Trophic dynamics in the fine-root based food web: integrating resource heterogeneity, root herbivores, and root foraging.

by

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ABSTRACT

Resources in the soil are heterogeneously distributed. We know that plant species differ in their root responses to nutrient patches and that these differences in foraging can influence plant competition. However, most studies of root-resource interactions overlook the potential top-down influence of root herbivores. While root herbivores can influence plant community structure, the extent to which they influence ecosystem-scale factors such as net primary production is unclear. In addition, little is known regarding root herbivore foraging behaviors and, more importantly, whether these foraging behaviors can actually influence species interactions. In this dissertation, I present a conceptual model of soil-root-herbivore interactions in which soil resource heterogeneity structures both root dynamics and the abundance and influence of root herbivores. I conducted two field and one greenhouse experiment examining this proposed model. The dissertation includes an introductory chapter (Chapter 1), a field study examining root responses to manipulations of soil fertility and root herbivory (Chapter 2), a greenhouse study that used plant species responses to heterogeneity to develop predictions about the role of root herbivores in mixed-species neighborhoods (Chapter 3), and a field study of planted communities examining soil fertility and fauna effects on above- and belowground structure and function (Chapter 4). In all cases, there were significant effects of root herbivores on community structure and components of net primary production. Resource distribution had a strong effect in studies conducted in sandy, nutrient-poor soils (Chapter 2 and 3), but had a reduced effect in the study conducted at Kentland Farm in loamy soils (Chapter 4). Interactions between resource availability and root herbivory were common. These results support the theory that the potential benefit of resource-rich patches may be constrained by root herbivores. This research complements recent findings that demonstrate other potential costs of species foraging behaviors (such as exposure to soil anoxia and increased drought stress), as well as potential effects of root herbivores and other soil fauna on plant diversity.

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CHAPTER 1:

INTRODUCTION

Soil nutrients are spatially heterogeneous. Variability of key nutrients such as nitrogen and phosphorous at scales of less than 1 m, well within the spatial scale of individual plant root systems, has been demonstrated in ecosystems as diverse as sagebrush steppe (Jackson and Caldwell 1993), deserts (Schlesinger et al. 1996), and tropical and temperate forests (Gonzalez and Zak, 1994, Gross et al. 1995, Farley and Fitter 1999a, Lister et al. 2000). These resource patterns both influence (Robinson et al. 1999, Bliss et al. 2002) and are influenced by (Guo et al. 2004) plant communities, and can have a strong effect on local trophic interactions (Hunter and Price 1992, Karr et al. 1992).

The spatial variability of soil resources (i.e., nutrient heterogeneity) has a clear influence on patterns of plant biomass allocation. Plants often increase the density of their root systems (i.e., forage) within nutrient-rich patches (Campbell et al. 1991, Einsmann et al. 1999, Farley & Fitter 1999b; Robinson et al. 1999). The biomass of some plants under heterogeneous conditions is greater than that under homogeneous conditions; such plants have been described as 'sensitive' to heterogeneity (Wijesinghe and Hutchings 1997, Einsmann et al. 1999). The extent of the root foraging response and plant sensitivity to heterogeneity varies between plant species (Einsmann et al. 1999, Bliss et al. 2002, Rajaniemi and Reynolds 2004), and may impact competitive interactions in plant communities (Robinson et al. 1999, Casper et al. 2000, Fransen and de Kroon 2001, Bliss et al. 2002).

Despite these potentially strong effects on plants, the majority of root foraging studies to date have overlooked potential interactions between resource fertility and root herbivory. Studies have generally been conducted either in highly controlled conditions where root feeders are never present, where they have been eliminated by soil fumigation, or where their response was not measured. However, root herbivores have shown strong potential to impact plant communities: reduction of soil herbivores can significantly impact individual species (Mitchell et al. 1991,

Strong et al. 1996, Maron 1998) and the diversity and structure of plant communities (Hendrix et al. 1988, Brown and Gange 1989, 1992, Masters and Brown 1997).

There are potentially strong interactions between fine root herbivory, microsite fertility, and fine root growth that can lead to important tradeoffs for individual plants. Plants that proliferate roots in nutrient-rich microsites in patchy environments can co-opt resources and grow larger than their non-proliferating competitors (Robinson et al. 1999, Bliss et al. 2002); this is considered to be the major benefit of root foraging in rich patches. In natural systems, however, root herbivores are likely to also respond to rich patches: root-feeding insects use CO_2 concentrations as a primary food-source cue (Jones and Coaker 1977, Brown and Gange 1990), and densities may be greater in nutrient-rich areas where roots proliferate and respiration rates are higher (Hogberg et al. 2001). Increased vulnerability of roots in these nutrient-rich microsites due to preferential herbivore foraging, therefore, is a significant potential cost of root foraging behavior.

In the following chapters, I present the results of experiments designed to examine how nutrient heterogeneity and root herbivores might interact to influence fine root dynamics and plant community structure and function. These experiments involved manipulations of total resource availability, resource distribution patterns, and soil organisms. I measured the impacts of these soil fertility and fauna treatments on root biomass, total plant biomass, growth of individual plant species, and the presence of root herbivores. These experiments included a field study examining root responses to manipulations of soil fertility and root herbivory (Chapter 2), a greenhouse study that used plant species responses to heterogeneity to develop predictions about the role of root herbivores in mixed-species neighborhoods (Chapter 3), and a field study of planted communities examining soil fertility and fauna effects on above- and belowground structure and function (Chapter 4).

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CHAPTER TWO:

PATTERNS IN SOIL FERTILITY AND ROOT HERBIVORY INTERACT TO INFLUENCE FINE ROOT DYNAMICS.

ABSTRACT

Fine scale soil nutrient enrichment typically stimulates root growth, but it may also increase root herbivory, resulting in tradeoffs for plant species and potentially influencing carbon cycling patterns. We used root ingrowth cores to investigate the effects of microsite fertility and root herbivory on root biomass in an aggrading upland forest in the coastal plain of South Carolina. Treatments were randomly assigned to cores from a factorial combination of fertilizer and insecticide. Soil, soil fauna, and roots were removed from the cores at the end of the experiment (8-9 months), and roots were separated at harvest into three diameter classes. Each diameter class responded differently to fertilizer and insecticide treatments. The finest roots (<1.0mm diameter), which comprised well over half of all root biomass, were the only ones to respond significantly to both treatments, increasing when fertilizer and when insecticide was added (each $P<0.0001$), with maximum biomass found where the treatments were combined (interaction term significant, $P<0.001$). These results suggest that root-feeding insects have a strong influence on root standing crop with stronger herbivore impacts on finer roots and within more fertile microsites. Thus, increased vulnerability to root herbivory is a potentially significant cost of root foraging in nutrient-rich patches.

KEYWORDS

Root ingrowth core, Scarabaeidae, Elateridae, heterogeneity, root foraging

INTRODUCTION

Although the relative strength of resource (bottom-up) and consumer (top-down) forces on primary producers has long been a central theme in terrestrial ecology (Hairston et al. 1960, Fretwell 1987, Matson and Hunter 1992), integrated

studies of their interactions in the soil are rare (Hunter 2001). The individual influences of soil resource heterogeneity on root density (Drew 1975, Robinson 1994), and of root herbivory on plant succession (Hendrix et al. 1988, Brown and Gange 1990) have been investigated. However, we have little knowledge of how spatial variability in soil fertility acts to structure root-based food webs. Since 50% or more of total ecosystem primary production may be allocated to roots (Jackson et al. 1997), it follows that interactions among resources, roots, and root herbivores can have important implications for both plant communities and ecosystem carbon and nutrient cycles.

Fine-scale (sub meter) spatial patterns in soil resources elicit foraging responses in plants. Roots tend to proliferate (i.e., forage) in soil patches of increased nutrient availability (Einsmann et al. 1999, Farley and Fitter 1999, Robinson et al. 1999); however, this response varies widely among species within a community (Einsmann et al. 1999, Wijesinghe et al. 2001, Rajaniemi and Reynolds 2004). Plants that selectively forage in nutrient-rich microsites can co-opt resources and grow larger than their non-proliferating competitors (Robinson et al. 1999, Hutchings et al. 2003). While foraging may confer benefits to intensively-foraging plants, there are costs involved, and a less precise forager may persist in a community at a place or time where the costs of foraging outweigh the benefits (Fransen and De Kroon 2001, Alpert and Simms 2002).

An overlooked potential cost that may increase with foraging is increased exposure to root herbivory. In natural systems, root herbivores are likely to respond to rich patches, as root-feeding insects use CO_2 concentrations as a primary food-source cue (Jones and Coaker 1977, Brown and Gange 1990). Therefore, root herbivore densities should be greater in nutrient-rich areas where roots proliferate and respiration rates are higher (Hogberg et al. 2001). Thus, root herbivores may act in such a way that imparts a significant cost to root foraging behavior, reducing the net benefit of foraging and influencing competition between plants with different foraging strategies.

Root herbivores may influence not only community-scale interactions (Brown and Gange 1990, Blossey and Hunt-Joshi 2003), but also root lifespans, rates of fine

root turnover, and ecosystem carbon cycling. In general, the distribution of root lifespans is understood to be strongly left-skewed; many roots live for as little as one to two weeks, most live for only a few months, while some may live for several years (Matamala et al. 2003, Trumbore and Gaudinski 2003). If herbivores focus on the youngest, finest-diameter roots, which are presumably the most palatable and least defended (Graham 1995, Eissenstat and Yanai 1997), but are also the most critical for resource uptake (Eissenstat and Yanai 1997), root herbivory may be one of the underlying causes of this skewed distribution.

The main objectives of this study were to determine how root herbivores affect fine root dynamics within the scale of small patches (microsites) in an aggrading pine-hardwood forest, and whether microsite fertility influences the magnitude of root herbivore effects. We installed root ingrowth cores at the beginning of the growing season, amended them with fertilizer and insecticide treatments, and returned at the end of the season to measure net fine root growth and presence of root herbivores. The data were used to test four hypotheses: (1) suppression of soil fauna would lead to increased fine root standing crop due to reduced levels of root consumption (root herbivory); (2) the influence of root herbivore suppression would be greater on more fertile microsites (i.e., a fertilizer and insecticide interaction) due to the proliferation response of roots in nutrient rich patches; (3) changes in root biomass in response to root herbivore suppression should be greater in finer-diameter roots (i.e., those < 1.0 mm in diameter), compared to coarser roots, since finer roots generally have reduced defenses against and increased risk of herbivory; and (4) root-feeding insect larvae would be found in higher densities in fertilized microsites because of preferential herbivore foraging in nutrient- or root-rich microsites.

METHODS

Study site

Research was conducted at the Savannah River Site (SRS), a National Environmental Research Park administered by the United States Department of Energy and located in the coastal plain of South Carolina. The climate is subtropical

with mean July maximum, January minimum and annual temperatures of 27, 9 and 24 ^{o}C, respectively and mean annual precipitation of 113 cm distributed relatively evenly throughout the year (Rogers 1990). Soils at the study site are Dothan and Fuquay series sands (loamy, kaolinitic, thermic Kandiudults, Rogers 1990).

The experiment was conducted during the 2002 growing season in an aggrading dry upland forest harvested in 1995. Prior to harvest, the stand was a 40-year old *Pinus elliottii* Engl. plantation. After harvest, vegetation was dominated by seedlings of a mix of tree species, including *P. elliottii, P. palustris* Mill., and several *Quercus* species, as well as a wide range of early successional herbs including various *Andropogon, Hypericum,* and *Rubus sp.* (Poaceae, Clusiaceae, and Rosaceae respectively).

Treatments and response measurements

Within a 50 m by 50 m plot, 200 sampling points were established (each representing a sampling microsite) on a 1m by 1m grid. Gridpoint locations for sampling sites were selected by randomly generating X-Y coordinates, with the restriction that sampling sites be separated by a minimum of 1.4 meters. Microsites were randomly assigned to treatments (50 per treatment) from a 2 x 2 factorial combination of ± fertilizer (Osmocote slow-release 15-9-12 plus minors) and ± insecticide (granular chlorpyrifos, Lorsban 15G). Ingrowth cores were installed in March of 2002 at each microsite by first removing a 10 cm diameter x 30 cm deep core of soil, then extracting the roots by sifting over a mesh sieve, amending the soil according to treatment (5.5 g fertilizer and/or 0.20 g Lorsban 15G), and, finally, repacking the soil to approximate the original bulk density. Roots removed from each core at the time of installation were returned to the lab, separated from residual soil, and divided into fine- and coarse-root fractions (< 2.0 and > 2.0 mm diameter, respectively). Root samples were then dried, weighed, and ash-corrected (10 minutes at 500 degrees Celsius) to estimate microsite ash-free root mass present at treatment installation, a potential covariate that may explain root production within ingrowth cores. Additional insecticide was incorporated into the top 1-2 cm of each insecticide-treatment microsite at approximately six-week intervals. During reapplication events, all microsite locations were visited and similarly disturbed.

Although observations of soil fauna at the time of installation were not recorded, no macro-organisms were observed other than termites (Isoptera), which were associated with woody debris in a limited number (< 10%) of samples. Woody debris was not returned to the cores when they were repacked.

Twenty-five randomly selected microsites from each of the four treatments were harvested in October and November of 2002 (seven and eight months after installation, respectively) by removing a 7.5 cm diameter x 30 cm deep core centered over the 10 cm diameter installation location. Soil samples were kept in coolers while transported to the Virginia Tech campus in Blacksburg, VA and stored at 3°C until processing. Fine roots and soil insects were removed from the samples by hand washing of roots over a 1mm mesh screen. Washed root samples were separated into three diameter classes (< 1.0 mm, 1.0 – 2.0 mm, and > 2.0 mm) mainly by eye, occasionally verified using dial calipers. Root samples were then dried, weighed, and ash-corrected (10 minutes at 500°C) to determine net production of microsite ash-free dry root mass (hereafter fine root biomass) per cm^3 in each size class over the period of the experiment. Soil insects from samples were stored in ethanol until identified to family using the keys of Stehr (1987). After identification, insects were dried to a constant mass at 60° C and weighed to determine insect biomass.

Test of direct effects of insecticide on plants

To examine our assumption that insecticide applications would have no direct influence on roots (i.e., insecticide-as-fertilizer effect), we conducted a greenhouse experiment using site-gathered soil and seed of three of the most common perennials at our site (two broad-leaved herbs, *Solidago altissima* L. and *Eupatorium compositifolium* Walt., and one grass, *Andropogon ternarius* Michx.). Plants were grown from seed in 15 cm diameter by 12.5 cm deep greenhouse pots in the spring of 2003 (20 pots per species), and allowed to grow for approximately four months. Beginning two weeks after plants were added to pots, 0.20 g of chlorpyrifos was added to ten of the pots at similar intervals to that used in the field experiment. At harvest, individual plants were separated into above- and belowground portions. Root samples were separated into < 1.0, 1.0 – 2.0, and > 2.0 mm diameter classes.

Root and shoot samples were dried to a constant mass, and then weighed to determine biomass in each pot.

Statistical analysis

All statistical comparisons were performed using Statistical Analysis System (SAS) software, version 8.01 (SAS Institute Inc., Cary, NC). Before addressing our hypotheses, we tested several underlying assumptions. First, to test for direct insecticide effects on plants, we used MANOVA (SAS Proc GLM) to analyze the responses of aboveground, belowground (each of three root diameter classes, <1.0 mm, 1.0 - 2.0, and > 2.0), and total plant biomass of each species to the application of soil insecticide in the single-plant greenhouse pots. Second, to assess the effectiveness of our insecticide applications, we compared insect census data from cores with and without insecticide. Third, potential differences in root and insect responses between October and November harvest dates were assessed using ANOVA.

To test our hypotheses that (1) herbivores had a significant impact on root biomass, (2) this impact increased with increased microsite fertility, and (3) root biomass responses varied between different diameter classes of roots, we compared the fine root biomass in each of our three root diameter classes (< 1.0 mm, 1.0 - 2.0, and > 2.0) from the four ingrowth core treatments (control, insecticide only, fertilizer only, and insecticide plus fertilizer) using multiple analysis of covariance (MANCOVA) with a 2 x 2 factorial design that included root mass in cores at the time of installation (i.e., February 2002) as a covariate. The data generally did not meet two of the statistical assumptions required for ANOVA (normality and homogeneity of variance), and standard transformations did not result in normality or homogeneity. Therefore, relationships between treatments and root biomass in each diameter class were tested using nonparametric statistics (Kruskal-Wallis, PROC NPAR1WAY). This required combining the two treatments into a single, four-level treatment variable. Significance among these four pseudo-treatments was assessed using a Kruskal-Wallis test for each diameter class of roots. If the latter test was significant, pairwise comparisons of groups (e.g., individual tests of fertilizer and pesticide effects) were performed using Mann-Whitney tests. In all cases, the

outcomes of the nonparametric tests agreed with the outcomes of parametric ANOVA (at the $P = 0.05$ level), and we present only parametric results in this paper. Our fourth hypothesis regarding the presence of insect herbivores in fertilized relative to unfertilized cores was tested using chi-square analysis due to the low occurrence of soil insects. We also analyzed treatment effects on root herbivore biomass using nonparametric ANOVA.

RESULTS

Insecticide-as-fertilizer and harvest date influences

In our potted-plant experiment testing for direct effects of the insecticide on plant growth, we observed no direct effects of insecticide on any component of plant biomass (overall insecticide effect, Wilks' lambda = 0.96, $F_{4,51}$ =0.52, $P = 0.72$, Table 2.1). Although the insecticide did not affect plant biomass, it effectively reduced insect biomass in the field, as we saw no insects in our insecticide-treated cores.

Harvest date did not influence root biomass. The main effect of harvest date ($F_{1,191} < 2.21$, $P > 0.14$) and its interaction with fertilizer (all diameter classes $F_{1,191} < 2.50$, $P > 0.11$) and insecticide (all $F_{1,191} < 2.6$, $P > 0.10$) were non-significant. As a result, harvest dates were combined for all further analyses of root biomass. The proportion of cores with insects did vary significantly between harvest dates ($\chi^2 = 11.96$, $P < 0.001$). Insects occupied cores more often in October than in November (18 cores with insects in October vs. 1 in November).

