

Ecology of Root-Feeding Insect Assemblages in Fire-Manipulated Longleaf Pine-Wiregrass Ecosystems

Matthew J. Dittler

Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State
University in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY
IN
BIOLOGICAL SCIENCES

Robert H. Jones (Co-Chair)
Erik T. Nilsen (Co-Chair)
Carlyle C. Brewster
Thomas R. Fox
Dorothea B. C. Tholl

April 16, 2013
Blacksburg, Virginia

KEY WORDS:

Root-feeding insect, fine root herbivore, belowground herbivory, longleaf pine,
fine root dynamics

Ecology of Root-Feeding Insect Assemblages in Fire-Manipulated Longleaf Pine-Wiregrass Ecosystems

Matthew J. Dittler

ABSTRACT

Root-feeding insects can have top-down influence on vegetative composition and ecosystem processes; however, they may respond to disturbance and bottom-up factors such as soil resources and site productivity. My research addressed the following questions: (1) Do disturbance (fire), vegetative composition, soil resources, and fine root standing mass influence the structure of root-feeding insect assemblages? (2) What types of roots do root-feeding insects eat, and do they forage selectively? (3) Do root-feeding insects influence fine root productivity? To address these questions, I studied root-feeding insect assemblages in longleaf pine wiregrass (*Pinus palustris-Aristida stricta*) ecosystems of southwestern Georgia, U.S.A. On a random basis, study sites were burned at least every other year (B), or left unburned (UB) for about 9 years. Fine root productivity and root-feeding insect abundances were sampled repeatedly across 54 random plots in UB and B sites. In Chapter 2, I characterized spatial and temporal patterns of root-feeding insect abundance, understory plant composition, soil resource availability, and fine root standing mass within each plot. Insect population densities were low overall, but abundance, patchiness, and diversity were greater in UB sites. Abundance patterns were significantly related to vegetative composition. In Chapter 3, I quantified the diet of root-feeding insects by measuring the natural abundance of carbon

(C) and nitrogen (N) stable isotopes in insects and fine roots. Using ^{13}C abundance, I examined the contribution of warm season grass roots to insect diet, relative to the proportion of warm season grass roots within adjacent root standing crop samples; ^{15}N abundance was used to detect omnivory. Overall, insects appeared to be non-selective herbivores and omnivores that may alter foraging behavior to maintain a mixed diet (i.e. reducing or increasing warm season grass consumption when its abundance was high or low, respectively). The extent of omnivory varied within and among taxa. In Chapter 4, I estimated the top-down influence of root-feeding insects on fine root productivity by comparison of ingrowth cores with or without an insecticide treatment. I detected a weak positive effect of herbivores on the productivity of non-grass fine roots (< 10% of fine root productivity).

ACKNOWLEDGEMENTS

First, I thank Bob Jones, my major advisor, for allowing me the opportunity to pursue graduate studies, even though I was fresh out of college and inexperienced. I thank him for his advice, comments, and especially for his kindness, patience, and encouragement. I appreciate the sense of critical thinking and independence that getting to work with Bob has fostered. He is also excellent as an administrator, teacher, editor, and ecologist.

This research was funded in part by grants or awards from USDA-NRI (grant # 2005-35101-15363), Joseph Jones Ecological Research Center (Ichauway), the Virginia Tech Graduate Research and Development Program, and Sigma Xi. These funding contributions were greatly appreciated.

I thank the dedicated Virginia Tech professors that served on my committee: Carlyle Brewster, Tom Fox, Erik Nilsen, and Dorothea Tholl. Their critical discussion and questioning, thoughtful suggestions and encouragement, were critical in the development of my research. I also thank each of them for their time and patience attending committee meetings, and for reading and commenting on my work. Thank you each for your unique contributions to my intellectual development. I also extend a special thanks to Erik Nilsen for being willing to step in as co-advisor.

I thank Dr. Bob Mitchell and the kind staff at Ichauway for sponsoring my work and providing access to resources and assistance. Bob Mitchell served as an adviser while I was at my field sites at Ichauway. He provided helpful advice and comments, critical questioning, and a sense of urgency and seriousness that helped fuel countless hours of productive field work and thinking. The administration, technicians, staff, and

field assistants that I encountered there were a pleasure to work with. It was great meeting so many interesting, kind, knowledgeable, and helpful people. Along with Bob, I thank Dr. Lindsay Boring, Jason McGee and Matt McCorvey for helping me to make things happen.

I give much thanks to those that helped with root sampling, root sorting, hole digging, and keeping sweaty company with the gnats and mosquitoes of South Georgia. These were mostly Virginia Tech undergraduate students, some of whom were paid as field and lab assistants, and many others who were even willing to volunteer. To Faunice R. Jackson, I express gratitude for the long hours of hard field work. And I thank Jenny Chung for her careful attention to detail, and for her endurance when it came to root sorting and weighing. Thank you each and all so much for all your hard work.

I thank Glen Stevens for helpful information and suggestions. I thank my other plant ecology-minded colleagues at VT -- Verl Emrick, Travis Belote, Amanda Lentz-Ronning, Wen Lin, and Zhe Bao -- for good paper discussions and scientific debates. I thank my many other colleagues and friends who have shared in good conversation and happy hours.

Finally, I would like to thank all my family and friends for continued love, support, and encouragement throughout my graduate experience. I am especially thankful for Emma Weatherley. Thank you for sharing your amazing love, unfailing friendship, and moral support.

ATTRIBUTIONS

Attributions of Authorship:

Dr. Robert H. Jones served as my major advisor throughout my time at Virginia Tech. For most of that time he served as Department Head of Biological Sciences at VT. He is currently Dean of the Eberly College of Arts and Sciences at West Virginia University. He is listed as a co-author on all of the data chapters and future publications from this work because of his intellectual and editorial contributions, and because he was instrumental in providing access to funding and additional resources.

Dr. Robert J. Mitchell, served in an advisory role while I resided at Ichauway during field seasons. He provided access to resources at Ichauway and made intellectual contributions that helped to shape my thinking in the work which I present in Chapters 3 and 5, and is therefore included as a co-author on that body of work.

TABLE OF CONTENTS

CHAPTER 1: *Introduction*

General Introduction.....	1
Belowground Herbivory in an Ecosystem Dynamics Context.....	4
Belowground Plant Herbivore Interactions.....	5
The Role of Fire Frequency, Bottom-Up, and Top-Down Forces in Structuring Plant and Herbivore Communities.....	7
Brief Life History of Root-feeding Insects.....	8
Literature Cited.....	11

CHAPTER 2: *The Role of Fire and Bottom-Up Forces in Structuring Root-Feeding Insect Assemblages in Longleaf Pine-Wiregrass Ecosystems*

Abstract.....	17
Introduction.....	19
Methods.....	24
Results.....	41
Discussion.....	49
Conclusions.....	58
Literature Cited.....	59
Tables.....	66
Figures.....	77

CHAPTER 3: *The Dietary Ecology of Root-Feeding Insect Assemblages in Longleaf Pine-Wiregrass Ecosystems*

Abstract.....	87
Introduction.....	88
Methods.....	93
Results.....	102
Discussion.....	105
Conclusions.....	112
Literature Cited.....	113
Tables.....	117
Figures.....	122

CHAPTER 4: *Top-Down Influences of Root-Feeding Insects on Fine Root Productivity
in Fire Manipulated Longleaf Pine-Wiregrass Ecosystems*

Abstract.....	130
Introduction.....	131
Methods.....	134
Results.....	142
Discussion.....	144
Conclusions.....	149
Literature Cited.....	150
Tables.....	154
Figures.....	160

CHAPTER 5: *Synthesis*

Summary.....	166
Implications for Ecosystem Dynamics.....	168
Significance of this Research.....	170
Literature Cited.....	170
Figures.....	172

APPENDICES.....	173
-----------------	-----

LIST OF TABLES

Table 2-1: List of root-feeding insect taxonomic groups.....	66
Table 2-2: Summary of results for Chi-square tests of insect count frequency distributions between fire treatments, sampling periods, and sites.....	67
Table 2-3: Summary of site vegetative composition, fine root standing crop, and root feeding insect population density.....	68
Table 2-4: Summary of site soil properties.....	69
Table 2-5: Summary of alternative generalized estimating equations (GEEs) for root-feeding insects.....	70
Table 2-6: Effect size summary for best fit experimental model of total insect abundance.....	73
Table 2-7: Effect size summary for predictive models of insect abundance.....	74
Table 2-8: Spatial auto-correlation indices for root-feeding insect counts.....	75
Table 2-9, a, b: Indices of spatial and temporal dispersion for insect count frequency data.....	76
Table 3-1: Quantitative list of the number of individual insects from each taxonomic group included in the dietary study.....	117
Table 3-2: Summary of minimum, maximum, and mean $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N ratio for fine root standing crops.....	118
Table 3-3: Mean $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N ratio of insect taxa.....	119
Table 3-4: Summary of Mann-Whitney U tests for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of insects in burned and unburned plots.....	120
Table 3-5: Average permil deviation of insect diet ($\delta^{13}\text{C}$) from local fine root composition.....	121

Table 4-1: Univariate statistical summary of fine root ingrowth estimates.....154

Table 4-2, a, b: Mixed model estimates of covariance components (**a**) and fixed effects (**b**) for Fire and Insect Removal Treatment.....155

Table 4-3: Univariate statistical summary of estimates for top-down effects from root herbivores (control minus treatment ingrowth).....157

Table 4-4: Summary of root-feeding insect counts.....158

Table 4-5: Summary of correlation coefficients for relationships among the top-down herbivore effects, total RFI biomass, RFI relative abundance, and fine root standing mass.....159

LIST OF FIGURES

Figure 2-1: General map of study area (Ichauway Property Boundaries).....	77
Figure 2-2: Conceptual diagram of sampling scheme.....	78
Figure 2-3: Conceptual diagram showing the layout of plots.....	79
Figure 2-4: Root-feeding insect population density (box-plots) for unburned and burned plots.....	80
Figure 2-5: Mean root-feeding insect population density for the unburned and burned treatment groups.....	81
Figure 2-6, a-d: Total insect biomass summary for unburned and burned treatments.....	82
Figure 2-7: Mean root-feeding insect population density for the 6 study sites.....	83
Figure 2-8: Mean root-feeding insect population density for the 6 sampling periods.....	84
Figure 2-9: Root-feeding insect population density for each unburned or burned study site at each time period.....	85
Figure 2-10: Standardized quantile variance for insect relative abundance.....	86
Figure 3-1: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of fine root standing crops.....	122
Figure 3-2, a-c: Box plots representing $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N of root-feeding insects and fine roots in burned and unburned plots.....	123
Figure 3-3: Trophic variability ($\delta^{15}\text{N}$) of insect taxa.....	124
Figure 3-4: Bivariate plot showing $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of insect taxa and fine root standing crop samples.....	125
Figure 3-5, a, b: Box plots comparing $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N of root-feeding insects across 3 sampling periods.....	126
Figure 3-6: Individual insect diet ($\delta^{13}\text{C}$) as a response to local fine root $\delta^{13}\text{C}$	127

Figure 3-7, a-c: Mean $\delta^{13}\text{C}$ for insect taxa ($\pm 95\%$ confidence interval) for white grub, wireworm, and other insect genera.....128

Figure 3-8: Local scale dietary selectivity (permil deviation from local fine root $\delta^{13}\text{C}$) as a response to fine root standing mass.....129

Figure 4-1: Conceptual diagram showing the layout of plots.....160

Figure 4-2, a-c: Box and whisker plots of total fine root ingrowth for unburned and burned plots, 3 ingrowth periods, and control versus insecticide treated cores.....161

Figure 4-3, a-c: Mean fine root ingrowth, for grass, other, and combined roots.....162

Figure 4-4, a, b: Box and whisker plots of the difference in control and insecticide treated ingrowth cores.....163

Figure 4-5, a, b: Root-feeding insect relative abundance and total biomass for unburned and burned fire treatments.....164

Figure 4-6, a, b: Bivariate plot of top-down herbivore effects in response to (a) cumulative herbivore biomass or (b) cumulative herbivore count.....165

Figure 5-1: Conceptual diagram showing potential interactions between root-feeding insects, fine root productivity, and other related components in fire-manipulated woodland ecosystems.....172

LIST OF APPENDICES

Appendix A: Site and plot locations; GPS coordinates of plots.....	173
Appendix B: Additional justification for Chapter 2 data analyses.....	174
Use of the Negative Binomial Distribution to Address Overdispersion of Count Data.....	174
Model Selection Using an Information Theoretic Approach.....	175
Use of the Geometric Mean for Indices of Spatial and Temporal Dispersion.....	176
Analysis of Spatial Autocorrelation.....	177
Literature Cited.....	178
Appendix C: Root-feeding insect raw count tally summary.....	180
Appendix D: Insect taxa-specific effect size summaries of parameter estimates based off generalized estimating equations.....	184
Appendix E: List of isotopic endpoints for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$	189
Appendix F: The C:N ratio as a response to $\delta^{13}\text{C}$ of fine root standing crop.....	190

1

2

CHAPTER 1

Introduction

Matthew J. Dittler

GENERAL INTRODUCTION

A great diversity of herbivores inhabits the earth, ranging from small (e.g. zooplankton and nematodes) to large (e.g. mammalian grazers). Though the green world hypothesis (Hairston, Smith and Slobodkin, 1960) raised the theoretical expectation that herbivore populations are controlled by top-down processes while plants are bottom-up regulated, there are many instances where this is not exclusively the case. For instance, large grazing herbivores can cause notable, sometimes long-lived shifts in plant community interactions and vegetative composition (Milchunas, 1988). There are many other examples, such as burrowing mammalian herbivores, which can alter spatial patterns in ecosystem structure, nutrient cycling, and vegetative composition (Villareal et al., 2008); and defoliating insects, which can alter ecosystem structure and function by transferring large amounts of carbon and nutrients from aboveground vegetation to soil biota (Lovett et al., 2002).

