hosting higher levels of an arbuscular mycorrhizal community that provides *Sapium* with a unique growth advantage from the acquisition of nitrogen compared to native plants but the species of AMF and specifics of the interaction may also be important.

The belowground soil community can have important consequences for the establishment and invasion of exotic species. Our results provide evidence that the belowground soil biota, potentially mycorrhizal mutualists, may not only contribute to, but also enhance the invasiveness of *Sapium* over natives. *Sapium sebiferum* receives unusual growth benefits in active, fertilized soils that may provide this species with a performance advantage over natives in the forest understory. A performance advantage would provide *Sapium* seedlings with a stronger chance of survival and establish-

ment in the forest understory over native species until gap formation, which may facilitate its further invasion into mesic, temperate forests. Further studies comparing the growth responses of *Sapium* in its native and introduced range are needed to generate a complete view of the magnitude of the soil biota's influence on *Sapium*'s invasive success.

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