Effect of treatments on roots

Fine root biomass in the smallest size class (i.e., diameter < 1.0 mm) ranged from 0.38 ± 0.037 mg/cm^3 (mean ± std. err) in control microsites to a maximum of 2.1 ± 0.15 mg/cm3 in microsites containing both insecticide and fertilizer additions, a more than five-fold difference in root biomass (Fig. 2.1, Table 2.2). Individually, both insecticide applications and fertilizer additions resulted in significantly greater root biomass (nearly two- and three-fold increases respectively) compared to control ingrowth cores ($F_{1,191} = 48$ and $F_{1,191} = 133$, respectively, both $P < 0.001$); additionally, we saw a significant interaction between fertilizer and insecticide ($F_{1,191} = 13.6$, $P < 0.001$, Table 2.2), a result of a greater positive effect of insecticide

application within fertilized than within unfertilized cores (Fig. 2.1). Together, the two variables (fertilizer and insecticide) and their interaction explained half of the variation in < 0.1 mm diameter fine root biomass observed at the time of harvest (R^2 = 0.50).

Biomass of the two remaining diameter classes of roots (1.0 – 2.0 mm and > 2.0 mm) was much lower than in the smallest diameter class, and these roots responded to treatments differently than did the finest roots. Mid-range roots (1.0 – 2.0 mm) had significantly ($F_{1,191}$= 5.28, P = 0.02, Table 2.2) more biomass in fertilized (0.088 ± 0.012 mg/cm^3) than unfertilized cores (0.14 ± 0.030, Fig. 2.1), but did not respond to pesticide application ($F_{1,191}$= 0.49, P = 0.48, Table 2.2). Biomass of the coarsest diameter roots (> 2.0 mm diameter) did not vary significantly in any of our treatments (Table 2.2).

Insect response to treatments

In insecticide-treated cores, we observed no insect adults or larvae. On the other hand, we found insects in 19 of the 100 cores where insecticide was not applied. Nearly all of the cores with insects contained only a single individual, although two cores contained two (21 insects in all cores). We found representatives from the orders Coleoptera, Diptera, and Blattaria (Table 2.3). Larvae were found from the families Scarabaeidae and Elateridae (Coleoptera) and Asilidae and Tipulidae (Diptera). We also found adult Scarabaeidae and Carabidae (Coleoptera) and Blattellidae (Blattaria).

Of those insects we observed, larvae from the families Scarabaeidae and Elateridae are well-known root feeders, and some larval Tipulidae feed on roots (Borror et al. 1989). These taxonomic groups represented 76% (16 of 21) of the insects found in our cores (detailed results in Table 2.3). Scarabaeidae larvae (commonly referred to as white grubs) were the dominant root herbivores in our cores.

Root herbivore counts varied between harvest dates, but did not differ significantly between treatments. We saw herbivores in cores more frequently in October (14 insects) than in November (only 2, χ^2 = 11.9, P < 0.001, Table 2.3). While we found root herbivores more often in fertilized cores (11 insects) than

control cores (5), and root herbivore biomass per unit area was 8 times higher in fertilized than unfertilized cores (Table 2.4), neither occurrence nor biomass were significantly different between treatments.

DISCUSSION

Root effects

Results supported our first three hypotheses that herbivory leads to reduced root biomass, herbivory is more intense in fertile than in relatively less fertile microsites, and root herbivory was focused on the thinnest diameter roots. To our knowledge, this is the first study that has demonstrated an interaction between root herbivory and fertility. Research on belowground herbivory is generally uncommon (Hunter 2001); however, there is ample evidence that aboveground, herbivory, nutrients and other factors such as producer biomass and natural enemies can interact (Hunter and Price 1992, Hunter et al. 1992). For example, in a study of black locust trees (*Robinia pseudoacacia* L.), fertilized trees initially suffered higher losses to herbivory, although they eventually appeared to gain some protection due to increases in foliar defenses through secondary compounds (Hargrove 1984). In *Spartina* marshes, increasing fertility results in increased plant growth but also increased herbivore (planthopper) colonization, survival, and fecundity; control by natural enemies was most significant in low-nutrient conditions (Denno et al. 2002). We saw a similar pattern in our study of belowground trophic dynamics, albeit at much different spatial scale—a positive plant response to increasing fertility that was constrained by increased root herbivory on more-fertile sites. Exploring both the responses of the roots themselves to increased root herbivore activity and the responses of the herbivores' natural enemies (including parasitoids such as entomopathogenic nematodes, Strong et al. 1996, Preisser 2003) is the next step in building an understanding of the role of nutrient heterogeneity on root-based food webs. We found larvae of the family Asilidae in two of our root cores; these larvae are potential predators of root-feeding scarabs in the pupal stage (Vittum et al. 1999).

Although the organophosphate insecticide we used to control soil fauna (chlorpyrifos) may theoretically stimulate production (i.e., act as a fertilizer), neither

our greenhouse experiment nor those carried out by other researchers using similar formulations of the same compound have provided evidence of such direct effects on soil fertility (Wells et al 2002) or on the biomass of plant roots (Coupe 2003) or shoots (Brown and Gange 1989, 1990, Coupe 2003). This supports our assertion that the increase in root mass we observed in insecticide-treated cores was due to a reduction in fine root herbivory.

Root herbivore responses and life cycles

While our hypotheses regarding root responses were supported, support for our fourth hypothesis that root herbivores would be more common on fertilized than 'control' microsites was mixed. Despite the clear influence of insecticide additions on root biomass, the proportion of samples containing root-feeding larvae was low (only 16% at the time of harvest). Although we did see higher total numbers of insect larvae in fertilized cores than unfertilized cores, the difference was not statistically significant, leading us to reject this hypothesis.

The incongruity between strong effects on roots and low frequency of root herbivores may have been caused by the timing of our observations relative to the life cycle of the dominant root herbivores (Scarabaeidae larvae, white grubs). White grub root herbivory is generally concentrated into two periods over a growing season. During a given year, the first of these feeding periods occurs in spring, from mid-March to early May; the second occurs between early August and late autumn, at which point the larvae descend to deeper soil horizons (> 30 cm depth) to overwinter. Given these life history patterns, larvae that fed in the cores may have emerged as adults in early summer, or descended beyond our sampling depth by the time of harvest.

Although we did not observe a large number of root herbivores in our samples, our density estimates match up well with those from other studies. In a multi-year study of soil fauna on the Konza prairie, Callaham et al. (2003) observed average white grub densities of approximately 5 individuals/m^2, and slightly higher average elaterid densities; these are much lower numbers than we observed. White grub densities of 46.3 and 47 individuals/m^2 were reported by Ueckert (1979) and

Lura and Nyren (1992) in infested areas of shortgrass and mixed-grass prairie, respectively; these densities are nearly equal to those observed in our October samples (45 larvae/m^2, Table 2.3). On the other hand, the rates of occurrence (percentage of samples containing a given taxa) we observed appear low when compared to samples taken near our site; a survey of soil fauna from Calhoun Experimental Forest, located in the South Carolina Piedmont (Callaham et al. in review), found Scarabaeidae and Elateridae in a larger percentage of samples (50 and 30%, respectively) than we did in this study (20 and 6% for October samples, Table 2.3). The lower occurrence rates we observed may reflect the reduced size of individual samples in our study (7.5 cm diameter cores vs. 30 x 30 cm soil pits used by Callaham et al.), or the fact that we sampled late in the year, at a time when many larvae may have descended to deeper soil depths to overwinter.

Costs and benefits of root foraging

Our study shows that increased exposure to root herbivores may be a significant cost of root foraging behavior. This has at least three important implications for interpretation of root foraging studies. First, it means that laboratory or greenhouse experiments, which usually have little or no root herbivory, probably overestimate benefits for precise foraging in the field, and fail to detect important indirect effects that herbivores may have on competitive interactions between plants in natural soil. In our experiment, those plants that foraged heavily in nutrient-rich patches were apparently exposed to higher levels of herbivory than were plants that foraged in areas of lower fertility. Previous studies have shown that fine root foraging in nutrient-rich microsites significantly influences inter- and intra-specific competition (Robinson et al. 1999, Fransen et al. 2001, Day et al. 2003), but the role that root herbivores may play has been overlooked. It is possible that herbivores that forage in rich patches may influence competitive outcomes in plant communities by more strongly affecting aggressively proliferating plant species. Second, increased rates of herbivory in nutrient rich patches may explain why some field studies have demonstrated a lack of root foraging precision in some plant species under some conditions, or have failed to see effects of heterogeneity on growth or competitive interactions in diverse communities (Casper et al. 2000, Bliss et al. 2002). In these

studies, root herbivores may be acting as a selective force or a modulator of plant-plant resource competition. Finally, short-term studies, even if conducted in the field, may be confounded if fewer root herbivores exist in the study site than are typical (due to slow movement through the soil, delayed site invasion after soil fumigation, etc).

The cost imposed by exposure to root herbivory, in addition to its implications for interpreting previously published studies, may also impact evolution of plant foraging behavior and coexistence of species. Fine-scale spatial structuring of nutrients, roots and herbivory may help to explain the range of foraging responses seen even between species with similar adult size and life histories (Einsmann 1999, Bliss et al. 2002, Hodge 2004). These potentially ultimate (evolutionary) influences on plants combined with more proximal influences of root herbivory may mediate coexistence of species in plant communities, particularly if spatially patchy herbivory suppresses aggressively foraging plant species, allowing species that forage less precisely (and presumably less efficiently) to persist where they would otherwise be outcompeted.

Implications for carbon cycling belowground

Root herbivory represents a rapid route of root turnover, one that is congruent with our improving understanding of the predominance of young age classes in root systems. Whereas previous models had assumed a normal distribution of fine root lifespans, the reality appears to be that root lifespans are strongly left-skewed (Tierney and Fahey 2002, Trumbore and Gaudinski 2003). Due to herbivory or other causes, many roots disappear within weeks to months of 'birth' (Stevens et al. 2002, Wells et al. 2002, this study), although some roots may live for as long as 9 years (Gaudinski et al. 2001, Matamala et al. 2003). What has been thought of as a large, homogeneous pool of roots is in truth a combination of different pools, more of which turn over more quickly and some of which live much longer than we have previously understood (Pregitzer 2002, Trumbore and Gaudinski 2003).

Root herbivory likely represents a significant cause of fine root turnover in many terrestrial systems, given the widespread distribution of the herbivores themselves. In the family Scarabaeidae, some 153 species from the genus

Phyllophaga have been identified across the United States (Forschler and Gardner 1990), and the Japanese beetle (*Popillia japonica*) is common to nearly all states east of the Mississippi River (Vittum et al. 1999).

Although this project focused on the responses of roots and soil macrofauna to fine-scale nutrient patches, soil organisms such as plant parasitic nematodes, mites, or Collembola likely also responded to our experimental treatments. Soil biota generally exhibit strong spatial heterogeneity, with many organisms exhibiting patchy distributions at fine (sub-meter) spatial scales (Ettema and Wardle 2002). The mechanisms controlling these distributions are variable, and may include oviposition effects, responses to patterns in soil moisture and resource availability, and responses to patterns in plant growth (Ettema and Wardle 2002). In this study, insecticide application likely led to reduced populations of all soil organisms, as chlorpyrifos applications generally result in reduced densities of earthworms (USDA 2001), Collembola (Frampton 1999, Pereira et al. 2005), mites (Cabrera 2004, Pereira et al. 2005, but see Michereff-Filho et al. 2004 for contrasting results) and soil arthropods in general (Wang et al. 2001, Dawson et a. 2003, Pereira et al. 2005). The effect of increased microsite fertility on soil fauna is more difficult to predict. Although organisms such as plant feeding nematodes are known to increase in density after fertilization (Smolik and Dodd 1983, Todd 1997), such responses are generally measured at the scale of large field plots, and overlook fine-scale effects. In a pot study examining the effects and responses of soil fauna, DeDeyn et al (2004) observed that the densities of plant parasitic nematodes increased with root biomass, and attributed this to bottom-up effects of resource supply on root herbivores. We saw a similar trend in this study, with increased losses to root herbivores in more-fertile patches. Although we limited our assessments of soil fauna to macroarthropods, it is very reasonable to assume that other types of root herbivores responded positively to the increased root biomass available in fertilized patches.

In sum, we have demonstrated that root-feeding fauna can have significant effects on fine root dynamics (and thereby carbon cycles) that increase with microsite fertility. This suggests that nutrient patterns in the soil may act as a template that structures root-based food webs, and further supports the need to consider root

herbivores in our models of plant community interactions (Bardgett and Wardle 2003, DeDeyn et al. 2004) and ecosystem carbon cycling.

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Table 2.1. Results of (a) MANOVA and (b, c, d, e, f) ANOVA analysis of greenhouse study, using root biomass from each of the three diameter classes (< 1.0, 1.0 – 2.0, and > 2.0), shoot biomass, and whole-plant biomass in relation to species and application of insecticide.

a) MANOVA				
	df	Wilks' lambda	*F*	*P*
Species	8, 102	0.096	28.340	<0.001
Insecticide	4, 51	0.961	0.520	0.719
Species x Insecticide	8, 102	0.935	0.430	0.899
b, c, d, e, f) ANOVA				
	df	MS	*F*	*P*
b) Biomass, roots < 1.0 mm diameter				
Species	2	42.071	48.390	< 0.001
Insecticide	1	0.210	0.020	0.877
Species x Insecticide	2	0.699	0.800	0.453
Error	54	0.870		
c) Biomass, roots 1.0 - 2.0 mm diameter				
Species	2	0.474	6.130	0.004
Insecticide	1	0.026	0.340	0.564
Species x Insecticide	2	0.012	0.150	0.862
Error	54	0.087		
d) Biomass, roots > 2.0 mm diameter				
Species	2	6.091	31.480	< 0.001
Insecticide	1	0.000	0.000	0.988
Species x Insecticide	2	0.154	0.800	0.455
Error	54	0.194		
e) Shoot biomass				
Species	2	28.251	14.840	< 0.001
Insecticide	1	2.521	1.320	0.255
Species x Insecticide	2	0.341	0.180	0.836
Error	54	1.903		
f) Whole plant biomass				
Species	2	27.009	6.870	0.002
Insecticide	1	1.625	0.410	0.523
Species x Insecticide	2	1.721	0.440	0.648
Error	54	3.914		

Table 2.2. Results of (a) MANCOVA and (b, c, d) ANCOVA, using root biomass at harvest from each of the three diameter classes (< 1.0, 1.0 – 2.0, and > 2.0mm) in relation to root biomass at the time of installation (covariate) and the application of fertilizer, soil insecticide, and their interaction.

a) MANCOVA				
	df	Wilks' lambda	*F*	*P*
Installation biomass	3, 189	0.99	0.58	0.630
Fertilizer	3, 189	0.58	45.25	<0.001
Insecticide	3, 189	0.79	15.92	<0.001
Insecticide x Fertilizer	3, 189	0.92	4.83	0.003
b, c, d) ANCOVA				
	df	MS	*F*	*P*
b) Roots < 1.0 mm diameter				
Installation biomass	1	0.62	0.78	0.460
Fertilizer	1	105.25	132.42	<0.001
Insecticide	1	37.65	47.38	<0.001
Insecticide x Fertilizer	1	10.68	13.45	<0.001
Error	195	0.79		
c) Roots 1.0 - 2.0 mm diameter				
Installation biomass	1	0.01	0.35	0.553
Fertilizer	1	0.19	5.35	0.022
Insecticide	1	0.02	0.50	0.481
Insecticide x Fertilizer	1	0.02	0.46	0.501
Error	195	0.04		
d) Roots > 2.0 mm diameter				
Installation biomass	1	0.05	0.60	0.440
Fertilizer	1	0.01	0.16	0.686
Insecticide	1	0.10	1.34	0.248
Insecticide x Fertilizer	1	0.00	0.03	0.865
Error	195	0.08		

Table 2.3. Insect data determined from ingrowth cores. As all insects were found in untreated (no insecticide) ingrowth cores, density data are based on untreated cores (50 per sampling date, 100 total, each 7.5cm diameter x 30cm deep). Families in ***bold italics*** represent those with known root-feeding larvae.

Order	Family	Number of individuals			Proportion containing			Density, larvae/m2		
		Oct.	Nov.	Total	Oct.	Nov.	Total	Oct.	Nov.	Total
Beetles	***Scarabaeidae (larvae)***	***10***	***1***	***11***	***0.20***	***0.02***	***0.11***	***45***	***4.5***	***24.8***
	Scarabaeidae (adult)	1	--	1	0.02	--	0.01	4.5	--	2.3
	Elateridae	***3***	***1***	***4***	***0.06***	***0.02***	***0.04***	***13.4***	***4.5***	***9***
	Carabidae	1	--	1	0.02	--	0	4.5	--	2.3
Flies	Asilidae	2	--	2	0.04	--	0.02	9	--	4.5
	Tipulidae	***1***	***--***	***1***	***0.02***	***--***	***0.01***	***4.5***	***--***	***2.3***
Roaches	Blatellidae	1	--	1	0.02	--	0.01	4.5	--	2.3
All root herbivores		***14***	***2***	***16***	***0.28***	***0.04***	***0.16***	***63.1***	***9***	***36***

Table 2.4. Root and root-herbivore data by fertilizer treatment, considering only cores without pesticide (50 cores per treatment). Data combine October and November harvest dates. Where available, biomass means are reported as mean (std. err.). Root biomass is ash-free dry mass of roots < 1.0 mm diameter; herbivore biomass is oven-dry biomass.