Less is known about the ecology of belowground insect herbivores due to a limited number of studies. In some systems, fine root herbivores have been shown to exert top-down influence on the structure and dynamics of plant communities (De Deyn et al., 2004; Brown and Gange 1990, 1989). They may also respond to fire (Sileshi and Mafongoya, 2006; Wikars and Schimmel, 2001) resource availability and site productivity (Stevens and Jones, 2006; Hunter, 2001; Hunter and Price, 1992). Fire is an important source of disturbance, the frequency and

intensity of which have important implications for the distribution and structure of plant and insect communities across a large extent of the Earth's terrestrial ecosystems (see Breshears, 2006 and Sileshi and Mafongoya, 2006). Root herbivores may respond to cues associated with more productive micro-sites or root volatile emissions, which may introduce a negative feedback influencing competitive (plant-plant) interactions associated with resource-targeted root proliferation (Stevens and Jones, 2006; Brown and Gange, 1990; Jones and Coaker, 1977).

Top-down effects of herbivores on net productivity and plant community dynamics are likely dependent on herbivore population density, and the evolutionary and ecological context of the system (Michunas et al., 1988). For instance, intermediate levels of grazing can lead to increases in plant diversity (see Connell, 1978) and productivity (McNaughton, 1985), while excessive herbivory can lead to reduced plant diversity, altered successional patterns (Brown and Gange, 1990, 1989), and significant declines in standing biomass and productivity (McNaughton, 1985). The magnitude of effects from disturbance, resource supply, and herbivory, are major determinates of the structure and dynamics of plant communities and ecosystems (Kambatuka et al., 2011; De Deyn et al., 2004; Hunter, 2001; Hunter and Price, 1992; Brown and Gange, 1990, 1989; also see Hairston et al., 1960). The research presented in this dissertation addressed several important, but poorly understood aspects of the trophic and population ecology of root-feeding insects, within a framework that took into account fire frequency, plant community structure, belowground productivity, and soil resources.

My objectives were to characterize the taxonomic structure, spatial distribution, population dynamics, diets, and top-down effects of root-feeding insect assemblages, and to investigate underlying relationships with disturbance frequency, vegetative composition, and soil

resource availability within fire-manipulated longleaf pine-wiregrass (*Pinus palustris*-*Aristida stricta*) ecosystems of southwestern Georgia, U.S.A.

My research addressed the following principal questions: (1) Do fire and bottom-up factors (vegetative composition, soil properties, and fine root productivity) influence the abundance, distribution, and population dynamics of root-feeding insect assemblages? (2) What types of roots do root-feeding insects eat, and do they forage selectively? (3) In what way, and by how much, do root-feeding insects influence ecosystem function (i.e. fine root productivity)? These questions address the ecology of root-feeding insects at multiple biological scales ranging from organisms to ecosystems. Each question is respectively addressed in Chapters 2, 3, and 4.

To determine the relationships among fire frequency, bottom-up factors, and the structure of insect assemblages (Chapter 2), I examined spatial-temporal, and taxonomic patterns of root-feeding insect abundances, along with understory plant community composition, fine root standing crop, and soil properties, across 54 study plots, evenly distributed across 6 sites, 3 frequently burned and 3 excluded from burning for about 9 years, and six sampling periods from 2008 to 2010. Root feeding insect abundance, patchiness, and diversity were measured, and relationships with fire frequency, sampling period, vegetative composition, soil fertility, and belowground production were estimated.

To quantify the diet of root-feeding insects (Chapter 3), I measured stable carbon and nitrogen isotope natural abundances from root-feeding insects collected in soil samples. I used carbon isotope abundance to estimate the proportionate dietary contribution of warm season grass (C_4) roots relative to C_4 abundance within fine root standing crop samples, across a fire-manipulated gradient in warm season grass abundance. Nitrogen isotope abundance was used to estimate the extent to which root-feeding insects were omnivorous.

To determine the top-down influence of root-feeding insects (Chapter 5), I measured fine root production in response to paired insecticide removal and control treatments within sample plots. Ingrowth cores were used to quantify fine root productivity in response to the herbivore removal.

BELOWGROUND HERBIVORY IN AN ECOSYSTEM DYNAMICS CONTEXT

Net primary production (NPP) is a key process in ecosystem dynamics. A large proportion of NPP, generally near 50%, is allocated to fine roots, due to their rapid rate of turnover (Eissenstat and Yanai, 2002, Fahey and Hughes, 1994; Vogt et al., 1986; Nadelhoffer and Raich, 1992). Though an important component of ecosystem function, fine root productivity is often absent or poorly estimated in ecosystem budgets. This is largely attributable to the methodological difficulties associated with making accurate measurements of fine root productivity belowground, and to a paucity of research addressing the multiple fates of fine root productivity (see Eissenstat and Yanai, 2002). An accurate assessment of fine root production (P_{FR}) over a specific time period should include the accrual of new fine root biomass (ΔB), natural mortality (ΔD), root exudation (ΔE) production of volatiles (ΔV), which may be associated with belowground symbiotic interactions, plant communication or plant defense, and herbivory (ΔH); therefore, $P_{FR} = \Delta B + \Delta D + \Delta E + \Delta V + \Delta H$; (modified from Eissenstat and Yanai, 2002).

Fine root herbivory is, in general, a poorly quantified, but potentially important fate of fine root NPP. Andersen (1987) estimates root consumption in the range of 6% - 30% of fine root NPP. Other studies demonstrate that the disappearance of fine roots between sampling intervals (presumably from herbivores) is common and may account for near 40% of fine root

mortality (Stevens et al., 2002; Fahey and Hughes, 1994; Hendrick and Pregitzer, 1992). If fine root herbivory is not accounted, then even otherwise careful estimates of fine root NPP may significantly underestimate belowground NPP.

BELOWGROUND PLANT-HERBIVORE INTERACTIONS

Herbivore Foraging and Diet

Foraging behavior and diet are important aspects of root-feeding insect ecology. To better understand interactions among belowground insect herbivores, vegetation dynamics, and ecosystem function, it is necessary to understand the degree to which herbivores exhibit dietary specialization. Herbivores that specialize on a small number of plant types should have very different outcomes for the plant community composition over time than herbivores that feed at random. Studies of aboveground systems show that selective herbivore diets can exert changes in vegetative composition (Schowalter, 2011).

Though belowground herbivores are generally described as either specialists or generalists, it is unlikely that all fall to one extreme of the specialist-generalist continuum. Many of the most common and widespread genera of belowground insect herbivores (e.g. white grubs and wireworms) are considered generalists (Stehr, 1991), but it is not necessarily the case that they forage randomly. There are several reasons to suspect they may not. Root feeding insects are considered pests of cool season (C_3) grasses (Fermanian et al., 2002), and scientific studies of root feeding insects tend to focus on cool season grass species, suggesting a possible food source preference (e.g. Bughrara, 2008 and Braman and Ramer, 2006). Plant tissue quality (e.g. C:N ratio, cellulose, lignin, nitrogen, and micronutrient content) varies among plants, and may influence digestibility and the overall dietary benefits for consumers (see Craine et al., 2003;

Jonas and Joern, 2003; Thaler et al., 2002). Furthermore, root herbivores may use CO₂ concentrations, and other volatile emissions to detect food sources (Jones and Coaker, 1977; Rasmann et al., 2005), which could lead to selective foraging behaviors and preferences for more productive or nutrient rich areas (e.g. Stevens and Jones, 2006).

The dietary ecology of root-feeding insects may reflect a set of tradeoffs. If no energetic costs were involved, a selective diet would likely benefit insects; however, this is rarely the case in nature. Optimal foraging theory (MacArthur and Pianka, 1966) assumes there is a tradeoff between the benefits incurred from selective foraging, and the energy or time spent searching for prey. The mobility of root-feeding insects is limited by the structure of the soil, which may increase the energetic costs associated with foraging; however, fine roots (prey) often occur at high densities. Belowground ecosystems offer a novel system in which to test hypotheses related to optimal foraging.

Plant Defensive Responses to Root Herbivores

Plants use a suite of direct and indirect strategies to defend themselves against insect herbivores (Bierre and van Dam, 2004; van der Putten, 2003; Whittaker, 2003; Sabelis et al., 1999; Faeth, 1985). One defensive strategy, which has been receiving increased attention from ecologists, is to attract the natural enemies of their herbivores by way of volatile organic cues (Rasmann, 2005; Rodriguez-Saona and Thaler, 2005; Turlings and Wackers, 2004; van Poecke and Dicke, 2004; van Tol et al, 2001), setting the stage for complex interactions and co-evolution (Whittaker, 2003). These types of plant-herbivore interactions are of further interest because they may influence spatial and temporal processes in community and ecosystem dynamics (De Deyn et al., 2004; van der Putten, 2003; Whittaker, 2003; also see Steven and Jones, 2006 and Brown and Gange, 1990). Therefore, in addition to direct consumption of fine root derived

carbon, herbivores may cause plants to alter their proportionate belowground allocation of carbon and nutrients toward root repair and defense (Biere and van Dam, 2004; Eissenstat and Yanai, 2002; Faeth, 1985).

THE ROLE OF FIRE FREQUENCY, BOTTOM-UP, AND TOP-DOWN FORCES IN STRUCTURING PLANT AND HERBIVORE COMMUNITIES

Disturbance, climate, soil properties, resource availability, and top-down pressure from herbivores, predators, and pathogens, are major determinants of the structure and dynamics of plant and insect communities in terrestrial ecosystems (Milchunas et al., 1988; Hairston et al., 1960). Fine root herbivores can exert top-down influence on the structure and dynamics of plant communities (De Deyn et al., 2004; Brown and Gange 1990), but they also respond to disturbances like fire (e.g. Sileshi and Mafongoya, 2006 and Wikars and Schimmel, 2001), bottom-up forces (e.g. Stevens and Jones, 2006; also see Hunter, 2001), and top-down forces (e.g. van Dam, 2009 and Aggrawal et al., 2005; also see Hairston et al., 1960). Many bottom-up forces interface with plant communities in the soil; however, few studies have examined interactions among fine root herbivores, vegetative composition, fine root productivity, and bottom-up factors (Hunter, 2001).