	Treatment	
	Control	Fertilized
Root biomass (mg/cm^3)	0.38 (0.037)	1.12 (.094)
Root herbivore biomass ($\mu g/cm^3$)	0.79 (0.56)	6.6 (2.4)
Number of root herbivores	5	11
Number of individuals		
Elateridae	3	1
Scarabaeidae	2	9
Tipulidae	0	1
Average individual biomass (mg)		
Elateridae	1.67 (0.67)	3.12
Scarabaeidae	24.50 (12.50)	48.88 (10.61)
Tipulidae	0	9.03

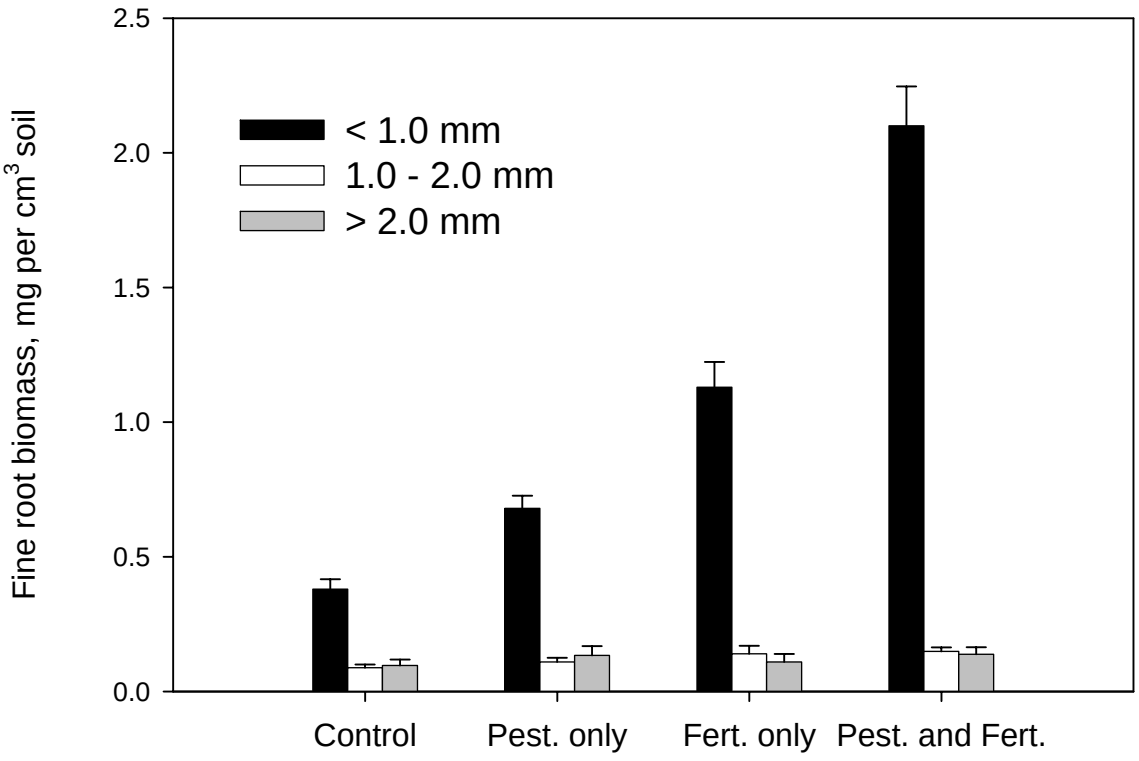

Fig. 2.1. Treatment effects on net ash-free fine root biomass in ingrowth cores. Results shown are from 200 cores, combining results from October and November harvests (harvest effect non-significant, $P > 0.14$). Responses of three diameter classes of roots are shown (< 1.0 mm, 1.0 – 2.0 mm, and > 2.0 mm). Bars show means + 1s.e.

CHAPTER THREE:

ROOT HERBIVORES, RESOURCE HETEROGENEITY AND ROOT FORAGING INTERACT TO INFLUENCE PLANT COMMUNITIES.

ABSTRACT

This experiment manipulated soil resource heterogeneity in greenhouse pots, and used differences between plant species' root responses to heterogeneity to make predictions about the influence of root herbivores on these plant species in competition. When root herbivores were added to two-plant mixed-species neighborhoods, *Eupatorium compositifolium*, the most precise forager of the three we examined, was the only species of the three to display a reduction in shoot biomass. Neighborhood composition, resource heterogeneity, and root herbivory all influenced total biomass in pots. Neighborhood composition had the greatest influence, followed by resource heterogeneity and then root herbivory which reduced total pot biomass by approximately 11% relative to pots without herbivory. As the most precise forager was the most influenced by root herbivores, these results point to a potential cost of morphological foraging behavior in roots. While top-down effects of root herbivores led to reduced standing biomass and influenced competition between plant species, they were not strong enough to overwhelm bottom-up effects of resource distribution.

KEY WORDS

Scarabaeidae, grubs, nutrient patches

INTRODUCTION

The distribution of nutrients in the soil is heterogeneous, and many greenhouse and some field studies have demonstrated plasticity in plant response to resource-rich patches. While many plants will proliferate roots (i.e., forage precisely) in nutrient-rich soil patches, the strength of this response varies widely between species in a community (Einsmann et al. 1999, Wijesinghe et al. 2001, Rajaniemi and

Reynolds 2004). Plants that aggressively forage in rich patches and simultaneously reduce root growth in poor patches can capture resources more efficiently (Hutchings and deKroon 1994) and grow larger than their non-proliferating competitors (Robinson et al. 1999, Hutchings et al. 2003). Despite this potential benefit, strong foraging responses have not translated into improved competitive ability or success in field studies (Casper et al. 2000, Bliss et al. 2002). This implies that the benefits of foraging may be offset by the costs or risks involved (Chapter 2, Neatrour 2005). This tradeoff may encourage the persistence of a less precise forager in communities, particularly when the costs of foraging outweigh the benefits (Alpert and Simms 2002).

Exposure to root herbivory may be a significant cost of root foraging behavior. In natural systems, root herbivores are likely to respond to rich patches, as root-feeding insects use CO_2 concentrations as a primary food-source cue (Jones and Coaker 1977, Brown and Gange 1990). Therefore, root herbivore densities should be greater in nutrient-rich areas where roots proliferate and respiration rates are higher (Hogberg et al. 2001). Exclusion of root herbivores by chemical insecticides has resulted in significant increases in root lifespan (Wells et al. 2002), net production of root biomass (Chapter 2), and significant shifts in plant community composition (reviewed in Brown and Gange 1990). Even so, it is unclear whether root herbivores influence competition between plant species based upon plant root foraging behaviors.

The central objectives of this research were to determine 1) if root herbivory can influence plant competition in heterogeneous soils; and 2) whether root foraging behavior is a key mechanism behind changes in competitive outcomes. To meet these objectives, we designed a greenhouse experiment using seedlings of three co-occurring perennials, planted two plants per pot in single- and mixed-species treatments. Single-species treatments were used to quantify the species-specific root foraging behavior or responses in homogeneous and heterogeneous conditions. Single-species foraging results were then used to develop hypotheses for our mixed-species treatments that involved manipulations of both nutrient heterogeneity and root herbivory. We hypothesized *a priori* that propensity to forage for nutrients may

not translate to enhanced growth because those same species that aggressively forage may be more susceptible to the detrimental influences of herbivory (Hypothesis 1). We further hypothesized that root herbivory should be concentrated in nutrient-rich patches (Hypothesis 2) because of root herbivore foraging. Finally, we hypothesized that the effect of root herbivores on total pot biomass would be greater under heterogeneous than under homogeneous conditions (Hypothesis 3).

METHODS

Experimental design

We chose three perennial species, one grass (*Andropogon ternaries* Michx.) and two forbs (*Solidago altissima* L. and *Eupatorium compositifolium* Walt.) to create competitive neighborhoods. These species are common, co-occurring early-successional perennials in the coastal plain of the southeastern United States. Seeds of these species were collected from an early-successional upland at the Savannah River Site, SC in fall 2002 (see Chapter 2 for further description of this field location).

The study was conducted in glasshouses located at Virginia Tech, Blacksburg, VA. Seeds were germinated in vermiculite in late February 2003, and planted into a low-nutrient potting soil on emergence of cotyledons. In mid-March 2003, seedlings were moved to 30 cm diameter x 28 cm deep experimental pots filled with construction-grade sand and inoculated with approximately 2 g of soil from the seed collection site. Two seedlings were planted per pot, 7.5 cm from the pot edge on opposite sides of the pot (Fig. 3.1). Pots were watered every other day over the course of the study to prevent drought stress. Fiberglass window mesh (1 mm grid) was inserted in the bottoms of all pots to prevent the loss of grubs (see below) and minimize soil loss. Pots were subjected to natural light and ambient photoperiod over the course of the study.

Pot treatments

Treatments were randomly assigned to pots in an incomplete factorial design (N = 198 pots). Pots were either planted with two seedlings from a single species (78 single-species pots) or one seedling from each of two different species (120 mixed-species pots). Single-species pots received either homogeneous or heterogeneous

nutrient distribution treatments (detailed below), and each species*fertility treatment combination was replicated 13 times (3 species x 2 fertility treatments x 13 reps = 78 pots). Mixed-species pots received either a homogeneous or heterogeneous nutrient treatment as well as a root herbivore treatment (with or without grubs, detailed below). In mixed species pots, each treatment combination was replicated 10 times (3 species pairs x 2 fertility treatments x 2 root herbivory treatments x 10 reps = 120 pots).

Nutrient distribution treatments involved addition of 5.0 grams of general purpose slow-release fertilizer (14-14-14 plus minors) to pots. In the homogeneous treatment, fertilizer was mixed uniformly into the upper 10 cm of the pot. In the heterogeneous treatment, 2.25 g of fertilizer was mixed into each of two plugs of soil (3.75 cm diameter x 10 cm depth), while the remaining fertilizer was broadcast uniformly over the surface of the pot. At the same time fertilized plugs were created, we removed and replaced soil from similarly spaced plugs to create unfertilized plugs. Plug patches were placed on an axis perpendicular to that of the seedlings (Fig. 3.1). These procedures resulted in two treatments with the same overall fertility, but one treatment provided approximately uniform nutrient supply while the other concentrated 90% of the available mineral resources into two nutrient-rich patches representing approximately 3% of the soil volume. Previous studies have shown little or no lateral movement of NO_3^-, the most mobile of our soil nutrients, from similar size patches (Einsmann et al. 1999); thus, we were able to compare root growth into fertilized and unfertilized patches at similar distances from plants, to estimate root proliferation within heterogeneous pots.

Root herbivores (white grub larvae, Coleoptera:Scarabaeidae, hereafter grubs) were collected beginning in late March of 2003 from home gardens in Montgomery County, VA. Grubs were removed by hand and stored in native soil at 5 degrees C until they were added to selected pots on April 4, 2003. Three grubs were added to the center of appropriate pots; any grub that did not dig itself beneath the soil surface within five minutes was discarded and replaced.

Harvest

Pots were harvested beginning on June 10, six weeks after the addition of larvae. Aboveground portions of individual plants were removed at the soil surface. Root biomass was sampled by first removing and compositing two 2.5 cm diameter x 15 cm deep cores of soil at the location of the fertilized and unfertilized (4 cm diameter) patches (Fig. 3.1, F.C. and U.C., respectively). Root biomass in homogeneous plots was harvested by removing and compositing two pairs of similarly spaced plugs. The remaining roots in all pots were processed as a single sample. Thus, each pot generated two aboveground samples (individual shoots) and three soil samples (e.g., in heterogeneous conditions we had samples of fertilized cores, unfertilized cores, and the remainder of the pot).

Roots and grubs were separated from the soil samples by washing over a 1 mm mesh screen. During washing, we recorded the vital status of each grub found in each sample as well as grub locations in pots (e.g., in fertilized plug, in pot outside of plug, etc). Washed root samples were moved to the lab and separated into < 2.0 and > 2.0 mm diameter classes mostly by eye, with occasional check using a dial caliper. All plant parts were dried to a constant mass at 60° C and weighed to determine biomass.

Data Analysis

Finalizing our hypotheses on competitive responses required ranking species by the strength of their foraging responses. To do this, we used single-species pots under homogeneous and heterogeneous conditions to compare root behaviors (i.e., precision of foraging) of each species. As previous research has shown that the finest roots (i.e., those with a diameter < 2.0 mm) are the most responsive to fertility and herbivory (Chapter 2), foraging responses were calculated using only roots < 2.0 mm in diameter. For individual heterogeneous pots, a root foraging index (RFI) was calculated by dividing the difference between root mass in the fertilized cores and the root mass in unfertilized cores by the sum of these two measures [i.e., RFI = (F.C. – U.C.) / (F.C. + U.C.), Fig. 3.1]. This calculation is a modified version of the relative fine root mass difference used by Mou et al. (1997) and Einsmann et al. (1999). In homogeneous pots, the RFI was calculated using similarly spaced plugs. We

analyzed root foraging responses using two-way ANOVA, with resource distribution pattern (homogeneous vs. heterogeneous) and species as main effects. Differences in root foraging precision (i.e., root foraging index) between the three species were investigated using Tukey's means comparison test (alpha = 0.10). These differences were used to rank species in terms of their propensity to forage, and to thereby specify our hypotheses about the influence of root herbivores on mixed-species pots

Prior to testing our hypotheses, we analyzed grub recovery rates to assess grub survival and thus the effectiveness of our herbivory treatments. Because harvest occurred over a two-week period coinciding with the period of adult beetle emergence, we predicted that the date a pot was harvested would influence the number of insects recovered. To test this prediction, we tracked both the number of insects recovered in each pot as well as the developmental stage (i.e., larva, pupa, or adult) and used linear regression to investigate relationships between harvest date and insect recovery.

To test our hypothesis that the strength of a plant species' root foraging response (measured as RFI) would be associated with increased exposure to root herbivory (i.e., Hypothesis 1), we compared the aboveground (shoot) biomass of individual species in mixed-species pots at the time of harvest. We used only shoot biomass (rather than whole-plant biomass) to assess these differences because we were not able to separate the roots of the different species. For each species, we analyzed the effects of the presence or absence of grubs, the species of competitor, the pattern of resource distribution, and the interactions on the shoot biomass of the target species using two-way ANOVA.

To test our hypothesis that root herbivores would selectively forage in nutrient-rich patches (Hypothesis 2), we used data on both grub distributions in the pots and the effects of the grubs on core biomass. Grub distributions were analyzed using a grub foraging index (GFI). This GFI used a similar formula to that shown above for RFI [(fertilized core – unfertilized core)/whole pot], but was based on numbers of grubs rather than root biomass. For heterogeneous pots, the GFI showed grubs in fertilized cores as a proportion of all grubs found in the pots. In homogeneous pots, the GFI showed the proportional distribution of grubs under

homogeneous conditions. We used nonparametric analyses to test for the separate influences of nutrient distribution (homo- and heterogeneous treatments) and plant neighborhood composition on grub foraging. To analyze the effect of root herbivory on root biomass in nutrient-rich patches, we calculated the difference in root biomass between fertilized and unfertilized patches of heterogeneous pots. We then compared this difference in pots with and without grubs, and tested the effect of herbivory using two-way ANOVA (with the presence of root herbivores and species composition as treatments).

To test Hypothesis 3, that root herbivore effects on total pot biomass would be greater under heterogeneous conditions, we tested the individual and combined effects of resource heterogeneity, root herbivory, and neighborhood composition on whole-pot (root + shoot) biomass using three-way ANOVA. All statistical comparisons were performed using Statistical Analysis System (SAS) software, version 9.1 (SAS Institute Inc., Cary, NC).

RESULTS

Species responses to heterogeneity

Nutrient heterogeneity resulted in a shift in root biomass patterns for each of the three plant species. Analysis of monoculture pots revealed significant treatment (homogeneous vs. heterogeneous conditions, $F_{1,77}= 235.29$, $P < 0.01$) and species ($F_{2,77}= 3.27$, $P = 0.05$) effects on our root foraging index. The mean root foraging index was never significantly different from zero in the homogeneous treatment. In the heterogeneous treatment, while each of the three species responded by proliferating roots in the nutrient patches, the response of *E. compositifolium* was significantly greater than that for both *A. ternarius* and *S. altissima* (Tukey's post-hoc analysis, $P = 0.087$ in both cases, Fig. 3.2). The strength of root foraging response did not differ between *A. ternarius* and *S. altissima* (Tukey's post-hoc analysis, $P = 0.99$).

These results indicated that *E. compositifolium* was the most precise forager of the three species analyzed. We used this result to predict that *E. compositifolium*, as

the most precise forager, should show the greatest negative response to the presence of grubs.

Grub recovery from pots

We recovered an average of 1.67 ± 0.12 (mean ± std. error) insects from each of the pots to which grubs were added. Recovery ranged from zero (no insects recovered) to a maximum of three (100% recovery). The number of insects found in pots at harvest declined significantly over the course of harvest (regression of harvest date on recovery significant, $P<0.01$, $R^2 = 0.43$, Fig. 3.3 A). Larvae represented the dominant developmental stage found at harvest (66 of the 99 recovered insects), and the number of larvae found in pots also declined significantly over the course of the harvest (Fig. 3.3 B). While pupae and adults were found in the pots, they were found less often than larvae (20 and 13 of each were recovered, respectively) and their recovery did not change through time (data not shown).

Grub effects on species in competition (Hypothesis 1)

As hypothesized, *E. compositifolium* was the most sensitive of the three species to root herbivory. Results of the ANOVA analyses on the response of each species to the presence or absence of grubs, as well as to the species of the competitor, showed varying trends for each of the species (Fig. 3.4). While the identity of the competitor had a consistent influence on shoot biomass of the target species, only *E. compositifolium* responded significantly to grubs. There were no significant interactions between the presence of grubs and the species of competitor for any of the species (interaction terms all non-significant, all $F_{1,36} < 0.15$, $P > 0.70$).

The shoot biomass of *A. ternarius* was significantly affected by the identity of the competitor in the pot, but was not significantly affected by root herbivory. *Andropogon ternarius* was twice as large on average when in competition with *S. canadensis* than with *E. compositifolium* (average shoot biomass of 2.02 g vs. 1.04 g, respectively, $F_{1,36} = 4.88$, $P = 0.03$).

The shoot biomass of *E. compositifolium* was significantly affected both by the presence of grubs ($F_{1,36} = 4.59$, $P = 0.04$) and by the identity of the competitor in the pot ($F_{1,36} = 10.19$, $P < 0.01$). *E. compositifolium* shoots were approximately 25% smaller in the presence of grubs (16.3 g without vs. 12.7 g with grubs), and were

approximately 30% smaller in pots with *S. altissima* than with *A. ternarius* (11.8 g vs. 17.2 g, respectively).

Solidago altissima was significantly affected by the identity of the competitor in the pot, but was not significantly affected by root herbivory. Shoot biomass was 27% lower, on average, when in competition with *E. compositifolium* than with *A. ternarius* (8.8 g vs. 12.1 g, $F_{1,36} = 5.65$, $P = 0.02$).

Grub responses to heterogeneity (Hypothesis 2)

Nutrient heterogeneity resulted in a statistically significant change in the grub foraging index (nonparametric test of heterogeneity on GFI, Kruskal-Wallis $\chi^2 = 6.11$, $P = 0.01$), with higher values displayed in the heterogeneous nutrient treatment. However, although the mean GFI values for each nutrient treatment were different from each other, neither was different from zero. Mean GFI values ± std. error for homogeneous pots were negative (-0.077 ± 0.057), while values for heterogeneous pots the values were positive (0.081 ± 0.052). Grub foraging did not differ between neighborhood types (Kruskal-Wallis $\chi^2 = 2.36$, $P = 0.31$)

Grubs significantly reduced the difference in standing root biomass between fertilized and unfertilized patches (grub effect $F_{1,54} = 3.85$, $P = 0.05$, Fig. 3.5). Although root biomass was always higher in fertilized cores than in unfertilized cores, grubs reduced the difference between fertilized and unfertilized cores by nearly 20%.

Effects on neighborhood biomass (Hypothesis 3)

Plant neighborhood biomass (i.e., whole pot, root + shoot biomass) in mixed-species pots was significantly influenced by neighborhood composition, the pattern in nutrient distribution (homogeneous vs. heterogeneous), and by the presence of grubs (all $F_{(1 \text{ or } 2),108} > 3.99$, $P < 0.05$, Fig. 3.6). Average community biomass was 26% greater in heterogeneous pots than in homogeneous pots (23.4 ± 0.84 g vs. 18.6 ± 0.84, respectively), and was reduced by an average of nearly 11% in pots where grubs were added (mean community biomass 22.18 ± 0.84 g in pots without grubs vs. 19.79 ± 0.844 g in pots with grubs). Although grub effects on community biomass appeared stronger in heterogeneous than in homogeneous pots (reductions of 16.1%

in heterogeneous vs. 3.3% in homogeneous pots, Fig. 3.7), there were no statistically significant interactions between treatments (all $F_{2,108} < 2.17$, $P > 0.12$).

DISCUSSION

We proposed a model of soil-root-herbivore interactions in which root herbivores would have a stronger effect on plant species with a greater tendency to forage for patchily distributed soil resources. The overall model is largely supported by the observed results. Most importantly, root herbivory appears to be a significant cost that can influence the net benefit of root foraging behaviors in plant communities. Plant species that aggressively forage for resources in nutrient-rich microsites may be at increased risk of root herbivory (Chapter 2), reducing the net benefit of root foraging.

The data also supported a prediction from our first hypothesis suggesting that a plant species' tendency to forage for soil nutrients should be associated with reduced yield when herbivores are present. *Eupatorium compositifolium* foraged more precisely than either *A. ternarius* or *S. altissima*, and was the only species of the three to show significant reductions in yield when grubs were present. However, *E. compositifolium* was smaller in pots with grubs regardless of the pattern of nutrient distribution, implying that the tradeoff between precision foraging and vulnerability to herbivory does not depend strictly on local resource conditions.

Although previous studies have shown that root foraging behaviors significantly influence inter- and intra-specific competition (Robinson et al. 1999, Fransen et al. 2001, Day et al. 2003), there have been few studies prior to this that have explored specific potential costs of root foraging behaviors. Despite the potential for increased resource capture (Hutchings and de Kroon 1994), rapid root foraging may not always be the most efficient approach over longer time periods. In an examination of the long-term rewards of foraging, Fransen and de Kroon (2001) observed that the more rapidly-growing and aggressively foraging species was not as competitive over a two-year study period as a slower-growing species that was not a precise forager. Our study is among the first to demonstrate that root herbivores can constrain the net benefit of root foraging by an individual species. By constraining

the benefits of root foraging, root herbivores may allow less-precise species that are presumably less efficient at nutrient capture, to persist in a community in situations where they would otherwise be outcompeted. Ultimately, the potential cost imposed by root herbivores may help to explain the range of foraging responses seen even between species from similar environments and with similar life histories (Einsmann et al. 1999, Rajaniemi and Reynolds 2004).

Support for our second hypothesis, that root herbivores would forage preferentially in nutrient-rich patches, was mixed. While we did see differences in grub distributions between homogeneous and heterogeneous treatments, and grubs were found in the fertilized cores of heterogeneous pots, they were found there infrequently. The grub foraging index in heterogeneous pots was not different from zero, and the significant difference between heterogeneous and homogeneous pots was due in large part to the concentrations of herbivores in one set of cores in the homogeneous pots. As both sets of cores in the homogeneous pots were theoretically equivalent, we assume that the differences in foraging between heterogeneous and homogeneous pots result from random chance. The lack of a foraging response to nutrient-rich cores was surprising, given that some root herbivores forage in response to CO_2 signals (Jones and Coaker 1977), which we presume were higher in fertilized patches due to the higher root biomass in these patches. However, while scarab larvae are fairly mobile and do tend to aggregate, the factors that control their behaviors are not well understood. Additionally, the design of our experiment, where nutrient patches represented only 3% of the soil volume, may have contributed to the small numbers of grubs we found in cores, regardless of fertility.

Although the grubs themselves were not concentrated within nutrient-rich patches, they significantly reduced root proliferation in these patches. In heterogeneous pots, grubs had no effect on biomass in unfertilized cores, but reduced the difference between fertilized and unfertilized cores (Fig. 3.5). It is possible that the concentration of fertilizer within the patches was high enough to cause grubs to avoid the inner areas of the patch, and to feed largely on the patch periphery. It is also possible that some of the grubs that fed in the patches had descended into deeper portions of the pots in advance of pupation. Indeed, one-third of the grubs we

recovered from the pots were either pupae or adults, and diapause generally takes place deeper in the soil profile than does feeding. Regardless of their final distribution, the reduction in root biomass in fertilized relative to unfertilized cores supports the notion that root herbivores were attracted to nutrient-rich microsites. Similar results were seen in field studies using root ingrowth cores (Chapter 2), where root herbivores reduced root biomass to a greater extent in fertilized cores than in unfertilized cores.

Our third hypothesis, that grub effects on neighborhood biomass would be stronger in heterogeneous pots than in homogeneous pots, was not supported by experimental results. The experiment involved manipulations of neighborhood composition, nutrient distribution, and root herbivory, and we observed significant effects of all three of these main treatments on neighborhood biomass (Fig. 3.6), but no interactions between main effects. Interestingly, although we did not observe the statistically significant interaction between herbivory and nutrient distribution that we predicted, grubs reduced whole-pot biomass an average of 16% in heterogeneous pots, but only reduced biomass 2.6% in homogeneous pots (Fig. 3.7).

This experiment involved manipulations of plant species composition, nutrient distribution, and root herbivory. Ranking these manipulations in terms of their effects on plant neighborhood biomass suggests that plant species composition is the most significant driver of total biomass production over the course of the study, as mean biomass at harvest for the different neighborhood types varied by approximately 60%. Nutrient distribution was the next strongest driver, with total pot biomass 26% greater in heterogeneous than in homogeneous pots. The effect of root herbivores was the weakest of the three treatments, as grubs reduced biomass by approximately 11% where they were present. These results suggest that, at least at the herbivore density we examined, bottom-up effects of community composition and nutrient supply dominate. While top-down effects of root herbivores reduce standing biomass and may influence competition between individual species, they do not overwhelm the bottom-up effects. This model agrees with recent experiments by De Deyn et al. (2003, 2004), where microcosm biomass was controlled largely by strong bottom-up

effects of resource supply, but soil fauna led to increased plant diversity by reducing the dominance of strong resource competitors.

In addition to affecting competition in plant communities, root herbivores may also alter soil microbial communities (e.g., Grayston et al. 2001), carbon assimilation by individual plants (Murray et al. 2002), and successional pathways (Brown and Gange 1990, De Deyn et al. 2003). The effects of the herbivores may be modulated by the activities of natural enemies such as entomopathogenic nematodes (Strong et al. 1996); it appears that some plants may use indirect defenses to attract these natural enemies (van Tol et al. 2001). Indeed, scarab densities are known to decrease with increasing soil organic matter between field sites (Ed Lewis, *pers. comm.*), despite the attraction of grubs to organic-rich patches (Stevens, unpublished data). It may ultimately be the local densities and behavioral responses of the natural enemies of grubs that determine the balance between costs and benefits of root foraging behaviors at a given point in time.

While it is plausible that we observed a causal relationship between the precise foraging of *E. compositifolium* and its vulnerability to herbivory, it is also plausible that the results we observed were driven by root herbivore preferences for the roots of *E. compositifolium*. When designing the experiment, we intentionally selected species that we hoped would be similar in terms of their palatability and phenology of root growth, and avoided species known to contain significant quantities of potentially toxic secondary compounds in their roots (e.g., *Asclepias* spp.). White grubs are polyphagous, and should be able to consume the roots of any of the species used in our neighborhoods. However, we did not conduct experiments examining the species feeding preferences of the grubs. Overall, these results demonstrate the potential for root feeders to influence plant species differentially based at least in part on their root foraging behaviors. This supports an emerging, complex view of resource-plant-herbivore interactions, and further supports the need to consider the role of soil fauna in plant community interactions (Hunter 2001, DeDeyn et al. 2004).

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Table 3.1. Results of ANOVA analysis of the influence of species and fertility treatment effects on root foraging index (RFI).

	df	MS	*F*	*P*
Root foraging index				
Species	2	0.07	3.27	0.044
Fertility treatment	1	5.39	235.29	<0.001
Species * fertility treatment	2	0.04	1.85	0.165
Error	77	1.65		

Table 3.2. Results of ANOVA analysis of whole-pot biomass responses to neighborhood species composition, fertility, and root herbivory treatments, as well as interactions between these main effects.

	df	MS	*F*	*P*
Whole pot biomass				
Species	2	1085.10	25.17	<0.001
Fertility	1	681.79	15.81	<0.001
Species * fertility	2	88.63	2.06	0.133
Grubs	1	163.37	3.79	0.054
Species * grubs	2	31.99	0.74	0.479
Fertility * grubs	1	88.30	2.05	0.155
Species * fertility * grubs	2	13.31	0.31	0.735
Error	107	43.11		

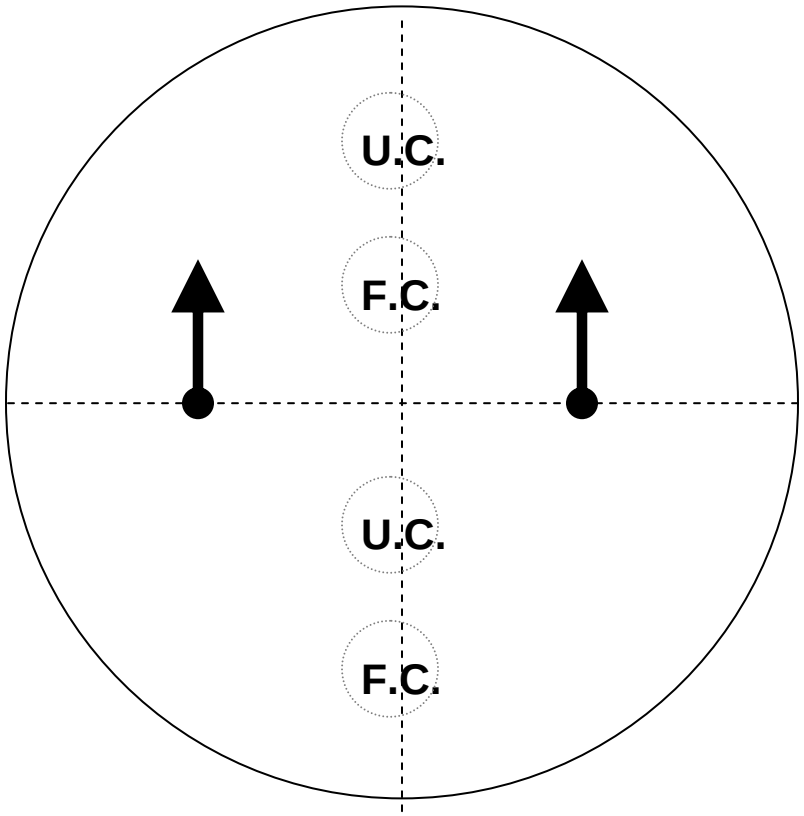

Figure 3.1. Pot layout for greenhouse experiments (not to scale), illustrating heterogeneous treatment design. Dashed lines indicate axes through pot (30 cm). Dark arrows indicate seedlings, 15 cm apart and 7.5 cm from pot edge). Locations of fertilized cores (F.C., 11 cm apart) and unfertilized cores (U.C., 11 cm apart) used for root sampling are shown as dashed circles. Locations of cores and seedlings were similar for all treatments.

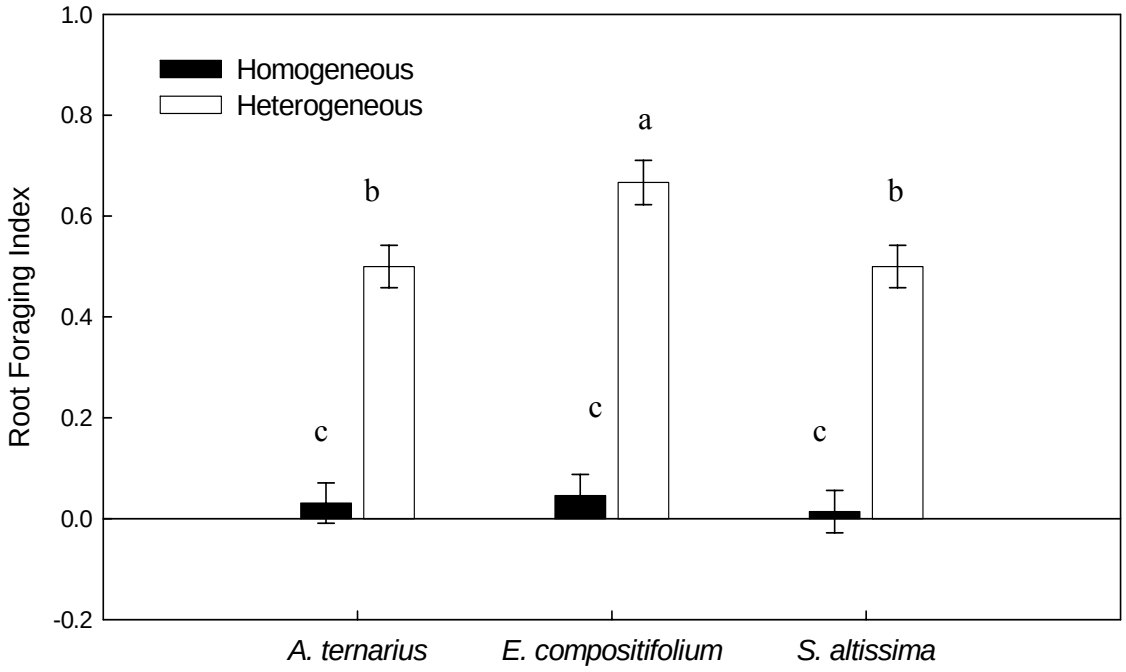

Figure 3.2. Root foraging index (RFI) calculated under homogeneous and heterogeneous conditions for each of the species in this experiment. Bars show means ± std. err. Different lowercase letters indicate significantly different values at the $P < 0.10$ level.

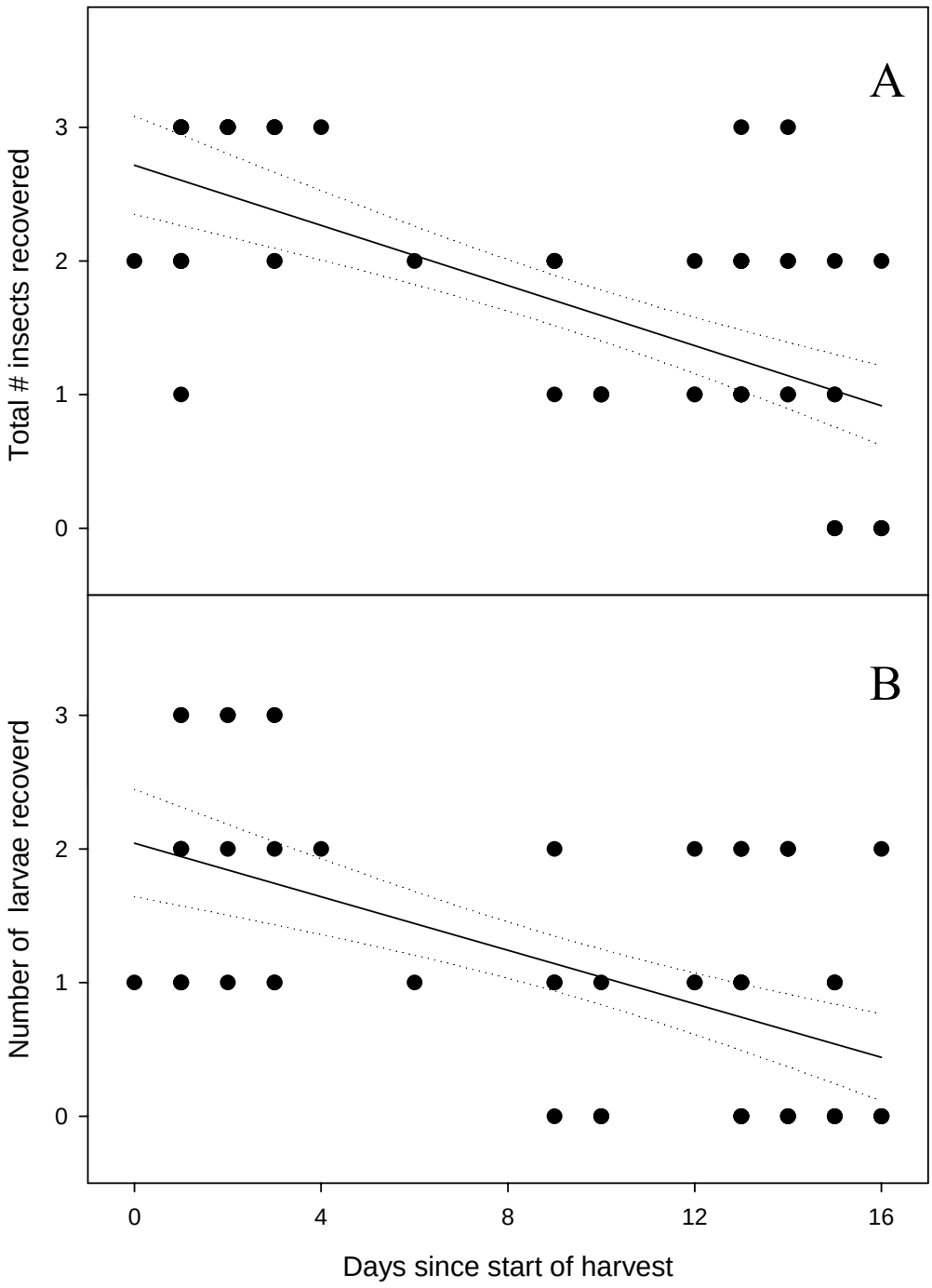

Figure 3.3. Regression examining the influence of pot harvest date (Julian date from beginning of harvest, June 10 2003) on the total number of insects recovered per pot (A) and the number of larvae (grubs) recovered per pot (B). Regression shown as a solid line, surrounded by a 95% confidence interval (dashed line).