Fire is an important disturbance that organizes the composition of vegetation along a continuum from grassland to forest (see Knapp et al., 2008 and Breshears, 2006). In fire-frequented woodlands such as the longleaf pine-wiregrass savanna, the influence of fire frequency on root-feeding insect abundance may be important for understanding the interactions of plant and herbivore communities. Fire driven changes in ecosystem structure are likely to impact root-feeding insect abundance (Sileshi and Mafongoya, 2006; Wikars and Schimmel,

2001). Structural redistribution of fine root biomass may impact herbivore foraging efficiency or the availability of suitable oviposition sites for aboveground adults. Increased forest floor litter associated with decreased fire frequency may also impact root-feeding insect population density by providing protection from predators to adults. Finally, altered fine root tissue quality associated with changes in species composition may impact fine root consumption rates and herbivore assimilation efficiencies, or result in altered herbivore foraging behavior. These impacts in turn, could have feedbacks for vegetative composition and ecosystem processes.

Top down effects of herbivores on net productivity and plant community dynamics are likely density dependent. For instance, intermediate levels of grazing can lead to increases in plant diversity (see Connell, 1978), and productivity (McNaughton, 1985), while excessive grazing can lead to altered plant community composition (Brown and Gange, 1990, 1989), decreased plant diversity, and significant declines in standing biomass and productivity (McNaughton, 1985).

BRIEF LIFE HISTORIES OF ROOT-FEEDING INSECTS

Root feeding insects, in particularly the larvae of Scarabaeidae (white grubs), Elateridae (wireworms), Curculionidae (weevils), and Cicadidae (cicadas) are widespread fine root herbivores. A basic understanding of the lifecycles and habits of these herbivores is important for understanding them in an ecological context. The following sections provide brief life histories for the insect taxonomic groups included in my research.

Larvae of Scarabaeidae (White Grubs)

There are several species of white grub, and the number of instars and length of time spent in the larval phase (generally 2 years or less) varies with species (Fermanian et al., 2002;

Stehr, 1991). Fermanian et al. (2002), Stehr (1991), and Ritcher (1966) summarize the general morphology, and natural history for white grub species in N. America. Scarabaeidae larvae generally overwinter deep in the soil (below the frost zone), and move up to the root zone early in spring to feed or pupate (depending on species lifecycle). It is important to note, however, that much of these observations were made in the northeastern United States. The study system described in the remaining chapters is located in Southwestern Georgia, U.S.A., and has a subtropical climate; therefore, hard freezes are infrequent and overwinter survival in such soils is not likely influenced by depth as they are at higher latitudes. Root feeding ceases prior to pupation, which is often observed between April and August in the Eastern U.S.A. (Fermanian et al., 2002; Stehr, 1991). Specific timing varies with species (Stehr, 1991), and possibly with climate and habitat quality. Adults emerge from the soil to mate and feed on aboveground vegetation, after which time, they return to the soil to lay their eggs (Fermanian et al., 2002). Oviposition is thought to occur within the roots of grasses, particularly in sod (Fermanian et al., 2002). After egg hatch, which in North America tends to occur in the summer, larvae feed on roots, growing in size until winter (Fermanian et al., 2002).

Larvae of Elateridae (Wireworms)

Larvae of a common genera of Elateridae, *Agriotes* pass through as many as 13 instars (Furlan, 2004). Larval development, as well as vertical migration in the soil, is thought to correlate with soil moisture, food availability, and soil temperature (Furlan, 2004). They are described as generalist feeders, but their intense feeding phases are thought to be near only 25% of whole development time (Stehr, 1991). Their lifecycle takes approximately 24-36 months, with shorter times for lower latitudes (Furlan, 2004). Larvae generally pupate between May and September, usually in the upper soil layer (Furlan, 2004). Wireworms may undergo deeper

vertical migration in response to low soil moisture or overwintering (see Stevens et al., 2008). For instance, they have been observed at depths near or below 40cm in response to cold winter temperatures (Furlan, 2004).

Larvae of Curculionidae (weevils)

Weevils are regarded as destructive agricultural pests. Almost all weevils are considered strict herbivores; some adults and larvae specialize on a particular species or type of plant (Stehr, 1991). Weevils cause significant above- and belowground agricultural damage every year, with serious economic consequences. Some species, such as black vine weevils, are notorious root feeders in their larval phase; other species feed on aboveground vegetation as larvae and then enter the soil to pupate (Stehr, 1991; May, 1966).

Larvae of Cicadidae (cicadas)

Cicadas live underground as nymphs for nearly the entirety of their lifespan (White and Strehl, 1978). They are thought to attain their diet from tapping into the sap of tree root xylem (White and Strehl, 1978). In their final instar as a nymph, cicadas emerge by tunneling up to the surface, where they molt to become adults (Williams et al., 1993). After mating, the adult female cuts slits into bark to deposit up to hundreds of eggs (Williams et al., 1993). After hatching, the nymphs drop back to the ground and burrow (Williams et al., 1993). Cicadas go through life cycles that last from 2 to 5 years, with some notable taxa, such as *Magicicada*, going through a 17 or 13 year life cycle, culminating in a synchronized emergence of adults, which may have evolved as a predator satiation strategy (Williams et al., 1993).

Larvae of Cebrionidae

Very little is known about these larvae, other than that they dwell in the soil (Stehr, 1991). We included them in some aspects of the studies described in later chapters because they belonged to the same super-family as wireworms, Elateroidea (Stehr, 1991), causing us to think it possible that they might have a similar diet (see Chapter 3). They resemble wireworms in general body shape, but were easily distinguished from the Elaterids based on conspicuous elongation of the prothorax.

LITERATURE CITED

- Andersen, D.C. 1987. Below-ground herbivory in natural communities: a review emphasizing fossorial animals. *The Quarterly Review of Biology* **62**:261-286.
- Breshears, D.D. 2006. The grassland-forest continuum: Trends in ecosystem properties for woody plant mosaics? *Frontiers in Ecology and the Environment* **4**:96-104.
- Biere, A., H. B. Mark, and J. M. M. van Damme. 2004. Plant chemical defense against herbivores and pathogens: generalized defense or trade-offs? *Ecophysiology* **140**:430-441.
- Braman, S.K. and P.L. Raymer. 2006. Impact of Japanese Beetle (Coleoptera: Scarabaeidae) feeding on Seashore Paspalum. *Journal of Economic Entomology* **99**:1699-1704.
- Brown, V. K., and A. C. Gange. 1990. Insect herbivory below ground. *Advances in Ecological Research* **20**:1-58.
- Brown, V.K. and A.C. Gange. 1989. Differential effects of above- and belowground insect herbivory during early plant succession. *Oikos* **54**:67-76.

- Bughrara, S.S., D.R. Smitley, D. Cappaert, and A.Krauchencko. 2008. Comparison of Tall Fescue (Cyperales: Gramineae) to Other Cool-Season Turfgrasses for Tolerance to European Chafer (Coleoptera: Scarabaeidae). *Journal of Economic Entomology* **96**: 1898-1904.
- Connell, J.H. 1978. Diversity in tropical rain forests and coral reefs. *Science* **199**:1302-1310.
- Craine, J. W. Bond, W. G. Lee, P. B. Reich, and S. Ollinger. 2003. The resource economics of chemical and structural defenses across nitrogen supply gradients. *Oecologia* **137**:547-556.
- De Deyn, G.B., C.E. Raaijmakers, and W.H. van der Putten. 2004. Plant community development is affected by nutrients and soil biota. *Journal of Ecology* **92**:824-834.
- Eissenstat, D. M., and R. D. Yanai. 2002. Root Life Span, Efficiency, and Turnover. Pages 221-238 *in* Y. Waisel, A. Eshel, and U. Kafkafi, editors. *Plant Roots: The Hidden Half*. Marcel Dekker, Inc., New York.
- Faeth, S. H. 1985. Quantitative defense theory and patterns of feeding by oak insects. *Oecologia* **68**:34-40.
- Fahey, T. J., and J. W. Hughes. 1994. Fine root dynamics in a northern hardwood forest ecosystem, Hubbard Brook Experimental Forest, NH. *Journal of Ecology* **82**:533-548.
- Fermanian, T.W., M.C. Shurtleff, R. Randell, H.T. Wilkinson, and P.L. Nixon. 2002. *Controlling Turfgrass Pests* 3rd Ed. Prentice-Hall, New York.
- Furlan, L. 2004. The biology of *Agriotes sordidus* Illiger (Col., Elateridae). *Journal of Entomology and Nematology* **128**:696-706.
- Hairston, N.G. F.E. Smith, and L.B. Slobodkin. 1960. Community structure, population control, and competition. *The American Naturalist* **94**:421-425.
- Hendrick, R., and K. S. Pregitzer. 1992. The demography of fine roots in a northern hardwood forest. *Ecology* **73**:1094-1104.

- Hunter, M.D. 2001. Out of sight, out of mind: the impacts of root-feeding insects in natural and managed systems. *Agriculture and Forest Entomology* **3**:3-9.
- Hunter, M.D. and P.W. Price. 1992. Playing chutes and ladders: Heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology* **73**:724-732.
- Jonas, J. L. and A. Joern. 2003. Host plant quality alters grass/forb consumption by a mixed-feeding insect herbivore, *Melanoplus bivittatus* (Orthoptera: Acrididae). *Ecological Entomology* **33**:546-554.
- Jones, O.T., and T.H Coaker. 1977. Oriented responses of carrot fly larvae, *Psila rosae*, to plant odors, carbon-dioxide and carrot root volatiles. *Physiological Entomology* **2**:189-197.
- Kambatuku, J.R., M.D. Cramer, and D. Ward. 2011. Savanna tree-grass competition is modified by substrate type and herbivory. *Journal of Vegetation Science* **22**:225-237.
- Knapp, A.K., J.M. Briggs, S.L. Collins, S.R. Archer, M.S. Bret-Harte, B.E. Ewers, D.P. Peters, D.R. Young, G.R. Shaver, E. Pendall, and M.B. Cleary. 2008. Shrub encroachment in North American grasslands: Shifts in growth form dominance rapidly alters control of ecosystem carbon inputs. *Global Change Biology* **14**:615-623.
- Lovett, G.M., L.M. Christenson, P.M. Groffman, C.G. Jones, J.E. Hart, and M.J. Mitchell. 2002. Insect defoliation and nitrogen cycling in forests. *Bioscience* **52**: 335-341.
- MacArthur R.H. and E.R. Pianka. 1966. On optimal use of a patchy environment. *American Naturalist* **100**: 603-609.
- May, B.M. 1966. Identification of the immature forms of some common soil-dwelling weevils, with notes on their biology. *New Zealand Journal of Agricultural Research* **9**:286-316.
- McNaughton, S.J. 1985. Ecology of a grazing ecosystem: The Serengeti. *Ecological Monographs* **55**:259-294.

- Milchunas, D.G., O.E. Sala, and W.K. Lauenroth. 1988. A generalized model of the effects of grazing by large herbivores on grassland community structure. *The American Naturalist* **32**:97-106.
- Nadelhoffer, K. J., and J. W. Raich. 1992. Fine root production estimates and belowground carbon allocation in forest ecosystems. *Ecology* **73**:1139-1147.
- Rasmann, S., T. G. Kollner, J. Degenhardt, I. Hiltbold, S. Toepfer, U. Kuhlmann, J. Gershenzon, and T. C. J. Turlings. 2005. Recruitment of entomopathogenic nematodes by insect-damaged maize roots. *Nature* **434**:732-737.
- Ritcher, P.O. 1966. *White Grubs and Their Allies: A Study of North American Scarabaeoid Larvae*. Studies in Entomology Number Four. Oregon State University Press, Corvallis, Oregon.
- Rodriguez-Saona, C., and J. S. Thaler. 2005. The jasmonate pathway alters herbivore feeding behaviour: consequences for plant defences. *Entomologia Experimentalis et Applicata* **115**:125-134.
- Sabelis, M. W., M. Van Baalen, F. M. Bakker, J. Bruin, B. Drukker, M. Egas, A. R. M. Janssen, I. K. Lesna, B. Pels, P. C. J. Van Rijn, and P. Scutareanu. 1999. The evolution of direct and indirect plant defense against herbivorous arthropods. Pages 109-166 in I. Olf, V. K. Brown, and R. H. Drent, editors. *Herbivores: Between Plants and Predators*. Blackwell Science Ltd., Cambridge.
- Schowalter, T.D. 2011. *Insect Ecology: An Ecosystem Approach*, 3rd ed. Academic Press, London.
- Sileshi, G. and P.L. Mafongoya. 2006. The short term impact of forest fire on soil invertebrates in the miombo. *Biodiversity and Conservation* **15**:3153-3160.
- Stehr, F.W. 1991. *Immature Insects* (Vol. 2). Kendall-Hunt Publishing Co., Dubuque, Iowa.