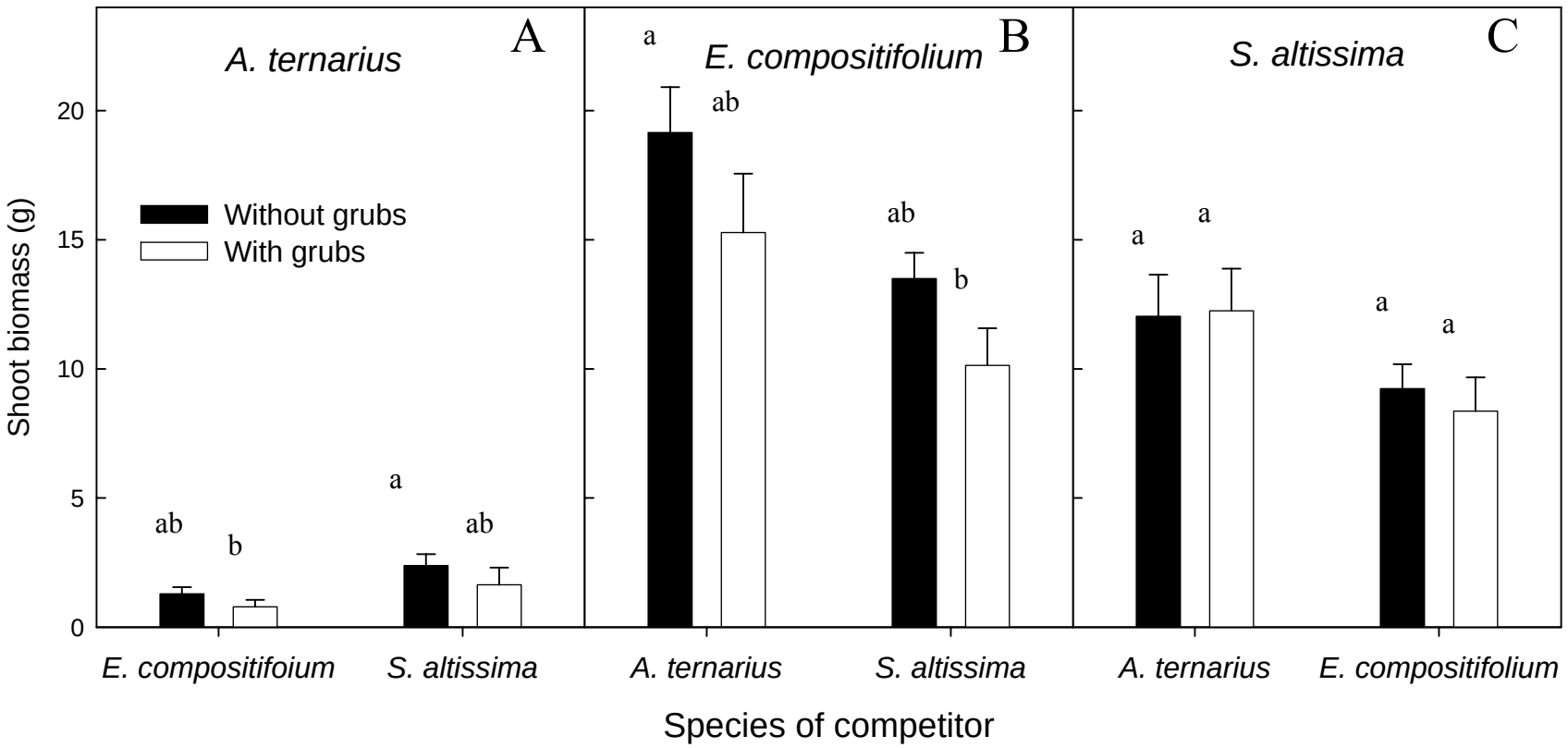

Figure 3.4. Shoot biomass change of individual plant species (panels A, B, and C) in response to grub additions in two interspecific neighborhoods. Bars show means + std. err. Within each panel, different lowercase letters indicate significantly different values at the $P < 0.05$ level, according to Tukey's post-hoc means separations.

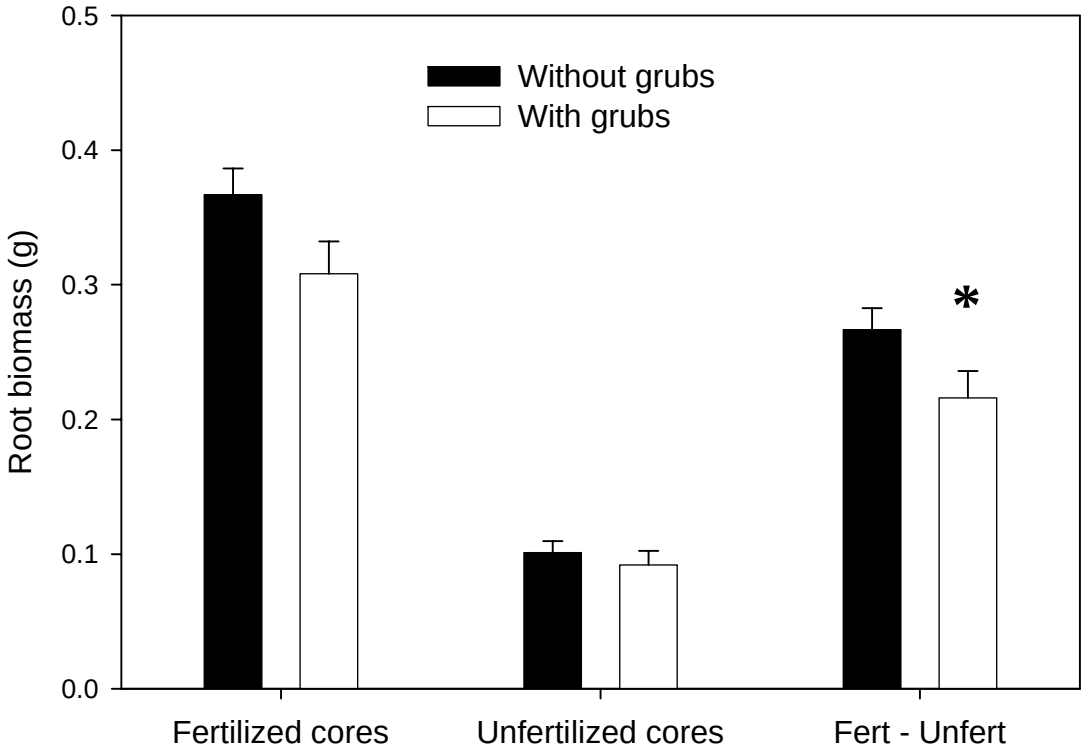

Figure 3.5. Influence of root herbivores on root biomass in fertilized patches, root biomass in unfertilized patches, and on difference in root mass between patches. Bars show means + std. err. Asterisk indicates a significant influence (alpha = 0.05) of grubs on root mass variability in plots.

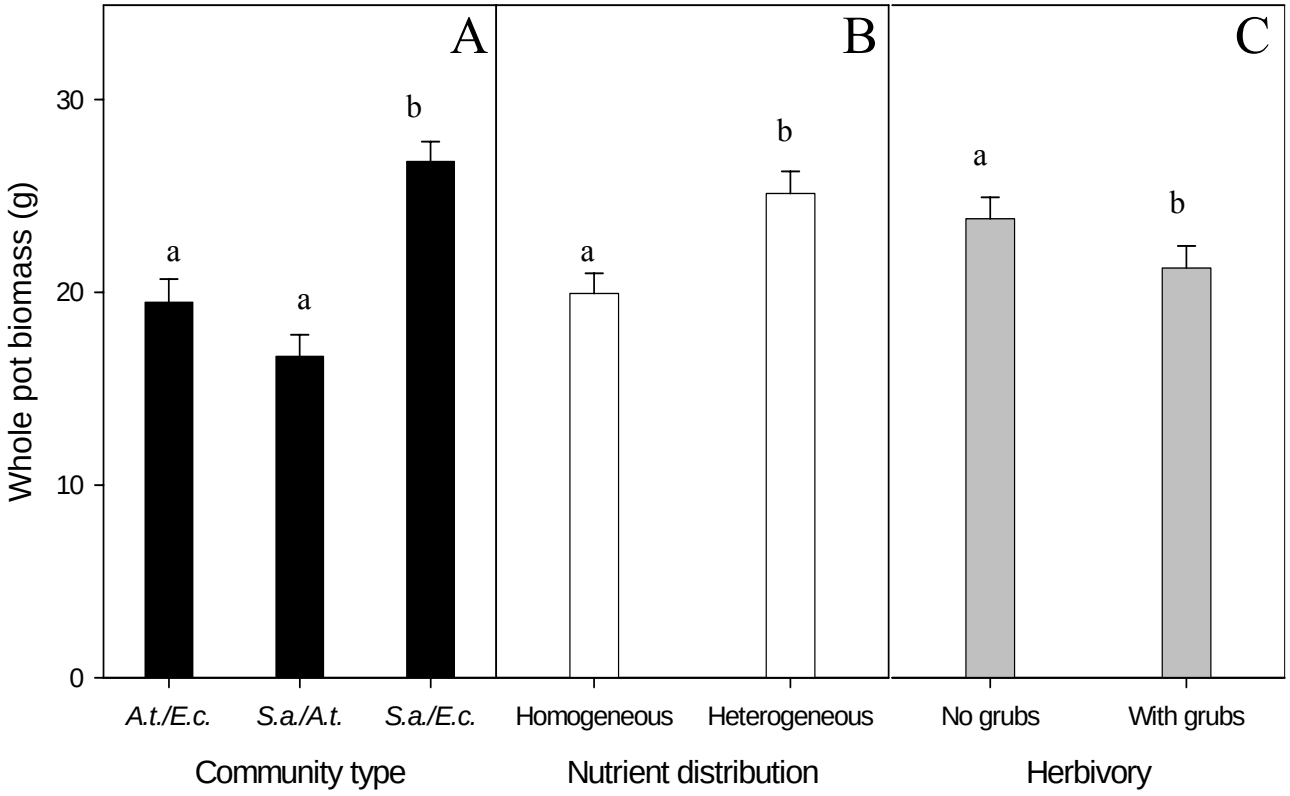

Figure 3.6. Influences of neighborhood composition (A), nutrient distribution (B), and the presence of root herbivores (C) on neighborhood biomass. Bars show means + std. err. Within each panel, different lowercase letters indicate significantly different values at the $P < 0.05$ level

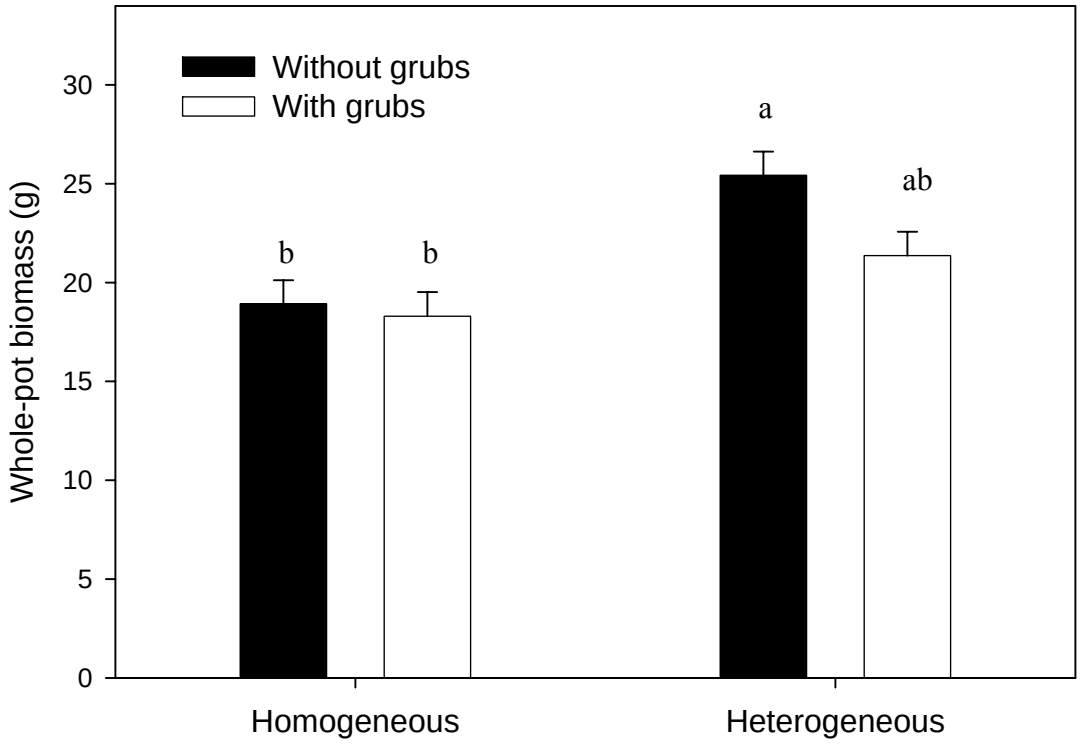

Figure 3.7. Influence of resource distribution and root herbivory on plant neighborhood biomass. Bars show means + std. err. Different lowercase letters indicate significantly different values at the $P < 0.05$ level.

CHAPTER 4:

INFLUENCES OF SOIL RESOURCE HETEROGENEITY ON SOIL FAUNA – PLANT INTERACTIONS

ABSTRACT

Although both resource heterogeneity and root herbivory are known to have important influences on plant communities, the degree to which spatial variability in resources might interact with soil resources to influence community structure and function is unclear. We designed a field experiment with a multiple-species community, and manipulated total resource availability, the distribution of resources in the soil, and soil fauna. Our fauna treatments included the removal of root herbivores as well as supplementing root herbivory by adding locally-collected white grubs. We measured treatment influences on growing-season aboveground biomass production of individual species as well as the combined community; in addition, we measured the responses of roots and root herbivores to treatments. We also considered the potential for differential effects on common, cool-season species and less common, warm-season species. Overall, while manipulations of soil fertility had little influence on above- or belowground dynamics, root herbivores increased the aboveground yield of cool season species. In some cases, the potential benefit of nutrient-rich patches depended on the level of root herbivory: where soil fauna were not manipulated, warm-season species responded positively to patches; the benefit of these patches was lost when root herbivory was increased by addition of grubs. Overall, these results support the concept that the influence of root herbivores on ecosystem structure and function varies with patterns of nutrient heterogeneity.

KEYWORDS

Scarabaeidae, grubs, nutrient patches, root production, ANPP, BNPP

INTRODUCTION

It is widely acknowledged that bottom-up forces (nutrient dynamics) and top-down forces (herbivory or natural enemies) interact to influence plant communities (Fretwell 1987, Hunter and Price 1992). However, despite the fact that much of the competition between plants occurs for resources belowground, few studies have considered how soil resources interact with soil fauna such as root herbivores to influence plant community structure and function (Hunter 2001). While the effect of mean resource levels on plant communities is widely recognized, researchers have demonstrated that the distribution of soil resources (i.e., resource heterogeneity) can influence competition between species in plant communities, and may ultimately play a major role in the regulation of plant community structure and function (Robinson 1994). Although the root systems of many plant species will preferentially forage in resource-rich patches, the strength of this response varies widely between co-occurring species (Campbell et al. 1991, Einsmann et al. 1999, Farley & Fitter 1999; Robinson et al. 1999, Wijesinghe et al. 2001), and does not appear to be conclusively tied to the size of a plant or its root system (Einsmann et al. 1999, but see Crick and Grime 1987 for contrasting predictions). Studies have demonstrated benefits of root foraging, including increased total resource capture (Hodge et al. 1999, Robinson 2001) and resource capture efficiency (Hutchings and deKroon 1994, Jackson and Caldwell 1996). However, the range of this behavior across different species implies that there are significant potential costs that may constrain its potential benefits.

In addition to direct effects on plant competition, the bottom-up effects of resource supply and distribution are likely to indirectly influence plant communities through interactions with soil fauna, including root herbivores. Root herbivores are known to have strong effects on communities, largely by selecting against dominant species (Brown and Gange 1990) and thereby increasing local species diversity (De Deyn et al. 2003). While the extent to which the effects of root herbivores varies within an ecosystem is unclear, most soil fauna exhibit clumped distributions in the soil (Ettema and Wardle 2002), and root herbivores can have influences on root dynamics that increase with resource availability and productivity at small scales (Chapter 2, DeDeyn et al. 2003, 2004).

We designed a field experiment to simultaneously assess the influence of resource availability, resource distribution, and soil fauna on plant community structure and function. Manipulations of resources were carried out by adding fertilizer either across an entire plot (homogeneous fertilization) or in multiple discrete resource patches in each plot (heterogeneous fertilization), while manipulations of fauna were conducted by either adding locally-collected root herbivores or reducing the densities of root herbivores and other fauna through application of soil insecticide. We measured the individual and combined effects of manipulations of fertility and soil fauna on aboveground plant community traits as well as on root systems. At the same time, we measured the responses of root herbivores to fertility treatments.

We analyzed the results of our manipulations with two overall goals. The first was to measure individual influences of soil fertility and soil fauna treatments on the plant community. Hypotheses in this case involved predictions based on previous research on the effects of heterogeneity and trophic dynamics on plant communities. Our second goal was to assess the strength of interactions that might emerge from the simultaneous manipulations of soil fertility and soil fauna, as these interactions may be of particular importance in plant communities. Because previous research has not yet shown trends in such interactions, we developed no specific hypotheses regarding potential interactions.

Hypothesized canopy and dominance responses

We hypothesized that relative to unfertilized plots, increasing fertility would increase the net production of aboveground biomass over the course of the study (Fig. 4.1). We further hypothesized that homogeneous fertilization would elevate soil fertility and favor dominance by a limited number of species, while patchy fertilization, by increasing overall resource heterogeneity in addition to fertility, would favor less dominant species (Fig. 4.1). We hypothesized that manipulations of soil fauna would also result in significant changes in patterns of aboveground biomass. Specifically, we hypothesized that reductions in root herbivores and other soil fauna would lead to increased aboveground yield and favor dominant species,

while additions of root herbivores would lead to reductions in aboveground yield, and favor less dominant species.

Root and root herbivore responses

We hypothesized that increasing fertility would decrease total root biomass regardless of nutrient distribution, but that the variability in root biomass would be increased in fine-scale fertilized plots relative to either homogeneous or untreated plots (Fig. 4.1). We hypothesized that increased fertility would also lead to increased densities of insect root herbivores, and that in fine-scale fertilized plots these densities would be higher in fertilized patches than unfertilized patches. Finally, we believed that manipulations of soil fauna would lead to shifts in standing root biomass; specifically, we hypothesized that reductions in root herbivores and other soil fauna would lead to increased root biomass (Fig. 4.1).