- Stevens, G.N., K.O. Spence, and E.E. Lewis. Root feeders in Heterogeneous Systems: Foraging Responses and Trophic Interactions. 2008. In: Root Feeders: An Ecosystem Perspective. CAB, Oxfordshire, UK.
- Stevens, G. N., and R. H. Jones. 2006. Patterns in soil fertility and root herbivory interact to influence fine-root dynamics. *Ecology* **87**:616-624.
- Stevens, G. N., R. H. Jones, and R. J. Mitchell. 2002. Rapid fine root disappearance in a pine woodland: a substantial carbon flux. *Canadian Journal of Forest Research* **32**:2225-2230.
- Thaler, J. S., M. A. Farag, P. W. Pare, and M. Dicke. 2002. Jasmonate-deficient plants have reduced direct and indirect defenses against herbivores. *Ecology Letters* **5**:764-774.
- Turlings, T. C. J., and F. Wackers. 2004. Recruitment of predators and parasitoids by herbivore-injured plants. *in* R. T. Cardé and J. G. Millar, editors. *Advances in Insect Chemical Ecology*. Cambridge University Press, Cambridge.
- van Dam, N.M. 2009. Belowground Herbivory and Plant Defenses. *Annual Review of Ecology, Evolution, and Systematics* **40**:373-391.
- van der Putten, W. H. 2003. Plant defense belowground and spatiotemporal processes in natural vegetation. *Ecology* **84**:2269-2280.
- van Poecke, R. M. P., and M. Dicke. 2004. Indirect defence of plants against herbivores: using *Arabidopsis thaliana* as a model plant. *Plant Biology* **6**:387-401.
- van Tol, R. W. H. M., A. T. C. van der Sommen, M. I. C. Boff, J. van Bezooijen, M. W. Sabelis, and P. H. Smits. 2001. Plants protect their roots by alerting the enemies of grubs. *Ecology Letters* **4**:292-294.
- Vogt, K. A., C. C. Grier, and D. J. Vogt. 1986. Production, turnover, and nutrient dynamics of above- and belowground detritus of world forests. *Advances in Ecological Research* **15**:303-377.

- Villarreal, D., K.L. Clark, L.C. Branch, J.L. Hierro, and M. Machicote. 2008. Alteration of ecosystem structure by a burrowing herbivore, the plains vizcacha (*Lagostomus maximus*). *Journal of Mammalogy* **89**:700-711.
- White, JoA. and C.E. Strehl. 1978. Xylem feeding by periodical cicada nymphs on tree roots. *Ecological Entomology* **3**:323-327.
- Whittaker, J. B. 2003. Root-animal interactions. Pages 363-385 in H. de Kroon and E. J. W. Visser, editors. *Root Ecology*. Springer, New York.
- Wikars, L-O. and J. Schimmel. 2001. Immediate effects of fire severity on soil invertebrates in cut and uncut pine forests. *Forest Ecology and Management* **141**:189-200.
- Willams, K.S., K.G. Smilth, and F.M. Stephen. 1993. Emergence of 13-year periodical cicadas (Cicadidae: Magicicada): phenology, mortality, and predator satiation. *Ecology* **74**:1143-1152.

CHAPTER 2

The Role of Fire and Bottom-Up Forces in Structuring Root-Feeding Insect Assemblages in Longleaf Pine-Wiregrass Ecosystems

Matthew Dittler, Robert H. Jones, and Robert J. Mitchell

ABSTRACT

Root-feeding insects can exert "top-down," predation-mediated, effects on plant communities. The direction and strength of these effects, however, depends on the abundance and distribution of the herbivores, the impacts of disturbance on ecosystem structure, and "bottom-up factors" such as soil resources and net productivity. We measured root-feeding insect abundances, understory vegetative composition, fine root standing crop, and soil properties across 54 study plots and 6 repeated sampling periods (2008 - 2010) to estimate the relationships among disturbance, bottom-up factors, and the structure of root-feeding insect assemblages in longleaf pine-wiregrass ecosystems of southwestern Georgia, U.S.A. Plots were distributed evenly between six sites, which were nested within two fire disturbance treatments: Burned (B) at least every other year, and unburned (UB) for approximately 9 years prior to our initial sampling period and throughout the experiment. Burning treatments were assigned on a random basis to a set of randomly selected stands that were dominated by > 60 year old longleaf pine (*Pinus palustris*) and also had three-awn pine wiregrass (*Aristida stricta*) abundant in the understory. Within plots, aboveground vegetative composition, fine root standing crop, and soil properties were also quantified. Relationships among these bottom-up factors, fire treatment, and herbivore

abundances were estimated using Marginal Longitudinal Generalized Linear Models, which accounted for repeated measures and the overdispersion (an excess of zeros and low-value integers) associated with herbivore count data. Model selection and inference followed an information theoretic approach. Finally, spatial and temporal patterns of dispersion were quantified, and analysis of quantile variance was used, to examine the spatial-temporal dynamics of root-feeding insect assemblages. Root feeding insect abundance and diversity were greater in the UB than the B fire treatment ($p = 0.062$). The mean population density (\pm standard error) for combined taxa was 5.1 ± 1.9 individuals per square meter; for UB and B treatments it was 6.5 ± 2.5 and 3.7 ± 1.8 , respectively. Two white grub and two wireworm genera were only found in UB sites. The cover of woody plants, soil nitrogen (NH_4^+), and fine root standing biomass were positively related to root-feeding insect abundance for combined taxa, while cover of wiregrass, other C_4 grasses, forbs, and surface organic matter were negatively related, as was the cation exchange capacity. This indicated that vegetative composition and to a lesser extent, soil fertility, influenced the structure of root-feeding insect assemblages. The relationships of individual taxa to specific variables varied in significance and direction. Calculation of dispersion indices in UB and B treatment groups revealed that root-feeding insect count distributions were spatially aggregated, and more so in UB sites. Finally, spatial aggregations of the highest and lowest herbivore densities tended to persist at similar densities over time, while intermediate densities were more variable. This pattern could follow from low rates of larval migration or consistent attraction of gravid adults or larvae to environmental cues.

INTRODUCTION

It is broadly accepted that herbivores can exert top-down forces, which along with bottom-up forces like nutrient dynamics and disturbance, influence plant community structure, succession, and ecosystem function (Hunter, 2001; De Deyn et al., 1994; Hunter and Price, 1992; Brown and Gange, 1990, 1989). Fine root herbivores alter fine root dynamics via direct consumption of fine root tissue, or by causing systemic mortality of low-order roots if higher order roots are damaged (Eissenstat and Yanai, 2002, Brown and Gange, 1990). Infestations of fine root herbivores can result in significantly reduced total productivity and increased plant mortality (Fermanian et al., 2002; Lura and Nyren, 1992, Brown and Gange, 1990). Furthermore, fine root herbivory can elicit a range of direct and indirect defense responses in plants, including some which have been shown to drive multi-trophic interactions (Rasmann, 2005; Arimura et al., 2004; Thaler et al., 2002; Price et al., 1980). At the same time, soil herbivory may be affected by factors that influence food quality and availability, such as vegetation structure, succession, soil properties, and disturbance (Stevens and Jones, 2006; Jonas and Joern, 2003; Hunter and Price, 1992). Therefore, bottom-up forces may not only alter vegetation structure directly, but also indirectly, by influencing the distribution and abundance of herbivores. Disturbances, bottom-up, and top-down forces interface with plant communities in the soil (Hunter, 2001); however, few studies have examined fine root herbivory in the context of these interacting factors (Stevens, 2005; Hunter, 2001).

Spatial and temporal variability of belowground herbivores, soil nutrients, and plant communities add complexities that can influence trophic interactions and make it difficult to assess community structure and function. Furthermore, spatial distribution is an important structural aspect of belowground herbivore assemblages, which is potentially influenced by

bottom-up factors. Non-random spatial organization is inherently common in nature, due to environmental heterogeneity and dispersal patterns of organisms during their life cycles (see Taylor, 1961). To date, most studies relating to spatial distributions of fine root herbivores have focused on heavily managed systems, such as vegetable crops, sod farms, or even golf courses (e.g. Dalthorp et al., 2000b), where environmental conditions are more uniform than in natural systems. These studies, however, have yielded important insights into the potential role of root feeding insects in non-agricultural and non-urban environments.

Root-Feeding Insects (hereafter RFIs) are economically important fine root herbivores that are distributed across a broad geographic area on all continents except Antarctica. These insects undergo their root-feeding phase as larvae or nymphs and then eventually emerge from the soil as adults to breed. Studies in agro-ecosystems have shown that the larvae of the Coleopteran families Scarabaeidae (white grubs), Elateridae (wireworms), and Curculionidae (weevils), and the Nymphs of the Hemipteran family Cicadidae (cicadas) are often abundant RFIs, potentially having significant economic impacts on ecosystem function. For instance, they are notorious pests of sod and turf-grass, often causing noticeable (aboveground) impacts on productivity and turf quality (Fermanian et al., 2002). Along with these groups, we collected and quantified the abundance of larvae belonging to family Cibrionidae (Coleoptera), because they were relatively abundant, and we taxonomically related to wireworms. Little has been published regarding their ecology, other than that they dwell in the soil (Stehr, 1991); but we considered the possibility that they could be root-feeders (see Chapter 3).

We used longleaf pine-wiregrass (*Pinus palustris* Mill.-*Aristida stricta* Michx.) ecosystems experiencing high and low frequencies of prescribed fire to examine the spatial-temporal patterns of belowground herbivores in response to disturbance frequency and

associated changes in vegetative composition. Having minimal horizonation, the sandy soils at our study sites were ideal for collecting and sorting soil insects and fine roots year-round (see Hendricks et al., 2006). Furthermore, burn exclusion treatments, which were randomly applied to half of our study sites for approximately 9 years provided us the opportunity to examine relationships between insect community structure, understory plant communities, and soil properties.

In fire-maintained woodlands, such as the longleaf pine-wiregrass ecosystem, fire frequency is negatively related to surface organic matter and influences plant community structure via a positive effect on the dominance of fire tolerant species (e.g. wiregrass), and a negative effect on fire sensitive species (e.g. hardwoods). Fire can alter the diversity and abundance of soil invertebrates through direct mortality due to lethal soil temperatures, or by indirect changes to vegetative composition, food availability (Sileshi and Mafongoya, 2006), and soil properties; therefore, it was important to account for both the direct and indirect effects of fire in the design and analysis of experiments involving fire manipulation.

Fire has been previously linked to direct mortality in many taxa of soil invertebrates, depending on the severity of the fire, in a boreal pine forest (Wikars and Schimmel, 2001) and in the miombo of Africa (Sileshi and Mafongoya, 2006). However, in the longleaf pine-wiregrass system, fire severity is relatively low due to frequent fires and reduced buildup of fuels (Jack et al., 2010; Boring et al., 2004); therefore, they may be less likely to cause widespread mortality to soil fauna. Indirect effects of fire may influence soil-dwelling insect abundance, distribution, growth rate, and diversity (Sileshi and Mafongoya, 2006). For instance, adults may aggregate around broad-leaved trees or in open, grass-dominated areas; and/or, surface organic matter may provide protective cover during copulation or oviposition.

Potential bottom-up influences on larval distribution include fine root biomass distribution, fine root chemistry (Sileshi and Mafongoya, 2006; Jonas and Joern, 2003), and soil properties (e.g. van Herk and Vernon, 2006), each of which are subject to change in response to fire frequency. Furthermore, fine root respiration, root exudates, and volatile emissions from fine roots may serve as orientation cues for RFI larvae, as well as their natural enemies (e.g. Rasmann, 2005; Jones and Coaker, 1977). In the southeastern U.S.A., repeated fires can lead to loss of soil nitrogen (N), organic matter content (depending on intensity), water holding capacity, and macropore space (Boyer and Miller, 1994). Also, since frequent fires lead to increased belowground inputs of organic carbon from fine roots of warm season grasses (which have relatively high carbon to nitrogen ratios [C:N]), the soil organic carbon tends to become more recalcitrant over time, and thus soil organic matter tends to accumulate (Knapp et al., 1998). Soil organic matter, nitrogen (N) availability, and soil moisture have previously been shown to relate to RFI abundance (Stevens et al., 2008; Stevens and Jones, 2006; van Herk and Vernon, 2006; Dalthorp et al., 2000b). Vegetative composition, soil properties, and fine root standing crop were therefore important variables to include in our study of RFI community structure.

Understanding the influence of environmental factors on the abundance and spatial-temporal distribution of RFIs will be an important step towards a broader goal of understanding them in an ecosystem dynamics context. The first objective of this study was to estimate the abundance of RFIs in a longleaf pine-wiregrass ecosystem. Second, we wanted to understand the relationships between disturbance, bottom-up factors, and the structure of RFI assemblages. Specifically, we asked how fire frequency and bottom-up factors like vegetative composition, soil properties, and fine root standing mass, related to RFI abundance, biomass, and diversity. The third objective was to understand how RFI population densities were spatially and

temporally distributed, and how stable the spatial distributions of the herbivores remained across time. The ecological effects of herbivores are likely heterogeneous, so understanding their spatial-temporal dynamics was an important part of understanding their role in ecosystem dynamics, and could have meaningful implications for their biological control, though this was not a focus of the study. Furthermore, spatial-temporal patterns could reveal ecological mechanisms underlying RFI distribution; for instance, a high degree of spatial-temporal stability could reflect low rates of larval dispersal from the site of oviposition, or consistent attraction of larvae or adults to environmental cues.

We made several hypotheses relating to our overarching premise that direct and indirect effects of fire disturbance frequency, resource availability, and fine root standing mass, would be important determinants of RFI abundances. Specifically, we hypothesized a negative relationship between RFI abundance and the frequency of burning because fires may cause some direct mortality, and because burning favors wiregrass, which has a high C:N relative to many other available plants (Jansen, 2007; Guo, 2004), potentially making it an unfavorable food source (see Chapter 3). Conversely, we predicted a positive relationship between RFIs and the abundance of herbs, shrubs, and hardwoods. Commensurate with this reasoning, wiregrass is considered of poor quality for grazing cattle (Grelen, 1978; Lewis et al., 1975). Based on findings from other studies, we hypothesized that RFI abundance would positively relate to soil organic matter, soil available N, soil moisture, and fine root standing biomass, because adults and/or larvae may be attracted to areas where these resources are abundant (Stevens et al., 2008; Stevens and Jones, 2006; van Herk and Vernon, 2006; Dalthorp et al., 2000b; Jones and Coaker, 1977). Finally, we hypothesized that RFIs would be spatially overdispersed (aggregated), and

that abundances within patches would remain relatively stable across time, because of low rates of larval migration, aggregation of adults during oviposition, or resource patchiness.

METHODS

Site Description

The study took place at the Joseph W. Jones Ecological Research Center (Ichauway), located near the town of Newton, in Baker County, Georgia USA (31° N, 84° W), which lies within the Dougherty Plain District of the East Gulf Coastal Plain. The district consists of roughly level to gently rolling land that gradually slopes toward the southwest; elevations range from 25 to 200 m (Drew et al., 1998). The climate of the region is humid subtropical and the precipitation averages 132 cm/year; average daily temperatures range from 5-17°C in winter, to 21-34°C in summer (Hendricks et al., 2006, Drew et al., 1998; Goebel et al., 1997). Ichauway comprises nearly 12,000 hectares of property (approximately 7,500 of which are forested), along 24 km of the drainage basin for Ichawaynochaway Creek, which is a major tributary to the Flint River. The Flint River forms 21 km of the property's eastern boundary (Figure 2-1). For further information about the study system, see Hendricks et al. (2006), Mitchell et al. (1999), and Drew et al. (1998).

Soils at the study sites are mostly Typic Quartzipsamments, but some Grossarenic and Aquic Arenic Paleudults also occur. Soils are underlain by karst Ocala limestone (Drew et al., 1998). These soils typically lack developed organic horizons, and exhibit minimal vertical stratification of the mineral soil (Hendricks et al., 2006; Drew et al., 1998). These soil properties allowed efficient sampling of root feeding insects and fine roots, and reduced the potential for

problems with fine root and insect sample contamination by fine soil particulates and organic matter, which tend to cling to roots during washing.

At the time of this study, overstory vegetation consisted primarily of 60-90 year old second growth longleaf pine, *Pinus palustris* (Miller), (Mitchell et al., 1999). Understory composition was dominated by the C₄ grass *Aristida stricta* Michaux (wiregrass), and to a lesser extent, also contained regenerating longleaf pine, shrubs, and hardwoods of varying developmental stage, C₃ forbs, legumes, and other predominantly C₄ grasses. Fire maintenance had been a regular part of the forest management at Ichauway. Individual stands on the property had been regularly burned on a 1-5 year interval (Hendricks et al., 2006). Frequent fire has promoted understory dominance of wiregrass and fire-adapted legumes, and a decreased abundance of fire-sensitive vegetation (including hardwoods).

Study Species

Immature insects of families Scarabaeidae (white grubs), Elateridae (wireworms), Curculionidae (weevils), Cicadidae (cicadas), and Cibrionidae, were sampled during the study, all of which were of order Coleoptera, except for family Cicadidae (order Hemiptera). White grubs, wireworms, and root-feeding weevil larvae have long been regarded as destructive soil-dwelling herbivores, frequently cited for economically significant damage to turf-grass and crops (Fermanian et al., 2002; Stehr, 1991). Cicadas have been thought to feed off of the phloem of tree roots throughout the majority of their lifespan as developing nymphs (White and Strehl, 1978). Little has been published about the ecology of Cibrionid larvae, other than that they dwell in the soil (Stehr, 1991; see Chapter 3). More information about the natural history and ecology of the study organisms is provided in Chapter 1. Table 2-1 lists all families and genera collected and identified. White grubs and wireworms were identified to the genus level; several

known root-feeding genera were recognized. Though some analyses relied on genus-level data, abundance estimates for white grubs and wireworms were based on combined tallies of respective genera. Unfortunately, the larvae of weevils have long been regarded as notoriously difficult to identify (Stehr, 1991), and so they were categorized broadly in this study as soil-dwelling weevil larvae. However, they were believed to be root-feeders based on morphological similarities to other root-feeding genera, and their consistent presence in the soil (see Stehr, 1991 and May, 1966). Cicadas were not identified to genera, but were known as root phloem feeders (White and Strehl, 1978; but see Chapter 3). Cibrionid larvae were not known root-feeders, but they were included in the investigation because they were abundant in the study system, belonged to the same super-family as wireworms, Elateroidea (Stehr, 1991), and had similar external morphology. They were not included with *Combined RFIs* because of uncertainties about their habits, but were considered separately. Encountered but not collected or quantified, were the non-root-feeding larvae of orders Diptera (frequently encountered) and Lepidoptera (occasionally), adult insects (occasionally), insect pupa (occasionally), arachnids (occasionally), annelids (frequently), cephalopods (occasionally), centipedes (occasionally), millipedes (occasionally), and other isopods (frequently).

Experimental Design

Fire Frequency Treatments:

Several forest stands were randomly selected to remain unburned (UB) prior to initiation of this experiment, to study how fire and N control vegetative composition, productivity, and nutrient cycling. A final burning in the fall of 2000 initiated the burn exclusion treatment (UB) in these stands. Three of these UB stands were randomly selected by drawing them blindly from a larger set of suitable stands. During an initial survey of the property, six frequently burned

stands were considered for the experiment because they met the following *a priori* criteria for inclusion in the experiment: Each had been burned at least every other year for at least 9 years prior to our study, exhibited dominance of mature longleaf pine in the overstory and dominance of wiregrass in the understory, and had no ongoing irrigation or fertilization. Three frequently burned stands were selected for use in our study of RFI distributions by drawing them randomly from the larger set of 6 available stands. Two of the selected B stands, underwent burning every other year (fall), while one was burned annually (summer). Both stand types (UB and B) contained significant wiregrass cover in the understory at the initiation of the study; however, UB stands had less wiregrass and more woody plant abundance than B stands, which was the expected result of burn exclusion in longleaf pine-wiregrass ecosystems. Stands did not undergo fertilization or irrigation during the course of the study.

Plot Establishment and Layout:

In each of the 6 selected stands, 9 rectangular plots (4 m x 14 m) were established by pacing along randomly drawn compass vectors (Figure 2-2). Coordinates of plot center locations were recorded using a Garmin[®] 12 handheld GPS; see Appendix A for map of plot coordinates. Plots were oriented with a compass to the cardinal directions, with the long dimension of the plot either running North to South, or East to West (chosen randomly). Insects and soils were sampled from the 4 m x 4 m center portion of plots; which were further divided into four quadrants (Figure 2-3). Fine roots were sampled from the peripheral portions (4 m x 4 m areas) of the rectangular plots (Figure 2-3). A 1 m boundary was placed between the center and peripheral portions of the plots (Figure 2-3). The spatial separation of root samples was needed to accommodate a tandem experiment because interference from the soil disturbances required to sample insects could bias estimates of fine root productivity (see Chapter 4).

Sampling Time Periods:

Plots were sampled for insects on 6 occasions: September 3 to 12, 2008 (Fall 08); May 23 to June 13, 2009 (Summer 2009); October 8 to November 9, 2009 (Fall 2009); March 8 to April 2, 2010 (Spring 2010), July 6 to July 29, 2010 (Summer 2010) and November 12 to 29, 2010 (Fall 2010). During Fall 2008, visual surveys of understory plant community composition were made within plots. During Summer 2009 fine roots standing crop samples and soil samples were collected from plots.

Determination of Sampling Scheme and Intensity:

Prior to establishment of experimental plots, a pilot study was conducted to establish an appropriate level of replication and sampling scale. To accomplish this, four 25 m x 25 m plots were selected randomly (2 in UB and 2 in B) and overlaid with a grid network on a 5 m spacing. One soil sample (40 cm x 40 cm x 20 cm depth) was excavated from each grid intersection and searched for larvae estimate insect relative abundance across the plot area. The relatively large 40 cm x 40 cm dimension was chosen to reduce sample variance. Samples collected from the grid intersections of the 25 m x 25 m plots revealed spatial aggregation of larvae. This suggested that it would be more representative of RFI abundances within sites to sample many small plots, than to use a few large plots with greater sampling intensity. Therefore, 3, 5 m x 5 m areas on the grid (randomly chosen) were intensively sampled (8 samples per 5 m x 5 m subplot). Aggregation was also observed at spatial scales less than 5 m x 5 m; for instance, it was common to find > 2 RFIs in one sample and zero in an adjacent sample. This issue was addressed in the full experiment by sampling in each quadrant of each plot to disperse individual soil samples within sample plots (stratified random sampling).

Arithmetic mean and variance were calculated sequentially as additional samples were included in the calculation. Analysis of the change in estimated mean RFI abundance with increasing sampling intensity, suggested that 4 soil samples were needed to accurately describe abundances within 5 m x 5 m subplots.

Sampling and Processing of Root-Feeding Insects

One soil sample (40 cm x 40 cm x 20 cm depth) was collected from each quadrant and searched for RFIs during each insect sampling period. To determine sample locations, 6 numbered pin flags were randomly placed throughout each quadrant during each sampling event (sample overlap was not permitted), and one flag number was randomly selected from each quadrant. Flags were placed at least 15 cm away from large obstacles (such as large tree trunks or stumps) that interfered with the ability to excavate the sample. A frame, used to consistently delineate the sample dimension during excavation, was centered on the selected pin flag prior to excavation.

The majority of fine root biomass occurs in the upper 20 cm of soil (active rooting zone); therefore, we targeted this depth in our sampling scheme. Though it is uncommon for soils in this region to undergo hard freezing, which has been associated with deeper vertical migration, we were concerned about the possibility of vertical migration in response to low soil moisture (see Stevens et al., 2008, Williams et al., 1993). Therefore, 8% of samples were further excavated to 40 cm depth, and 2.5% to 60 cm depth, during the summer 2010 sampling period, which was a relatively dry part of the year.