METHODS

Study site and species

The study was conducted at Kentland Farm, which is part of the Virginia Agricultural Experiment Station, operated by Virginia Tech's College of Agriculture and Life Sciences. Kentland Farm fronts the New River near Whitethorne, in the northwestern portion of Montgomery County, Virginia. Field plots were located on a 900,000-year-old terrace of the New River in a mown grassy field. The terrace soils are Low Shottower Series, a Fine, kaolinitic, mesic Typic Paleudult. These are well-drained loams with a 200+ year history of agriculture. We established 108 sites (each containing a 0.8 m by 0.5 m plot surrounded by a 0.4 by 0.3 m buffer) on a grid within an approximately 50 x 50 m area in May of 2003. Individual sites were separated by 1.5 m of natural vegetation that was mown intermittently over the course of the study. Before treatments were established, each site was covered with 1.2 x 0.8 m 8-mil black plastic sheeting for four months to kill existing vegetation. After the plastic was removed, individual plots were tilled to a depth of approximately five centimeters.

We chose eight species to create our planted communities, including six herbaceous species and two trees (Table 4.1). The herbaceous species included *Festuca arundinacea* Schreb. var. Kentucky-31 (tall fescue), *Achillea millefolium* L. (yarrow), and *Chrysanthemum leucanthemum* L. (ox-eye daisy), which are common in cool-season grasslands and in unmanipulated areas around the plot, and *Andropogon gerardii* Vitman (big bluestem), *Rudbeckia hirta* L. (black-eyed susan), and *Solidago canadensis* L. (goldenrod), which are later-blooming, warm-season species. The two tree species were *Acer rubrum* L. (red maple) and *Pinus virginiana* P. Mill. (Virginia pine), locally common early-successional trees.

Plot preparation and establishment

Seedlings of each of the eight species were germinated in a greenhouse at Virginia Tech beginning in May of 2003. When seedlings had attained sufficient size to survive transplantation, they were planted into 2.5 x 2.5 cm plugs in Metro Mix 200 (Scott's Sierra Horticulture Products, Marysville, OH), a low-nutrient potting mixture. Plants were watered twice daily and fertilized twice with a balanced liquid fertilizer to ensure adequate growth. Seedlings were moved outdoors in October of 2003 and hardened off prior to planting in field plots.

In addition to the herbaceous plants that we grew from seed, we also divided local mature clumps of *F. arundinacea, A. millefolium, A. gerardii*, and *S. canadensis* in the summer of 2003 to produce plants from divisions. These divisions were graded by eye to approximately uniform root mass and then planted in 7.5 cm diameter pots filled with Metro Mix. These pots were stored outdoors at the Virginia Tech greenhouse complex until planted in experimental plots. One-year old bare-root nursery-grown seedlings of *P. virginiana* and *A. rubrum* were planted in plots in spring of 2004.

Herbaceous species were planted in a 0.8 by 0.5 m plot inside the 1.2 by 0.8 m vegetation-free areas beginning in late October 2003. Plants were planted in an 8 by 5 arrangement on a 10 by 10 cm grid (0.8 m by 0.5 m plots, Fig. 4.2). To encourage more uniform representation of species within plots, random seedling locations were generated separately for the inner and border areas of the plot (Fig. 4.2). Inner areas were planted with a total of 18 plants. This 18-plant community was composed of

two individuals of each of the six seed-grown herbs, one individual of each of the four species that were propagated by division, and one nursery-grown seedling from each of the two tree species. The 22 plants in the border area were haphazardly planted with 2 – 3 seed-grown individuals of all eight species.

Plot treatments

Treatments were randomly assigned to plots from a factorial combination of nutrient treatment (3 levels) and soil fauna treatment (3 levels). Although the initial design designated twelve replicates for each of the nine treatments, a numbering error resulted in a non-uniform distribution of replication. As a result, each treatment was replicated between 8 and 15 times across the site (mean = 12, median = 13, mode = 13.5).

Nutrient availability treatments were applied in March 2004, and included 3 categories: control, homogeneous fertilization, and patchy fertilization (Fig. 4.1). In control plots, soil resources were not manipulated. Both coarse- and fine-scale fertilized treatments involved addition of similar amounts of fertilizer. Fertilizer was applied as a combination of 405 g of composted cow manure (0.5-0.5-0.5 NPK, Black Kow Composted Cow Manure, Oxford, FL) and 13.5 g of slow release fertilizer (15-9-12 NPK + minors, 8 – 9 month release Osmocote, Scott's Miracle-Gro Co., Marysville, OH). Fertilizer was either uniformly incorporated into the soil between seedlings to a depth of 2.5 cm (homogeneous) or concentrated into fourteen 5.0 cm diameter by 5.0 cm deep patches located equidistant from seedlings (heterogeneous fertilization, Fig. 4.2). This resulted in two nutrient-addition treatments, a homogeneous fertilization in which fertilizer was incorporated approximately uniformly within the plot, and a fine-scale fertilization in which the same amount of fertilizer was concentrated into patches representing approximately 7% of the total plot area.

Soil fauna treatments included a control treatment, a soil fauna suppression treatment (insecticide/nematicide), and a root herbivore supplementation treatment (Fig. 4.1). Soil fauna were suppressed via application of soil insecticide (2 g granular chlorpyrifos, Lorsban 15G, Dow AgroSciences, LTD, Indianapolis, IN) to plots at six week intervals beginning in March 2004. Root herbivore supplementations were

made by adding locally collected white grub larvae (Coleoptera:Scarabaeidae) to plots in both April and August of 2004 (30 grubs added to each plot at each date). Grubs were collected by hand from Lavery Sod Farm (Elliston, Virginia) and stored in native soil at 5º C they were added to plots. Grubs were distributed uniformly across each plot; any grub that did not dig itself beneath the soil surface within five minutes or that was attacked by ants was discarded and replaced.

Plots were weeded by hand throughout the experiment. Small weeds were removed entirely from the soil, while large weeds were clipped at the soil surface. The matrix of vegetation surrounding the plots was mowed at approximately monthly intervals; any clippings produced by mowing were removed from experimental plots.

Plant and soil insect responses

We assessed canopy biomass using both non-destructive sampling and destructive harvests. Canopy leaf area was estimated non-destructively in June and August 2004 using a Li-Cor LAI 2000 leaf area meter (Li-Cor, Lincoln, NE, USA). Leaf area was measured at five points between plant seedlings along the center row in each plot (Fig. 4.2). Senescent herbaceous flowering stalks were removed from plots throughout the growing season, and all remaining herbaceous (non-tree) biomass aboveground was harvested in November 2004. Aboveground herbaceous vegetation was separated by species, dried to a constant biomass at 60^{o}C, and weighed to determine aboveground dry mass production for each herbaceous species over the course of the experiment. These species-specific measures were combined to measure total aboveground yield per plot. Growth of nursery trees and survival and growth of seed-grown trees was low and no trees were included in the November harvest. Growth of nursery-grown tree seedlings was estimated from measurements of basal diameter and height of seedlings taken in March of 2004 and again in November 2004; these measurements were converted to an allometric index of growth using the standard equation [net growth = Δ (height * diameter^2)]. Changes in this index were used as surrogate measurements of tree seedling growth, but were not included in estimates of aboveground biomass yield.

Root and root herbivore responses to treatments were measured by harvesting soil cores from plots in June and October 2004. Cores were harvested from areas

between seedlings; core locations were randomly selected prior to planting, but were the same for all plots. In June, four 7 cm diameter by 20 cm deep cores were removed from each plot with a bucket auger; June cores were taken from the area between the border row and the inner area of the plot (Fig. 4.2). In heterogeneous fertilized plots, two fertilized cores and two unfertilized cores were harvested and composited by treatment; locations were the same for homogeneous and control plots. In October, six 7.5 cm diameter by 20 cm deep soil cores were removed from each plot using a 7.5 cm diameter steel sleeve; October samples were removed from within the inner area of the plot (shaded area, Fig. 4.2). In heterogeneous plots, three fertilized and three unfertilized cores were harvested and composited by treatment; locations were similar for homogeneous and control plots. All soil samples were stored at 3^{o}C until processing. Fine roots and soil insects were separated from the soil by hand-washing samples over a 1-mm sieve (June samples) or using a hydropneumatic root elutriator (Gillison's Variety Fabrication, Benzonia, Michigan) to wash samples over a 1-mm sieve (October samples). Washed roots were separated into three diameter classes (< 1.0 mm, 1.0 – 2.0 mm, and > 2.0 mm) mainly by eye, occasionally verified using dial calipers. Roots were not separated by species. Separated root samples were then dried, weighed, and ash-corrected to determine ash-free dry root mass per cm^3 of soil (hereafter fine root biomass) in each size class at each date. As we removed two sets of composite cores at each sample date, two measures of fine root biomass for each size class were generated from each plot. These measures were combined to determine total fine root biomass per plot, but were also compared to assess variability in root biomass within plots. Soil insects from samples were stored in ethanol until identified to family using the keys of Stehr (1987). After identification, insects were dried to a constant mass at 60°C and weighed to determine insect biomass.

Analyses

We used ANOVA to analyze our 3 x 3 factorial design and test our predictions concerning treatment effects on net growing-season production of canopy biomass, dominance (the distribution of aboveground biomass between species), root biomass, and abundance of root-feeding insects. To control for variability between

plots in the site that pre-dated our manipulations, we included plot row and column locations in all measurements as plot covariates, reflecting an observed linear gradient of productivity across the study area.

To test our hypotheses regarding canopy biomass, we analyzed treatment effects on plot canopy area and total aboveground biomass using MANOVA. Potential differences between treatments were assessed using Tukey's least-squares means separation ($P < 0.05$).

We investigated potential changes in dominance between treatments using two lines of evidence. These were (1) analyzing treatment effects on the mean plot biomass of individual species in the community using individual ANOVA, and (2) analyzing treatment effects on the mean plot biomass of the two species groupings (locally dominant cool-season species and locally uncommon warm season species) as well as the ratio of cool season:warm season biomass using MANOVA. Potential differences in each of these measures between treatments were assessed using Tukey's least-squares means separation (at $P < 0.05$).

To address our hypotheses regarding root responses, we tested treatment effects on (1) whole-plot measures of standing root biomass and (2) root biomass variability within plots. Standing root biomass in each of our three root diameter classes (< 1.0 mm, 1.0 - 2.0, and > 2.0) was measured by combining the root biomass from both sets of cores, while the variability in root biomass was measured as the difference in root biomass between the two sets of cores taken per plot. In heterogeneous plots, this compared nutrient-rich patches to patches without fertilization, and thus indicated the root foraging response exhibited by the community. In homogeneous and control plots, this distribution indicated the variability in root mass under conditions of more uniform nutrient supply. We analyzed treatment effects on root biomass in each diameter class (< 1.0 mm, 1.0 - 2.0, and > 2.0) using two MANOVA analyses. Because previous results (Chapter 1) showed that the finest diameter roots (< 1.0 mm diameter) were the most responsive to our treatments, we conducted one MANOVA for < 1.0 mm diameter roots (June and October measures) and conducted a separate MANOVA for the other two diameter classes (again, both June and October measures). We also analyzed

treatment effects on the variability of root biomass using ANOVA. In this case, separate analyses were conducted for both our June and October samples; at each date, potential differences between treatments were assessed using Tukey's least-squares means separation (at $P < 0.05$).

To assess our hypotheses regarding root herbivore responses to treatments, we tested treatment effects on (1) total numbers and biomass of larval Elateridae and Scarabaeidae (wireworms and white grubs) observed between plots using MANOVA, and (2) variability in numbers and biomass of these groups within plots. Variability in root herbivore densities and biomass was measured as the difference between the two sets of cores taken per plot; in heterogeneous plots this difference indicated root herbivore foraging behavior in response to fine-scale fertility. Effects of treatments on variability in root herbivore density and biomass within plots was also tested using MANOVA.

RESULTS

Canopy biomass

We observed a range of responses to treatments between the three measures of canopy biomass (Fig. 4.3). Plot measures of leaf area in June were not significantly influenced by either fertility or fauna treatments ($F_{2,78} < 0.68$, $P > 0.61$). September measures of leaf area were not influenced by soil fauna treatments ($F_{2,77} = 1.58$, $P = 0.21$), but were influenced by fertility treatment ($F_{2,77} = 12.81$, $P < 0.0001$), with leaf area nearly 50% higher in heterogeneous plots than either control or homogeneous plots (Fig. 4.3). Measures of aboveground yield showed a different relationship to treatments. Yield did not vary between fertility treatments ($F_{2,78} = 0.52$, $P = 0.60$), but was influenced by soil fauna. Yield was greatest in plots where root feeders had been added. Yield in these grub-added plots was 20% greater than in control plots, and 10% greater than in plots where we added pesticide. Overall MANOVA results indicated an overall effect of soil fertility treatment on these responses (Wilks' lambda = 0.72, $F_{6,150} = 4.42$, $P < 0.001$), but no overall effect of soil fauna manipulations and no overall interaction between the two (Wilks' lambda = 0.86 in both cases, $F < 1.90$, $P > 0.08$).

Measures of dominance

Although individual species varied in growth (Table 4.1), and varied substantially in their responses to treatments, only 3 of the 16 comparisons we made (8 species, 2 main factors for each) showed significant responses to individual treatments. *Pinus virginiana* responded significantly to the fertility treatment ($F_{2,77}$=3.89, $P < 0.05$), showing nearly 70% greater growth in homogeneous plots relative to the other two treatments (Fig. 4.4). *Achillea millefolium* and *F. arundinacea* both responded to the soil fauna treatments, though they exhibited different responses. In the case of *A. millefolium*, biomass was greater by half in plots where grubs were added than in either control or pesticide-treated plots. *Festuca arundinacea* responded to either manipulation of soil fauna (additions or removals), and was lowest in unmanipulated plots.

In addition to these single-factor responses, we saw significant interactions between the soil fertility and soil fauna treatments (i.e., $P < 0.05$ for interaction term) for both *F. arundinacea* and *S. canadensis* (Fig. 4.5A,B). In the case of *F. arundinacea*, where soil fauna were unmanipulated, there was a uniform response to soil fertility treatments. The response to homogeneous fertility, however, differed markedly based on the specific manipulation of soil fauna. Where fauna were reduced with pesticide, *F. arundinacea* biomass was greater in the homogeneous plots; however, where grubs were added, *F. arundinacea* biomass was lowest in these same homogeneous plots (Fig. 4.5A). *Solidago canadensis* exhibited a different interaction pattern. In this case, biomass was largely uniform where fauna were reduced, and was maximum in ambient fauna plots under the fine-scale nutrient treatment. In plots where grubs were added, however, biomass appeared lowest in the heterogeneous plots (Fig. 4.5 B).

Overall, multiple analysis of variance techniques suggest that soil fauna treatments had a consistent effect (Wilks' lambda = 0.85, $F_{6,152} = 2.14$, $P = 0.05$) but do not support a consistent fertilizer effect (Wilks' lambda = 0.86, $F_{6,152} = 1.99$, $P = 0.07$) or an interaction between the two (soil fauna * fertility treatment interaction, Wilks' lambda = 0.79, $F_{12,201} = 1.6$, $P = 0.09$). Comparing responses to treatments between our species groupings (dominant, cool-season species and warm-season

species) showed that the two groups did not respond uniformly to treatments (Fig. 4.6). Biomass of cool-season species varied significantly between soil fauna treatments, and appeared highest in plots where grubs were added (Fig. 4.6B). Warm-season species, on the other hand, responded to patterns in soil fertility, and biomass in homogeneous plots was nearly 20% greater than in unfertilized plots (Fig. 4.6A). In addition, we observed significant interactive effects of soil fertility and fauna in the case of warm season biomass (interaction term significant, $P < 0.05$, Fig. 4.5C): while fertilizing generally had no effect in plots where fauna were reduced with pesticide, plots with fauna showed differences between fertility treatments. Maximum biomass in control-fauna plots occurred with fine-scale fertilization; in plots where grubs were added, however, biomass in fine-scale plots was comparable to that in unfertilized plots, and maximum biomass was found with homogeneous fertilization (Fig. 4.5C).

Root biomass

Multivariate analysis of > 1.0 mm diameter root responses (i.e., both 1.0 – 2.0 mm and > 2.0 mm diameter) showed no significant responses to soil fertility (Wilks' lambda = 0.91, $F_{8,148} = 0.92$, $P = 0.50$, Table 4.2) or soil fauna (Wilks' lambda = 0.92, $F_{8,148} = 0.76$, $P = 0.64$) treatments and no interactive effects of fertility and fauna treatments (Wilks' lambda = 0.85, $F_{16, 227} = 0.75$, $P = 0.74$).

In contrast, multivariate analysis of < 1.0 mm diameter root responses (i.e., the finest class of roots) showed significant responses to the soil fauna treatment (Wilks' lambda = 0.85, $F_{4, 154} = 3.24$, $P = 0.01$, Table 4.3). There were no significant effects of the fertility treatment, and no interactive effects between fertility and fauna treatments (in both cases, $F < 1.10$, $P > 0.36$, Table 4.3). Root biomass in general was greater in plots where insecticide had been added, although the difference was only significant in the case of samples taken in June (Tukey's post-hoc analysis, $P < 0.05$). While root biomass in this diameter class also appears greatest in this treatment in the fall sampling, the overall effect of fauna treatment was only significant at a reduced alpha level (alpha = 0.10).

No treatment effects on root system heterogeneity were detected. Combined multivariate analysis demonstrated no overall effect of soil fertility treatment (Wilks'

lambda = 0.96, $F_{4,154} = 0.76$, $P = 0.55$) or soil fauna manipulations (Wilks' lambda = 0.95, $F_{4,160} = 0.91$, $P = 0.46$) on root heterogeneity (data not shown).

Root herbivores

MANOVA analysis of root herbivore responses to treatments showed no overall responses to soil fertility and no interactive effects of fertility and fauna treatments (both Wilks' lambda > 0.84, F < 1.21, $P > 0.29$), but did show a significant overall response to soil fauna treatments (Wilks' lambda = 0.69, $F_{8,192} = 4.96$, $P < 0.0001$). The overall pattern was higher numbers and biomass of root herbivores in plots where grubs were added (Fig. 4.8B). In October samples we observed greater numbers of root herbivores and greater root herbivore biomass in ambient and grub-added treatments than in plots to which we added insecticide.