Soil samples were excavated using a narrow, round-ended shovel (Sharpshooter™). During excavation, soil was directly transferred to a large plastic bin or placed on top of plastic landscaping cloth to prevent escape of insects. Soil was added to the sieve surface one handful at

a time to aid in spotting larvae before sieving, which we noted caused occasional damage to some larvae, making identification and washing more difficult. Soil was then sieved in the field (3 mm mesh) in manageable increments over a wheelbarrow. Any material that did not pass through the sieve was combed through by hand and searched for larvae.

Larvae were identified in the field to the extent possible, and then stored on ice inside small plastic jars until the end of each workday, at which time they were frozen. Insects were later thawed, and gently washed with distilled water to remove soil or other debris attached to their outer surface. Each was then re-examined in the lab under a dissecting microscope, and identified using Stehr (1991) and Ritcher (1966). White grubs and wireworms were identified to the genus level and other larvae were identified to the family level.

Following identification, larvae were either preserved in ethanol for future record, or dried for weighing. Insects used for dry weight estimates were sampled on four occasions: May 23 to June 13, 2009, October 8 to November 9, 2009, March 8 to April 2, 2010, and November 12 to 29, 2010. Many of the insects sampled during September 3 to 12, 2008, and July 6 to 29, 2010, were preserved in alcohol as a record of specimen taxonomy, and are currently stored at the Virginia Tech Insect Collection (Insect Identification Lab, Department of Entomology, Price Hall). Specimen jars are labeled (Dittler, Matthew / Insect Family / 2008 - 2010 / Baker Co. Georgia). Insects used for weight estimates were placed in tin weighing dishes, dried for at least three days at 60°C, weighed to the nearest 0.1mg, and stored individually in paper envelopes. Estimates of total biomass and mean individual biomass were based on these samples.

Sampling and Processing of Fine Roots

During the summer of 2009, fine root standing biomass was sampled from the periphery of the plots (4 m x 4 m subplots located on opposing ends of each 4 m x 14 m rectangular plot).

Peripheral subplots were each further subdivided longitudinally, as two halves, to increase spatial representativeness (Figure 2-3). One root sample (10 cm diameter x 20 cm depth) was taken within each 4 m x 2 m subsection (4 samples total per 4 m x 14 m plot). Locations of fine root standing crop samples within each 4 m x 2 m area were determined in the same manner as for insect samples. Root standing biomass was sampled by first removing aboveground vegetation and forest floor litter to reveal the mineral soil around an area about 5 cm larger diameter than the core. A 10 cm (interior diameter) coring pipe was then driven in (perpendicular to the ground) to 20 cm soil depth with a sledgehammer, and then retracted with the soil intact. Soil was then removed from the core and sieved in the field (3 mm mesh) to remove the bulk of soil. Root losses through the mesh were assumed to be well below 10% of total standing mass. Remaining soil and roots were then stored in plastic zip bags and frozen at the end of each work day.

Each root sample was later thawed, rinsed from the sample bag onto a sieve (1 mm mesh), and washed free of soil and organic debris, by gently showering the sample with a garden hose and a nozzle with numerous small output holes, using low to moderate water pressure. A shallow water trap, located below the sieve, was used to observe, and attempt to recover, any roots that washed through the sieve (root loss did not appear to be a problem). After washing, each root sample was transferred to a paper blotter, folded to contain the roots, sealed inside a small plastic zip bag and frozen for future sorting.

Root samples were later transferred to a shallow plastic tray filled with distilled water to about 5 cm depth for sorting. Roots and small gravel remaining on the blotter were rinsed into the tray. The bottom of the tray was transparent and placed respectively over a white and a black surface to provide increased contrast for finding light and dark colored roots during sorting.

Roots were freed from any remaining debris by swishing the roots in the tray, and gently massaging the roots between the fingers and thumb to remove clinging soil particles (if necessary). They were then sorted into 3 diameter classes: < 0.5 mm, 0.5 mm to 1.0 mm; > 1.0 mm roots. Roots were snipped with scissors as needed to place them into the correct diameter class. Root length was estimated for each diameter class using a modified Newman grid-intersect method (as described in do Rosario et al., 2000). Fine roots were placed in a tray marked with a grid (5 cm grid unit) and the number of grid intersections was tallied with a mechanical counter. For samples with relatively few roots, a 1 cm grid unit was used. Root length (cm) was estimated as $(\pi/4) \times (\# \text{ of intersects} \times \text{the grid unit})$. Fine roots were then removed from the tray, dried at 70°C for at least 3 days in tin weighing dishes, and weighed to the nearest 0.01 g to estimate fine root standing crop (grams of oven dry mass per m², to the 20 cm depth).

Soil Properties

To estimate soil nutrient parameters and organic matter content for each plot, soils were sampled from the 4 m x 4 m plot centers. During the summer of 2009, a soil auger (2 cm diameter) was plunged to 20 cm depth, once within each quadrant, and these 4 subsamples were composited to form a representative soil sample for each plot. The composited sample from each of the 54 study plots was air dried, sieved (2 mm mesh), and mixed prior to analysis at both the Virginia Tech Ecosystem Science Laboratory (NH₄⁺ and NO₃⁻) and the Virginia Tech Soil Testing Laboratory (all other soil parameters). The proportion of coarse fragments which did not pass through the sieve were minimal, so they were not weighed.

The concentrations of soil NH₄⁺ and NO₃⁻ were estimated by anaerobic incubation followed by extraction using a strong KCl solution, as in Maynard et al. (2008). Soils (5 g

subsamples) were mixed with distilled water, in test tubes, capped, and shaken to disperse soil. Soil suspensions then underwent anaerobic incubation for 7 days at 40°C. Contents of test tubes were then quantitatively transferred to 100 ml centrifuge tubes using 3M KCl, until the total weight of soil-KCl suspension was approximately 55 g. Centrifuge tubes and contents were shaken with a mechanical shaker for 1 hr. The suspension was then allowed to settle for about 10 min. The leachate was then decanted into acid washed sample bottles, over funnels fitted with Whatman #1 glass-fiber filter paper, and refrigerated until further analysis (less than 1 week later). Nutrient concentrations were quantified using high pressure liquid chromatography (analysis performed by Virginia Tech Ecosystem Science Laboratory). Final concentrations were adjusted for background noise using soil-free KCl blanks which were otherwise processed identically.

Soil water content (volumetric) was estimated based on *Time Delay Refractometry* (TDR), (see Topp et al., 1984). We used a portable TDR by HydroSense™ with a hand held probe (20 cm length). Soil water measurements were made on three dates: July 18, 2010, November 25, 2010, and January 4, 2011. On each sampling date, approximately 50 data points were collected within each forest stand, at random intervals ranging between approximately 2 and 20 meters, along four transects (starting points and bearings were chosen randomly).

All other procedures were conducted at the Virginia Tech Soil Testing Laboratory. Procedures that were used are provided in Maguire and Heckendorn (2011). Soil organic matter content (%) was estimated by the *Loss On Ignition* method (LOI), which works on the assumption that the mass lost after exposure to 360°C for 3 hours was due to the loss of organic matter (Combs and Nathan, 1998; see Heiri et al., 2001). Soil pH was determined in distilled water (see Kalra, 1995) using a LabFit Pty Ltd, model AS-3000 automated dual H Analyser. All

other soil ions were measured using an Inductively Coupled Plasma Atomic Emission Spectrometer (see Vela et al., 1993), CirOS VISION model by Spectro Analytical Instruments, and equipped with a CETAC ASX520-HS autosampler. Cation exchange capacity was estimated by the Virginia Tech Soil Testing Laboratory by summation of basic and acidic ions (see Sumner and Miller, 1996): Estimated Soil CEC = Acidity + Ca + Mg + K (meq/100 g soil or cmol/kg). Base saturation and acidity were based on the concentrations of basic or acidic ions.

Estimation of Vegetation Cover in Plots

During the fall of 2008, prior to any excavation, the percent cover of the following five functional groupings of aboveground vegetation were estimated visually within each plot: Forbs (including legumes), woody shrubs and hardwoods, wiregrass, other grasses, and young pines (saplings less than 5 cm diameter at breast height). The percent cover of litter and bare soil was also visually estimated by standing at a plot boundary, looking at the various functional groups one by one, and estimating the percentage of ground area covered by those plants up to the canopy height of young pine saplings (approximately 3 m). Estimates of percent cover greater than 7% were rounded to the following increments: 10%, 20%, ... 100%. When cover was equal to or less than 7%, estimates were rounded to the following increments: 0%, 1%, 3%, 5%. Estimates were made in increments to promote accuracy over precision, given the low-precision associated with visual assessments of plant cover (as in the Braun-Blanquet scale for cover-abundance estimates). Arithmetic means were calculated for sites and rounded to the nearest increment. Cover of mature trees was not estimated within plots, but basal area across the broader study system ranged from 9 to 15 m²/ha, and most trees were greater than 60 years old

(Hendricks et al., 2006; Mitchell et al., 1999). Stocking levels appeared relatively similar within the six sites.

Data Analysis

There were several challenges associated with the analysis of count data (Sileshi, 2008, 2006). These included the overdispersion associated with large numbers of zeros typical of count-based datasets, distributions that were skewed towards lower counts, and variance heterogeneity (Sileshi, 2008, Taylor, 1961). Exploratory analyses of data early in our experiment revealed that overdispersion, resulting from patchy spatial distributions, was inherent in the study system because the variance to mean ratio (VMR) was greater than 1. The negative binomial distribution was used to account for the overdispersion and variance heterogeneity (see e.g., Ismail and Jemain, 2007; Sileshi, 2006; and White and Bennets, 1996). To test the appropriateness of the negative binomial distribution, data from the first few sampling events were used to compare model fit under different distributional assumptions for a set of generalized log-linear models (see Bolker et al., 2008), using the GENOD procedure in SAS[®]. The response variables considered were the plot count tallies for combined root-feeding taxa and individual taxa. Independent variables included fire treatment and percent vegetation cover parameters (wiregrass, woody shrubs and hardwoods, herbaceous forbs, other grass species, surface organic matter, and bare ground). The AIC (*Akake's Information Criterion*) and BIC (*Bayesian Information Criterion*) values for the following distributions were compared with these theoretical distributions: Normal, Poisson, Negative Binomial. The Negative Binomial distribution generally resulted in either better or equivalent model fit (AIC and BIC) and was used thereafter as the theoretical distribution in the remainder of the analysis.

After sampling across all sampling periods was completed, the generalized linear models were adjusted to incorporate an additional covariance matrix to account for lack of sample independence associated with repeated measures (*Marginal Longitudinal Generalized Linear Models*). Specifically, we used *Generalized Estimating Equations* (GEEs), which solved a system of equations iteratively to produce empirical parameter estimates of means and standard errors for each effect included in a set of alternative models (Sileshi, 2006). GEEs were constructed using SAS[®] software (2011 release) using the *GENMOD* procedure (LINK=log; DIST=NB). A REPEATED statement was included in the protocol to invoke the use of GEEs. In our case, plot was designated as the repeatedly measured variable.