DISCUSSION

We saw strong effects of soil fertility and soil fauna on different components of plant community structure and function. Most interestingly, when we considered the responses of warm-season species, we observed a tradeoff between the patchy availability of nutrients and exposure to root herbivory. Where soil fauna were not manipulated, warm-season species in general and *S. canadensis* in particular were larger in heterogeneous plots than in homogeneous plots, despite similarity in overall plot fertility. This suggested that where soil fauna were not manipulated, root foraging in small, resource-rich patches allowed these species to increase their biomass. The benefit of nutrient rich patches was lost, however, where root herbivore numbers were increased by addition of grubs. In the latter case, biomass of the warm-season species decreased to the level of unfertilized plots (Fig. 4.5 B, C). In contrast to their negative effects in fine-scale plots, grubs appeared to increase the biomass of warm-season species in coarse scale fertilized plots. This tradeoff is similar to that observed Chapter 3, in which the more precise foraging species suffered a greater reduction in growth than did species with less precise foraging when root herbivores were common. Together, these results imply that spatial variability in soil fertility can interact with the behavior of root herbivores to influence plant community structure.

Our first hypothesis, that plant canopy biomass would increase in response to additions of fertilizer and removal of soil fauna, but would decrease where root herbivores were added, was not supported by results. We only saw significant responses to soil fertility in one instance (leaf area measured in September, Fig. 4.3A), and this relationship did not extend to changes in canopy biomass. This lack of response to fertility is surprising, given that our experiment included manipulations of total resources (i.e., fertilization) in addition to comparisons of resource distribution. The lack of total yield differences between heterogeneously and homogeneously fertilized plots, however, was less surprising since other studies have reported little effect of resource heterogeneity in mixed-species plots (Cahill and Casper 1999, Bliss et al. 2002).

Plant canopy responses to soil fauna were significant, but were different than hypothesized. Although June measures of leaf area did not differ between soil fauna treatments, a trend developed by September in which either manipulation of fauna appeared to increase canopy area. By the end of the study, maximum aboveground biomass was observed in plots where root herbivore levels were increased by adding grubs, and there appeared to be a stimulation of aboveground biomass in pesticide-treated plots as well (Fig. 4.3B).

Our second hypothesis, that treatments would influence the relative dominance of individual species or functional groups, was also unsupported by the study results. Although some species (such as *S. canadensis* and the warm-season species discussed above) responded to fertility treatments, the response was influenced by soil fauna, and in fact, the fertility by fauna interaction had stronger effects than did the spatial pattern of fertility alone. The addition of grubs appeared to increase the biomass of dominant cool-season species (*F. arundinacea* and *A. millefolium*, Fig. 4.4B, Fig. 4.6B), and reduce the positive responses of warm-season species to fine-scale resource patches (Fig. 4.5).

The predictions laid out in our third hypothesis related to root responses to treatments. Contrary to our prediction, we observed no responses of roots to increases in fertility, regardless of the scale of fertilization. We also observed no effect of our treatments on the spatial variability in the root system within plots.

Although the lack of a fertilizer effect on roots parallels aboveground results in this study (Fig. 4.7), the lack of an effect of rich patches was particularly surprising. Increases in root biomass in nutrient rich patches have been observed in greenhouse conditions (Drew 1975, Einsmann et al. 1999, Farley and Fitter 1999, Chapter 3) and in the field (Bliss et al. 2002, Chapter 2). The lack of a response in this case may reflect the influence of a diverse, crowded community with a dense rooting system. Previous work has indicated a potential 'carrying capacity' for roots (Jones et al 2003), in which community root biomass remains constant despite shifts in the relative contribution of plant species, and it may have been that root biomass was more limited by space than by fertility in this case. In addition, it is possible that there were root foraging responses to patches early in the study, but that roots drew down resource levels in the patches over the course of the growing season to the levels in background soil; this would likely result in a more uniform root distribution pattern and mask short-term root proliferation responses. It is also possible that, given the high levels of fertility in the unamended soil (data not shown), applications of fertilizer did not result in a meaningful increase in soil fertility.

In contrast with the lack of soil fertility effects, root biomass did change as a result of our manipulations of soil fauna. Root biomass in the finest class of roots (< 1.0 mm in diameter) increased in plots where soil insecticides were added. This agrees with the trends seen in previous studies (Chapter 2, Chapter 3). The soil insecticide we used likely led to reduced populations of all soil organisms, as chlorpyrifos applications generally result in reduced densities of earthworms (USDA 2001), Collembola (Frampton 1999, Pereira 2005), mites (Cabrera 2004, Pereira 2005, but see Michereff-Filho et al. 2004 for contrasting results) and soil arthropods in general (Wang et al. 2001, Dawson et a. 2003, Pereira et al. 2005). Given the decrease in standing root biomass where we applied insecticide, root herbivory may have a greater effect on root dynamics in this system than do organisms such as soil conditioners or detritovores, which were also likely reduced by insecticide.

Our final hypothesis, that root herbivores would respond positively to increasing soil fertility, was not supported by our results. None of our soil fertility treatments resulted in differences in the numbers or biomass of root herbivores

observed between plots, and soil fertility had no effect on the distribution of root herbivores within plots. We did see a significant reduction in root herbivore numbers and biomass where we applied soil insecticide, as well as a general increase in root herbivore biomass and numbers in plots where we supplemented levels of root herbivores (Fig. 4.8B). These results indicate that our manipulations of soil fauna were generally successful; however, we added white grubs to plots in a density of 75 grubs/m^2, yet levels in grub-added plots were not significantly higher than in control plots.

Overall, we saw that although some species responded significantly to resource treatments, soil resource distribution and supply had little effect on plant community biomass above- and belowground. Manipulations of soil fauna had a greater influence on community structure and function. Most interestingly, fauna and fertility combined to influence the biomass of less-common, warm-season species; in this case, root herbivores reduced the benefit otherwise seen in plots with resource-rich patches. We have shown that the influences of soil fauna vary significantly with patterns in soil fertility, and support the need to further integrate the responses of soil fauna into our understanding of plant-resource interactions.

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Table 4.1. Brief description and mean plot biomass of species used in this study. Measures show total aboveground biomass production (yield, grams per plot over the study period) of herbaceous species, and net change in growth measured using an allometric relationship (growth, cm^3). Values are mean (std. err.).

Species name	Common name	Family	Bloom period	Cool/warm	Yield, g
Achillea millefolium L.	Yarrow	Asteraceae	Early	Cool	117.62 (6.67)
Chrysanthemum leucanthemum L.	Ox-eye daisy	Asteraceae	Early	Cool	9.34 (1.26)
Festuca arundinacea Schreb. var. Kentucky 31	K-31 Tall fescue	Poaceae	Early	Cool	116.10 (6.45)
Andropogon gerardii Vitman	Big bluestem	Poaceae	Late	Warm	8.63 (0.92)
Rudbeckia hirta L.	Black-eyed susan	Asteraceae	Late	Warm	89.64 (4.66)
Solidago canadensis L.	Goldenrod	Asteraceae	Late	Warm	151.60 (6.27)
					Growth
Acer rubrum L.	Red maple	Aceraceae	n/a		2063.24 (109.86)
Pinus virginiana P. Mill.	Virginia pine	Pinaceae	n/a		2001.01 (242.77)

Table 4.2 Results of (a) MANCOVA and (b, c, d, e) ANCOVA analysis of field study, using 1.0 – 2.0 and > 2.0 mm diameter fine root biomass from June and October samplings in relation to fertilizer and fauna treatments, with plot and column location within the overall site as covariates.

a) MANCOVA				
	df	Wilks' lambda	*F*	*P*
Fertilizer treatment	8, 148	0.908	0.92	0.504
Fauna treatment	8, 148	0.922	0.76	0.641
Fert x Fauna	16, 227	0.854	0.75	0.738
Row	44, 285	0.713	0.60	0.980
Column	40, 282	0.579	1.09	0.331
b, c, d, e) ANCOVA				
	df	MS	*F*	*P*
b) June roots 1.0 - 2.0 mm diameter				
Fertilizer treatment	2	0.005	0.11	0.897
Fauna treatment	2	0.003	0.58	0.559
Fert x Fauna	4	0.004	0.66	0.623
Row	11	0.004	0.66	0.773
Column	10	0.004	0.74	0.686
Error	77	0.005		
c) June roots > 2.0 mm diameter				
Fertilizer treatment	2	0.136	1.89	0.158
Fauna treatment	2	0.004	0.05	0.951
Fert x Fauna	4	0.077	1.06	0.379
Row	11	0.036	0.51	0.892
Column	10	0.046	0.64	0.773
Error	77	0.072		
d) October roots 1.0 - 2.0 mm diameter				
Fertilizer treatment	2	0.050	0.65	0.526
Fauna treatment	2	0.026	0.33	0.717
Fert x Fauna	4	0.041	0.52	0.720
Row	11	0.031	0.40	0.953
Column	10	0.073	0.93	0.510
Error	77	0.078		
e) October roots > 2.0 mm diameter				
Fertilizer treatment	2	0.276	0.38	0.687
Fauna treatment	2	1.420	1.94	0.151
Fert x Fauna	4	0.662	0.90	0.467
Row	11	0.648	0.88	0.560
Column	10	1.449	1.98	0.048
Error	77	0.734		

Table 4.3 Results of (a) MANCOVA and (b, c) ANCOVA analysis of field study, using < 1.0 mm diameter fine root biomass from June and October samplings in relation to fertilizer and fauna treatments, with plot and column location within the overall site as covariates. Bold entries indicate factors significant at alpha = 0.10.

a) MANCOVA				
	df	Wilks' lambda	*F*	*P*
Fertilizer treatment	4, 154	0.96	0.84	0.504
Fauna treatment	**4, 154**	**0.85**	**3.24**	**0.014**
Fert x Fauna	8, 154	0.90	1.09	0.374
Row	22, 154	0.71	1.28	0.190
Column	**20, 154**	**0.57**	**2.17**	**0.004**
b, c) ANCOVA				
	df	MS	*F*	*P*
b) June roots < 1.0 mm diameter				
Fertilizer treatment	2	0.08	1.14	0.324
Fauna treatment	**2**	**0.31**	**4.49**	**0.014**
Fert x Fauna	**4**	**0.16**	**2.25**	**0.071**
Row	**11**	**0.12**	**1.72**	**0.084**
Column	10	0.08	1.19	0.311
Error	78	0.07		
c) October roots < 1.0 mm diameter				
Fertilizer treatment	2	0.26	0.63	0.536
Fauna treatment	**2**	**1.12**	**2.76**	**0.070**
Fert x Fauna	4	0.01	0.03	0.998
Row	11	0.38	0.93	0.516
Column	**10**	**1.38**	**3.40**	**0.001**
Error	77	0.41		

Soil fertility treatments

Added

Control Coarse Fine

Soil fauna treatments

Removal Control Add Herbivores

Response variables

0	+	+	Canopy biomass (yield)	+	0	-
0	+	-	Dominance	+	0	-
0	-	-	* Root biomass	+	0	-
0	+	+	* Root herbivores	-	0	+

Figure 4.1. Illustration of plot treatments and predicted effects on response variables, showing three levels of fertility treatment (control, homogeneous, and heterogeneous) and three levels of fauna treatment (root herbivore/soil fauna reductions, control, and increased root herbivory). Asterisks indicate an additional prediction of increased heterogeneity in heterogeneous plots.

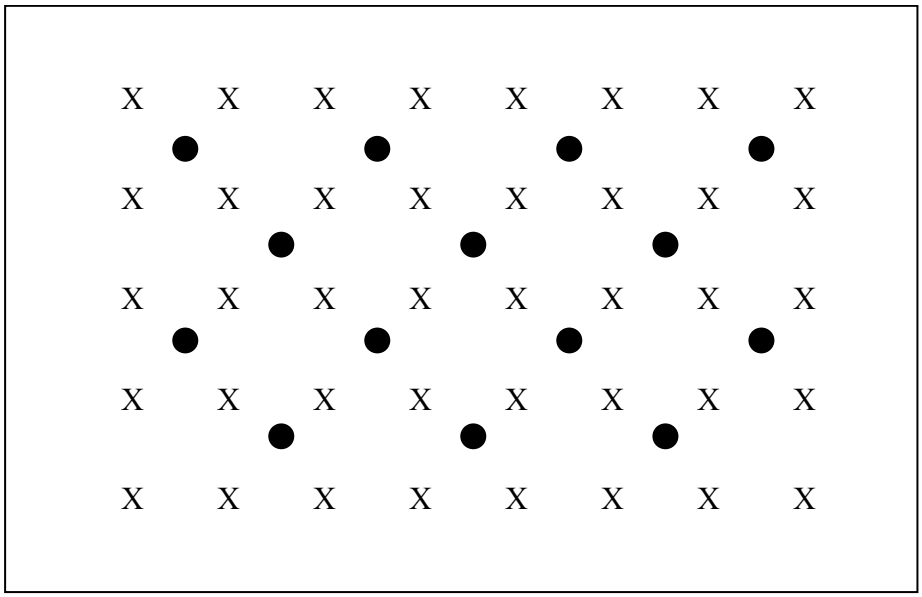

Figure 4.2. Illustration of 8 x 5 plant plot, consisting of an inner 6 x 3 plant area (shaded) and an outer border row, planted as described in text (plants shown as X). Plots received no fertilizer, a homogeneous application of fertilizer (control and homogeneous treatments not shown), or a heterogeneous treatment in which fertilizer was concentrated into fourteen discrete patches (shown as dark circles). Patches were placed equidistant from plant locations.

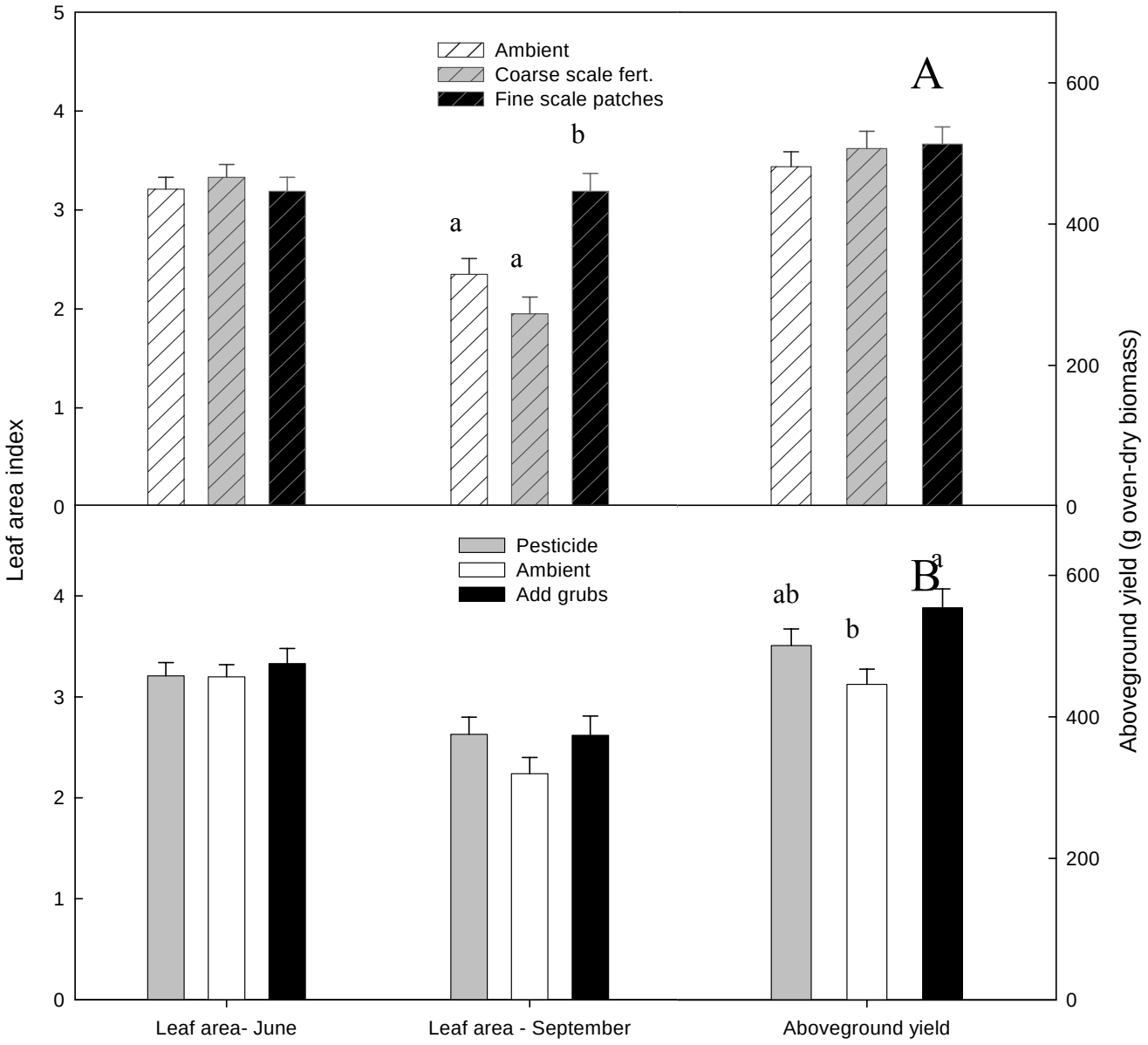

Figure 4.3. Influence of soil fertility (A) and soil fauna (B) treatments on average plot measurements of leaf area and canopy biomass. Bars show means + std. err. Within each three-bar group, different lowercase letters indicate significantly different values at the $P < 0.05$ level.

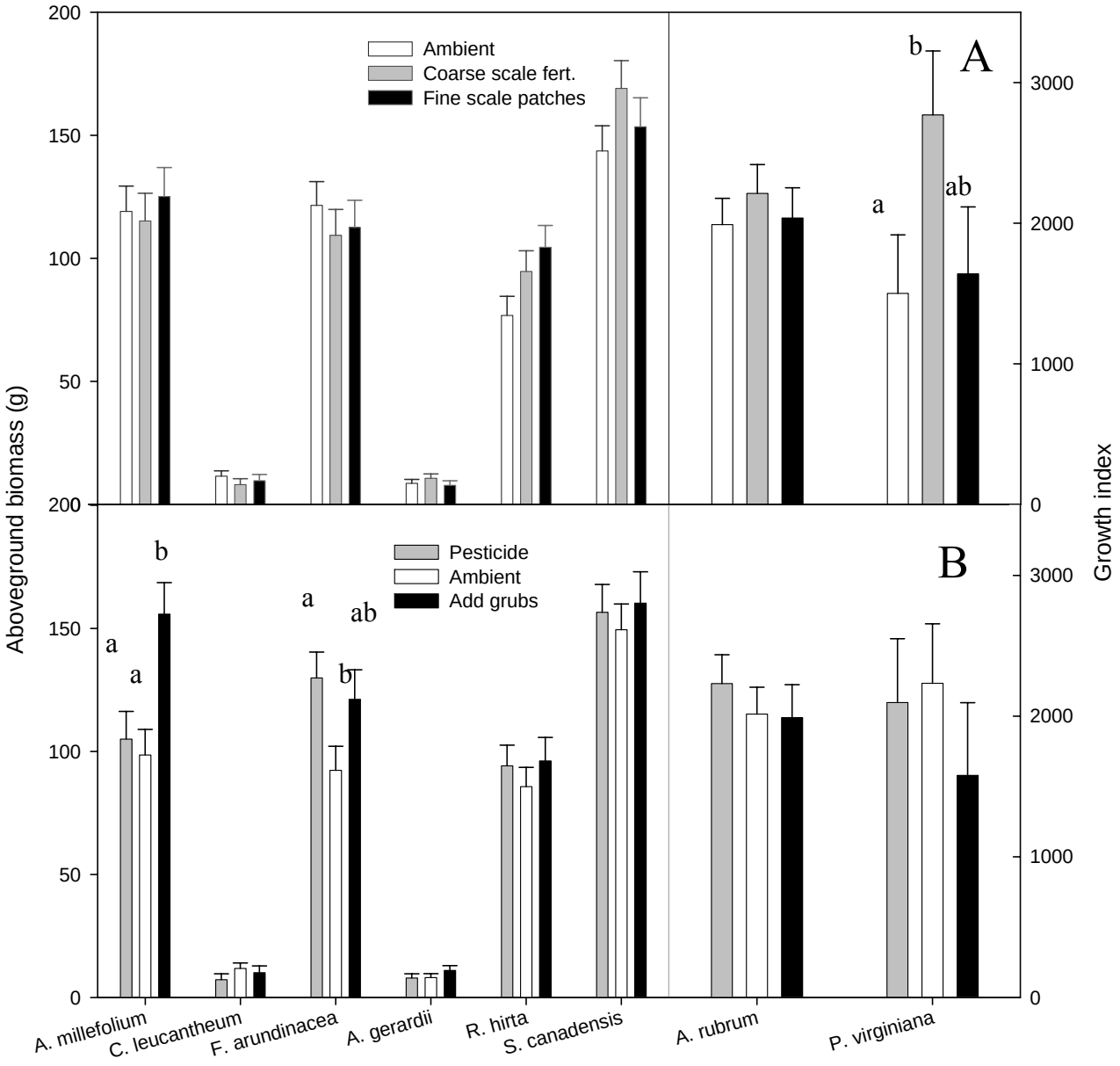

Figure 4.4. Influence of soil fertility (A) and soil fauna (B) treatments on individual species. Bars indicate average aboveground biomass of each species; each bar represents mean plot biomass + std. err. Within each three-bar group, different lowercase letters indicate significantly different values at the $P < 0.05$ level.

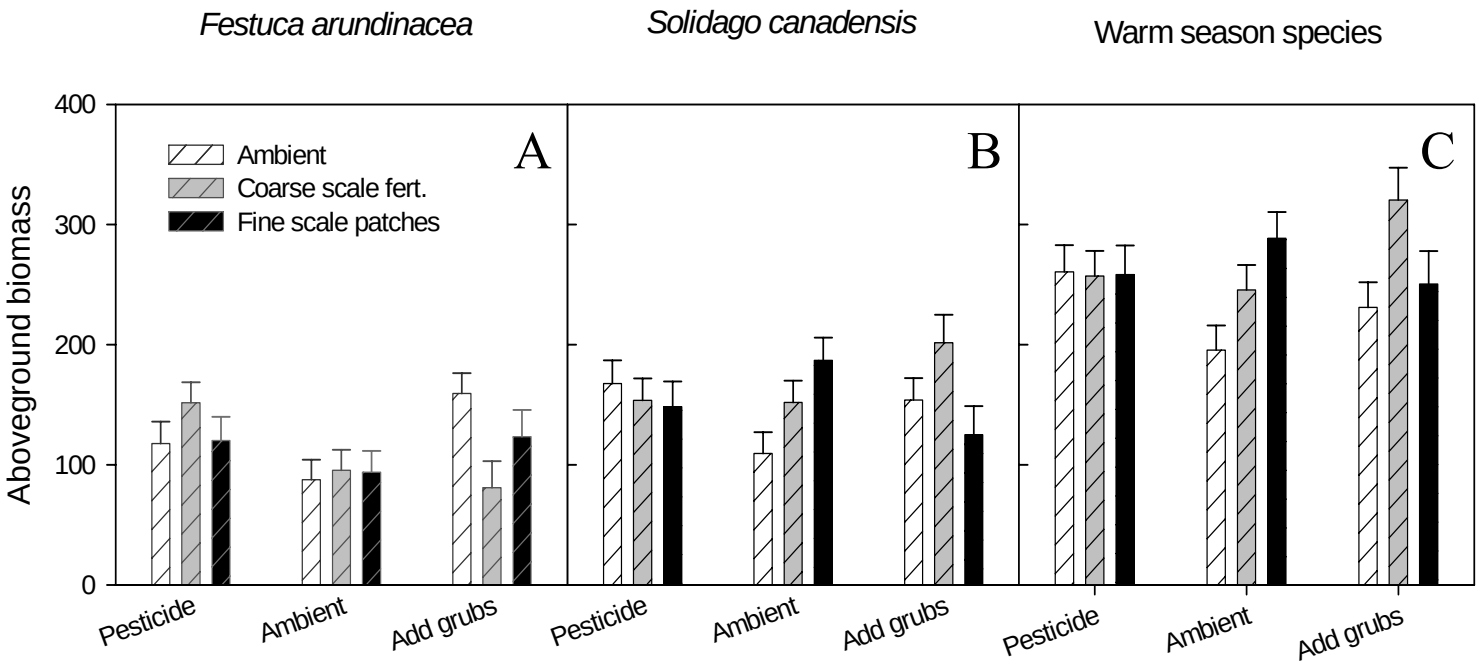

Figure 4.5. Interactive responses of *F. arundinacea* (A), *S. canadensis* (B), and warm-season functional group (C) to soil fertility and fauna treatments (soil fertility x soil fauna interaction significant in all cases, $P < 0.05$). Bars show mean aboveground biomass + std. err.

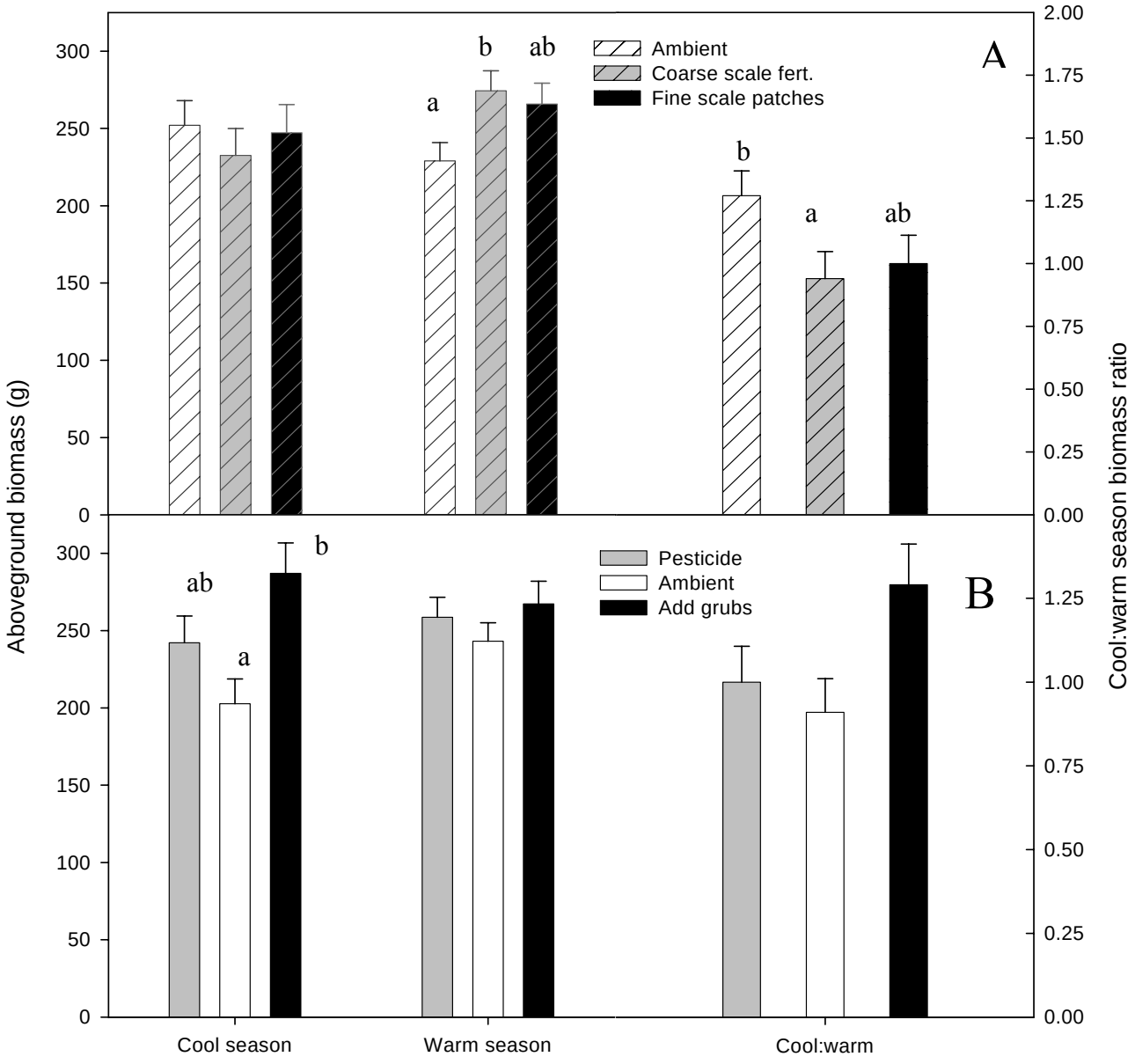

Figure 4.6. Influence of soil fertility (A) and soil fauna (B) treatments on aboveground biomass of cool- and warm-season functional groups. Bars indicate mean average aboveground biomass of group or mean ratio of cool:warm biomass. Bars show means + std. err. Within each three-bar group, different lowercase letters indicate significantly different values at the $P < 0.05$ level.

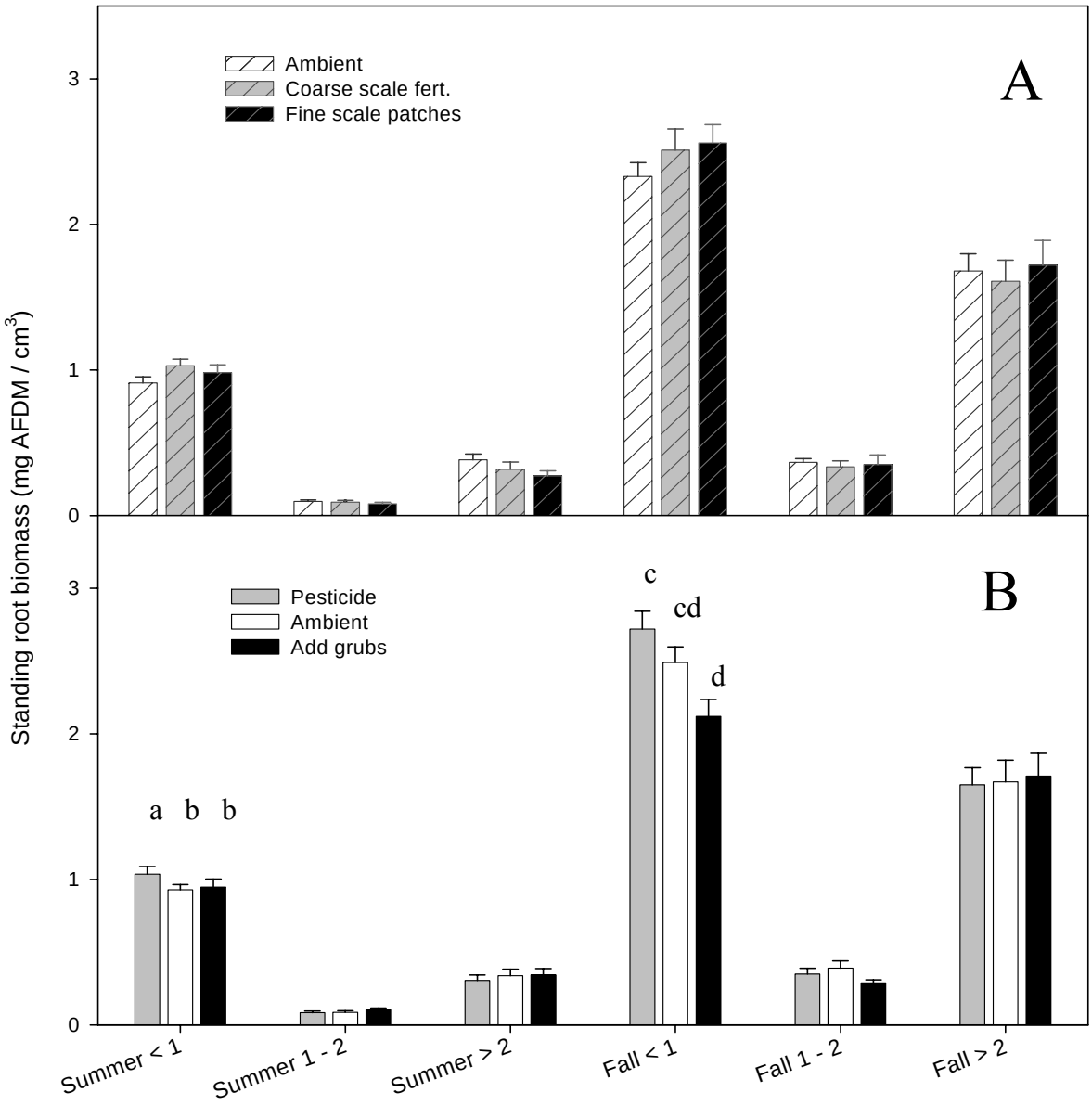

Figure 4.7. Soil fertility (A) and soil fauna (B) treatment influences on root biomass in each of three diameter classes (< 1.0mm, 1.0 – 2.0 mm, and > 2.0 mm) in June and October. Bars shows mean standing root biomass + std. err. Within three-bar groups, different letters indicate significant differences between means at alpha = 0.05 (letters a, b) or at alpha = 0.10 (letters c and d).

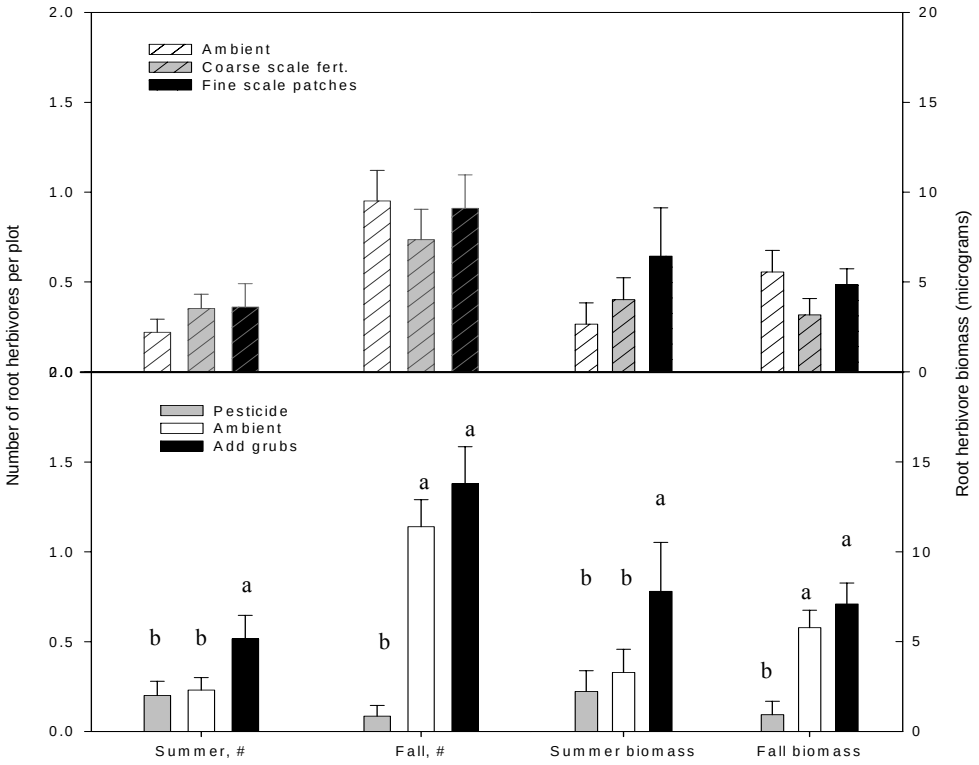

Figure 4.8. Effects of soil fertility (A) and soil fauna (B) treatments on root herbivore numbers and biomass. Bars show means per plot + std. error. Within three-bar groups, different small letters indicate significant differences at $P < 0.05$.

SUMMARY

The previous chapters examined how nutrient heterogeneity might serve as a bottom up template for influencing root dynamics, behaviors and influences of root herbivores, and plant community structure and function. Several key findings emerged from this research:

1. Root herbivores appear to consume significant quantities of fine root carbon, and this effect increases with microsite fertility (Chapter 2).
2. Plant species differences in root foraging behaviors are associated with their responses to root herbivores (Chapter 3). In this case, the most precise forager was the only species that suffered reduced growth in the presence of root herbivores.
3. Both reductions in soil fauna through insecticide application as well as increases in root herbivory stimulated production of canopy biomass and the balance between locally common, cool-season species and less common, warm-season species (Chapter 4).
4. While patterns in soil fertility and root herbivory did interact to influence community structure and function (Chapters 2 and 3), in some systems the overall influence of soil fertility was low (Chapter 4). Even in this case, however, there were interactive effects of resource heterogeneity and root herbivores on the growth of warm-season species, suggesting that these interactions may influence the persistence of species in communities prone to competitive exclusion.

Overall, my data show that while resource heterogeneity may influence root foraging patterns and interspecific competition in plant communities, root herbivores respond to resource patches as well. By foraging in these resource-rich patches, root herbivores appear to constrain the potential benefits of root foraging. Thus, root herbivores may be a selective force that balances potential gains by precisely-foraging plant species. This influence may help to explain the range of root behaviors that are commonly seen in plant communities. In addition, these results support the need to consider both the responses and effects of root herbivores in community interactions and ecosystem carbon cycling.