For combined RFIs and specific taxa, two series of alternative statistical hypotheses in the form of model statements for GEEs were produced. The first set of hypotheses all included the basic parameters relevant to the underlying design of the experiment: Fire treatment (UB, B), Site (1-6), and Sampling Period (1-6). The second set of hypotheses all contained fire treatment effects, but not site or sampling period effects. The purpose of these models was to examine vegetation, soil properties, and fine root standing crop, blind to spatial and temporal covariates, to evaluate their potential as predictors of RFI abundance in longleaf pine-wiregrass ecosystems. For each of the two sets of hypotheses (later referred to as experimental and predictive, respectively), blocks of variables chosen to represent (a) aboveground vegetative composition, (b) soil properties, and (c) fine root standing crop, were added to each sequential hypothesis as a parameter set. Soil nutrients, except for N, P, K, and Fe, were not included in the variable set, though average values are reported for each site, because variability among plots was minimal and exploratory analyses revealed no indication of potential relationships. Additional parameters related to soil chemistry, and the percent of ground covered by young regenerating pines

(defined as < 5 cm max. diameter and < 4 m tall), were discarded from the parameter sets, because effects were consistently insignificant in exploratory statistical models. Remaining variables in each parameter set were then checked against each other for collinearity ($p < 0.1$). For parameters which exhibited collinearity (e.g. base saturation and acidity), the variable with the lowest significance for combined RFI abundance was removed. Soil moisture was not used as a covariate because only one average value was measured for each site, introducing collinearity with sites. The final variables included for parameter set (a) were percent cover of (i) wiregrass, (ii) woody shrubs and hardwoods, (iii) forbs, (iv) other grasses, and (v) surface organic matter. Variables for set (b) were (i) mineralized NH_4^+ (mg/kg), (ii) organic matter (%), (iii) base saturation (%), and (iv) cation exchange capacity (CEC, meq/100 g). Variables for set (c) were (i) standing biomass and (ii) standing length of roots < 1.0 mm diameter.

An *Information Theoretic* (IT) approach, was used to choose an appropriate correlation structure for the covariance matrix (associated with the longitudinal component of the study design), and to compare the information yield across the set of alternative hypothetical models (see Johnson and Omland, 2004 and Royall, 1997). Use of quasi-likelihood-based information criterion (QIC and QIC_u) allowed a comparison of the strength of evidence for the competing models, given the overdispersed data (Burnham et al., 2011; Pan, 2001). QIC_u (a modification of QIC) has been suggested to be a better indicator for model parameter inclusion; whereas, QIC has been considered more appropriate for evaluating correlation structures (Pan, 2001). QIC and QIC_u values were estimated in the SAS[®] system automatically for GEEs. The independent, exchangeable, and first-order autoregressive (AR1) correlation structures were compared, and the AR1 structure was chosen because of modestly lower values for QIC. This correlation structure assumed that observations taken closer together in time were likely to be more related.

Backwards selected models for combined and separate taxa were constructed for additional comparison between the set of hypothesized models and models constructed via an automated selection procedure. Backwards selection began with all variables included and then removing the least significant terms sequentially until no non-significant terms ($p > 0.15$) remained. P-values between 0.1 and 0.25 are considered practical during model selection procedures because less conservative criterion for the removal of model parameters could result in a less informative final model (see Whittingham et al, 2006). Inference about the importance of including particular parameter sets in the models, and final choice of a best-fit model, was based on change in QIC_u relative to an intercept-only model (Δ), which allowed comparisons of each model with the predictive value of mean abundance alone.

In addition to the IT analysis, GEEs were used to generate empirical estimates for specific categorical effects, GEEs were also used to empirically estimate the effect sizes (Mean \pm 1 standard error) used in figures that illustrated patterns in abundance between fire treatments, among sites and sampling periods, and for the site by sampling period interaction of combined RFIs.

To also test the overall significance of fire treatment (UB, B) in a more traditional way, contingency tables and Chi-square tests for independence were used (calculations performed in Microsoft[®] Excel[™], 2007). The count bins (ranges of counts assigned a categorical identity) used for the contingency tables and subsequent Chi-square tests comparing across UB and B treatments, across sites (6), and across sampling periods (6), were as follows: For comparisons of all taxa combined, 0, 1, 2 to 3, 4 to 5, 6 to 10, and > 10 ; for UB v B comparisons of grubs, wireworms, and weevils, 0, 1, 2 to 3, 4 to 5, and > 5 ; for comparisons across sampling periods of grubs, wireworms, and weevils, and for all comparisons of cicadas and Cibrionids, 0, 1, 2 to 3,

and > 3 . Bins were chosen to avoid empty bins while maintaining a minimum expected value greater than 1; actual minimum expected frequencies for bins are reported along with Chi-square statistics, degrees of freedom, and p-values generated from the tests. This analysis was thought to be of use for considering site and sampling period effects, and the combined direct and indirect effects of the fire treatment, in isolation from other variables.

Multiple indices of spatial autocorrelation, and count data dispersion were calculated to better describe the spatial and temporal distribution of RFIs. Moran's I and Geary's C, two measures of spatial autocorrelation (see Fortin et al., 2002), were calculated separately for each sampling period, using the VARIOGRAM procedure (experimental) in SAS[®] (2011 release). The following statements were included after the PROC statement: *Compute* novar autoc, (weights = distance), *Coordinates* xc=Eastings yc=Northing (from GPS data). Additional dispersion indices calculated were the variance: mean ratio (VMR), the coefficient of variation (CV), the range, and K (dispersion factor for NB distribution), (see Southwood, 1978). These parameters were calculated across plots (within each site) to represent spatial dispersion for each time period; and across time periods, to represent temporal dispersion of each plot. Geometric means were used to represent average values for combined time periods (spatial dispersion) and combined plots within each site (temporal dispersion). In the few cases where the range of values contained either zero or negative values, 0.1 was substituted in order to compute the geometric mean, as can reasonably be done at low frequency to circumvent the inability of the geometric mean to include negative or zero values (see Martin-Fernandez et al., 2003). The mean of any dataset consisting of all zeros was assumed to be zero. Calculations of dispersion parameters and geometric means were performed in MicroSoft[®] Excel[™] 2007.

The tendency for low, moderate, or high density patches to occur in the same locations over time was measured by the *Quantile Variance* (QV). Dalthorp et al., (2000, a, b) provided examples and specific explanations of calculations and interpretation of QV analysis. QV is often presented in a standardized form, to aid in interpretation. To calculate Standardized Quantile Variance (SQV), observed QV was divided by expected QV, which for a given quantile (p) was assumed to be p(1-p), (Dalthorp et al., 2000, a). We calculated SQV for total RFI abundances across the six sampling periods. As published in Dalthorp et al. (2000, a), for a given quantile (p) of a population density, the observed QV was calculated as

$$QV_p = \frac{\text{var (number of time periods each plot was in quantile p)}}{\text{number of time periods}}.$$

Therefore, for a given quantile (p),

$$SQV_p = \log \left(\frac{QV_p}{p(1-p)} \right).$$

Specifically, we used the following quantiles for our data set: 0.1, 0.25, 0.5, 0.75, 0.9.

Univariate statistical summaries of individual insect biomass, soil parameters, aboveground vegetative composition, and fine root standing biomass for each site and burn treatment were computed with Microsoft® Excel™, 2007. Statistical analysis of individual insect biomass between fire treatments (taxa specific basis) was by way of two sample t-tests using Systat® software; unequal sample variances were assumed and a Bonferroni adjustment was applied. Root-feeding insect diversity was reported as the number of taxa identified within each fire treatment (UB or B). Basic data organization, data storage, and all other basic calculations not otherwise described were performed in Microsoft® Excel™, 2007. All data graphics presented were generated with Sigma-Plot® software. Additional justification for the analyses used is provided in Appendix B. A summary of the raw count data collected during the experiment was provided in Appendix C.

RESULTS

Root-Feeding Insect Abundance, Biomass, and Diversity

Unburned sites had greater population density, herbivore biomass, and taxonomic diversity than did burned sites. Over the six sample dates, a total of 1,105 RFIs were sampled, 696 in UB sites and 409 in B sites (Table 2-1). Insects were identified from 2 insect orders, and 5 families. Within the Scarab family (white grubs), 5 distinct genera were identified; likewise, 6 distinct genera were identified in the Elaterid family (wireworms). A comprehensive list of the taxa collected, the number of individuals sampled, population density, and average biomass of individuals (taxa specific basis) for UB, B, and combined treatments, is provided in Table 2-1.

Combined RFI abundance as well as the statistical distribution of counts differed among fire disturbance treatments. The mean population density (\pm standard error) for combined taxa was 5.1 ± 1.9 individuals per square meter; for UB and B treatments, it was 6.5 ± 2.5 and 3.7 ± 1.8 , respectively (Table 2-1). Higher median relative abundances, a higher degree of skew towards higher relative abundances, and an increased prevalence of high-count outliers characterized UB, relative to B plots (Figure 2-4). A chi-square (χ^2) test indicated a significant difference between UB and B treatments for combined RFIs ($\chi^2 = 14.4$, d.f. = 5, min. expected = 7.0, $p = 0.013$, Table 2-2). On a taxa-specific basis, χ^2 tests on fire treatment effects were most significant for grubs, wireworms, and Cebionids (Table 2-2). However, χ^2 tests for the independence of frequency distributions had limited precision and did not account for additional covariates. The GEE-based empirical estimates of mean and standard error showed weevils and wireworms with the largest overall differences due to fire (Figure 2-5). Differences in combined abundances between UB and B treatments were driven by weevils and wireworms (combined genera), although subtle increases in abundance (UB) were also present in Cebionids and grubs

(combined genera), but not cicadas (Figure 2-5). The grub genus *Cyclocephala* and the wireworm genera *Agriotes*, *Melanotus*, and *Sericus* were present in both UB and B sites, but were relatively more abundant in UB plots (Table 2-1). Two grub genera, *Phyllophaga* and *Anomala*, were present in both UB and B sites, but were relatively more abundant in B sites (Table 2-1).

The individual biomass of insects was generally not influenced by fire disturbance treatment. Body size generally did not differ between UB and B stands, but weevils were a notable exception ($t = -4.8$, d.f. = 200.5, $p < 0.0001$). The total biomass of RFIs was 33.2 g (sampled during 3 of 6 sampling periods and summed); it was 21.0 g in UB and 12.2 g in B treatments. The corresponding estimates on a unit area basis average across all time periods were 241 mg/m² (overall), 304 mg/m² for UB, and 177 mg/m² for B treatments. Total biomass was considerably higher in the UB treatment (Figure 2-6, a) due to greater abundances of multiple taxa and to increased body size of weevils in UB stands (Table 2-1). Figures 2-6, b-d illustrates total biomass patterns in UB and B treatments on an area basis for each RFI taxa. Total biomass on a taxa-specific basis was detectably higher in the UB treatment for all RFI families, except for Scarabaeidae (white grubs) and Cicadidae (cicadas), (Figure 2-6, b). *Phyllophaga* was the only white grub genus out of 5 to follow this pattern, but it was also the most abundant genus by a substantial margin, driving the increased abundance of white grubs in the B treatment. The family Curculionidae (weevils) exhibited the most significant difference in total larval mass between treatments due to the combined effects of increased abundance and increased body mass of individuals in the UB treatment group.

Diversity was higher in UB sites, based on the total number of taxa found in each burn treatment (14 identifiable taxa in UB and 10 in B). The diversities of wireworm and white grub

genera were responsible for the overall increase in diversity in the UB treatment (Table 2-1). The grub genera *Diplotaxis* and *Maladera*, and the wireworm genera *Limonius* and *Hemirhipis* were found only within UB sites (Table 2-1). Relative abundance of less common species was also higher in UB sites. The *Maladera* species sampled, which was relatively abundant in UB sites (Table 2-1), was thought to be the Asiatic Garden Beetle, *Maladera castanea* (Arrow), which is an exotic species. Our observation of *M. castanea* was south of the published species range, but this species was recently found in Auburn, Alabama, which is also south of the published range (Held and Ray, 2009).

Site-Specific Summary of Environmental Variables Measured

Compared to unburned plots, the burned plots had approximately double the cover of wiregrass and bare ground, while unburned plots had several times greater cover of woody plants and litter (Table 2-3). A site-wise summary of the measured soil parameters is provided in Table 2-4. Although this study was not set up to conduct statistical tests of site differences (because they were treated as a blocking factor), some substantial variation across sites did occur, which may explain some of the influences of sites as covariates in several models (see below). For example, soil moisture varied among sites from wettest to driest as listed: Site 6 (B) > 4 (B) > 3 (UB) > 2 (UB) > 1 (UB) > 5 (B). Burned sites 4 and 6 had the greatest mean percent organic matter. Burned site 5 had the greatest base saturation (%) and least acidity (%). Burned sites 4 and 6 had a higher, and burned site 5 had a lower CEC than the unburned sites. All burned sites had higher mineralized nitrogen (NH₄⁺) than any of the unburned sites. Finally, soils in site 4 (B) had the highest concentrations of P, and sites 1 (UB) and 2 (UB) had lower concentrations of K compared with all other sites.

Environmental Parameters and Root-Feeding Insect Abundance: Information-Based Inference

After accounting for co-variation due to site and sampling period, the information gain for inclusion of additional parameter sets for fire disturbance, vegetative composition, soil properties, and fine root standing crop, were evaluated using QIC_u (Table 2-5). The inclusion of fire treatment, vegetative composition, and soil parameters improved model fit (QIC_u) for combined RFI abundance (Table 2-5). After accounting for other covariates in the best-fit model, the burn exclusion treatment (UB) was not significantly related ($p = 0.89$) to total RFI abundance (Table 2-6); however it was meaningful for certain taxa (Appendix D). Cover of woody shrubs and hardwoods, herbaceous forbs, other grass species, and surface organic matter had the greatest significance of all continuous covariates included in the model ($p < 0.1$ for all), (Table 2-6). The estimated cover (%) of woody shrubs and hardwoods was positively related to combined RFI abundance, while cover of herbaceous forbs, other grasses, surface organic matter (forest floor litter), and wiregrass (not significant) were negatively related to combined RFI abundance (Table 2-6). Soil organic matter (%) and NH_4^+ was positively related to RFI abundance, while CEC was significantly, and base saturation (%) non-significantly, negatively related to the abundance of combined RFIs (Table 2-6). Sampling period was also a significant source of co-variation for combined RFI abundance. The fall 2008 and summer 2010 periods had negative or neutral co-effects in the model, while all other sampling periods had positive co-effects (Table 2-6). Site 1 (UB, Red Dirt Woods) and Site 5 (B, Turkey Woods) had weak negative or zero co-effects in the model, while all other sites had weak positive co-effects (Table 2-6).

The best fitting (QIC_u) predictive models of combined RFI abundance only included the parameter sets for aboveground vegetative composition and fine root standing crop (Table 2-7).

This model substantially improved the predictability of RFI abundance over the intercept-only model ($\Delta = -363$). The percent cover of wiregrass, herbaceous forbs, other grasses, and surface organic matter, was negatively related to RFI abundance, while percent cover of woody shrubs and hardwoods was positively related. Fine root standing biomass was positively related to RFI abundance, while fine root length was negatively related. This seemingly contradictory pattern appeared driven by site 5 (B), which had the lowest RFI abundance and a fine root standing crop characterized by a substantially lower mass to length ratio than other sites. The effects due to percent cover of herbaceous forbs and other grasses had the highest significance in the model ($p < 0.1$, Table 2-7).

Experimental and predictive models for individual taxa suggested that environmental factors influenced RFI abundances across the study system (Table 2-5). Spatial and temporal variation appeared important for many, but not all, of the RFI taxa studied, based on the difference in ΔQIC_u for the best fitting experimental and predictive models (Table 2-5), and on the significance of site and sampling period covariates in the best fit experimental models (Table 2-6; Appendix D). Based on the change in QIC_u (Δ), the abundances of white grubs and wireworms were not predicted well by the parameters included in either set of models, but weevil and Cibrionid abundances were well predicted (Table 2-5).

The fit (QIC_u) of experimental models for white grubs was improved by adding vegetation, soil, and fine root parameter sets to the model (Table 2-5). However, QIC_u for this model was greater than (not as good as) QIC_u for the intercept-only model, which indicated that accounting for the influence of different sampling periods and sites (inherent in the experimental design) reduced model parsimony without providing a significant amount of additional

information. The best fit predictive model of white grub abundance accounted for fire alone (UB or B treatment) and improved predictability over the intercept-only model (Table 2-5; $\Delta = -13.7$).

Wireworm abundance, in the best fit experimental model, was best modeled by including fine root standing crop in addition to the site, sampling period, and fire treatment effects (Table 2-5). The fire treatment was significant after accounting for significant co-variation due to site and time period (Appendix D). For predictive models, the best fit model included only soil parameters; however, QIC_u for this model was not as good as the intercept-only model, so mean wireworm abundance provided a more parsimonious prediction than the predictive model that accounted for fine root standing crop (Appendix D).

Weevils were the most effectively modeled taxa in the study system (based on Δ). In the experimental models, which accounted for site and sampling period effects, model fit was not improved by including vegetation, soil, or fine root parameter sets. The best fit model, which only included fire treatment, site, and sampling period, greatly increased predictability for weevil abundance over the intercept-only model ($\Delta = -405.9$). The best fit predictive model only included vegetation and fine root standing crop parameter sets (Table 2-5; $\Delta = -213.9$). This indicated that above- and belowground vegetation parameters were correlated with, and could be important to the distribution of weevil abundance.

The inclusion of vegetation, and particularly soil and fine root standing crop parameter sets, improved QIC_u of experimental models for Cibrionid abundance over the intercept only model (Table 2-5; $\Delta = -68.9$). The effect sizes and significance levels attributed to fire, site, sampling period, and specific vegetation, root, and soil variables are summarized in Appendix D. Effect size of sampling period covariates were significant in the experimental models. The best fit predictive model for Cibrionid abundance included fire treatment, vegetative composition,

soil, and fine root parameter sets; this improved QIC_u over the intercept-only model (Table 2-5; $\Delta = -47.3$). The majority of improvement was from the inclusion of soil and fine root parameter sets, whereas vegetation was less important (Table 2-5).

Cicada abundance was very low overall, which decreased our confidence in more specific abundance patterns due to limited number of samples. Cicada abundance did not differ significantly due to fire treatment or site. Temporal fluctuations in abundance could have been important but not detectable; for instance, cicadas were not found during each sampling period, and therefore sampling period was not included in the experimental models for cicadas because at least one observation was required for use of GEEs to generate effect size estimates. The inclusion of soil and fine root parameter sets increased information yield in the experimental models, but QIC_u for the intercept-only model was still better due to the loss of parsimony from including site and fire effects (Table 2-5). The best fit predictive model of cicada abundance included only the fine root standing crop parameter set. This model improved QIC_u markedly over the intercept-only model ($\Delta = -91.0$).

Spatial-Temporal Dispersion of Root-Feeding Insects

Various measures of spatial autocorrelation and dispersion indicated that distributions of RFIs (combined taxa) were spatially aggregated within sites. Analysis of spatial autocorrelation across all study plots (during each sampling period) indicated that relatively proximate plots were more likely to have similar combined RFI abundance than relatively distant plots: Geary's c was significant for most sampling periods (Table 2-8). However, Moran's I was not significant except during one sampling period (Table 2-8), which indicated there was not an explicit pattern of spatial aggregation across the entire study system (e.g. no specific directional gradient or hot spot). Significant variation due to site, as exemplified in χ^2 tests (Table 2-2), and the plot

showing RFI population density for each site (Figure 2-7), indicated that insects may be aggregated at a landscape scale. However, since site was considered as a block or random effect, we were unable to draw conclusions about the significance of site differences. Spatial patchiness was also detected at the within-site scale via several indices of dispersion, most of which indicated that RFIs were spatially aggregated within each site (Table 2-9, a). Aggregation was also observed within individual plots because variance of the 4 samples taken within each plot commonly exceeded the mean count (unpublished data). For example, large differences in RFI counts were commonly observed among 4 adjacent samples within the same plot, during the same time period.

The vast majority of RFIs appeared to dwell in the active root zone (top 20 cm of soil). Sampling deeper in the soil suggested that larvae were not migrating below 20 cm due to low soil moisture. We never encountered grubs, weevils, wireworms, or cicadas below 20 cm depth in our system. Only one Cebrioid larva was ever encountered below 20 cm depth.

Temporal variability of combined and specific RFI taxa was evident within the study area because effects due to sampling period were significant both in χ^2 tests and GEEs (Table 2-2, Figure 2-8). Indices of dispersion for specific sites indicated that count data were not overdispersed across time, which contrasted with the spatial overdispersion observed (Table 2-9, a, b).

Spatial-temporal interactions were also considered at two scales (combined RFIs only). At the larger scale (among-sites), effect sizes (generated by modeling the site by sampling period interaction) indicated that some sites were more temporally variable than others, and that peak abundances for different sites occurred during different sampling periods (Figure 2-9). The spatial-temporal distribution of combined RFIs was also evaluated by way of Standardized

Quantile Variance (SQV), which summarized the extent of temporal variability within each sample plot across the range of abundance quantiles observed in the study system (Figure 2-10). The SQV provided evidence of non-random spatial-temporal aggregation, ($SQV > 0$), but only for the highest and lowest quantiles (0.9 and 0.1), (Figure 2-10). This indicated that plots with very high or very low abundance were likely to maintain a similar abundance across sampling periods. Plots with overall RFI abundance between the 0.25 and 0.75 quartiles tended to fluctuate within the quartile range at a near random temporal basis ($SQV \sim 0$), or were not likely to maintain abundance at the same quartile between sampling periods ($SQV < 0$), (Figure 2-10). These trends in SQV were similar for the UB and B treatment groups across the entire quartile range examined (Figure 2-10).

DISCUSSION

Root-Feeding Insect Population Densities

Root feeding insect population density, and thus the potential for top down effects on roots, was low in our study sites. Overall, mean RFI density was approximately 5 individuals per square meter. In contrast, Stevens and Jones (2006) reported densities of 36 individuals per square meter in a mixed pine forest at the Savannah River site, South Carolina, U.S.A. In another study in longleaf pine, investigators were unable to detect an effect due to pesticide, which may have resulted from low densities of root-feeding insects and nematodes (however densities were not quantified). Population density estimates for root-herbivores in non-agricultural systems are uncommon. The two studies previously mentioned were the only publications that we found for comparison with other pine forests in the Southeastern U.S.A. But in both of these studies, densities were far below the high densities (> 100 individuals per

square meter) that have been commonly reported in studies of turf, or used in greenhouse experiments (e.g. Dalthorpe et al., 2000, b). The relatively low population density in our study sites, even in those unburned for 9 years, may be related to the long history of frequent fire in the study system prior to this research.

Factors Related to the Abundance, Biomass, and Diversity of Root-Feeding Insects

Effects of Fire on RFI Abundance:

Fire exclusion altered several elements of ecosystem structure and function. Chi-square tests accounting only for fire treatment, suggested that combined direct and indirect effects of fire influenced all root-feeding taxa except for cicadas (Table 2-2). Model fit statistics and output from "best" models revealed that indirect effects of fire (changes in understory vegetative composition) were related to root-feeding insect abundances (Tables 2-5, 2-6; Appendix D); however, effects from fire not attributable to vegetative composition were non-significant (Table 2-6; Appendix D). This suggested that indirect effects from fire were more important than direct mortality in this system.

Available evidence from the literature suggested that fires in other forested ecosystems have caused direct mortality of larvae, reducing population densities for several months or longer. Sileshi and Mafongoya (2006) concluded that fire in the miombo (Zimbabwe, Africa) reduced abundance of soil arthropods by way of direct mortality or by effects on available food resources; however, the authors were unable to distinguish between direct and indirect effects of fire in their analysis, because soil arthropod abundance was only compared between patches of forest that had or had not been impacted by a stand-replacing fire, and no covariates were measured. Wikars and Schimmel (2001) measured soil arthropod abundance before and after prescribed fires in Scots Pine (*Pinus sylvestris*) forests in central Sweden, and observed greater

than 50% mortality. Mortality effects increased in relation to the depth to which soil organic matter was consumed during the fire. In longleaf pine-wiregrass ecosystems, soil organic matter is not usually concentrated in the uppermost soil horizon (Hendricks et al., 2006) where combustion of the organic layer would transfer more heat deeper into the soil; furthermore, fires occur regularly, preventing fuel buildup and reducing fire intensity and duration (Boring et al., 2004; Jack et al., 2010). These factors may explain the apparently lower importance of direct mortality effects from fire, relative to indirect changes in plant community structure.

Nevertheless, even though direct mortality did not appear important in our data, it is conceivable that with a long history of frequent fire, cumulative mortality effects may also be related to the low population densities found throughout the study system.

Vegetative Composition and RFI Abundance:

Inclusion of vegetative parameters in GEEs generally increased information yield as predicted, suggesting that aboveground vegetative composition was an important factor relating to the population density and spatial distribution of RFIs. Also, as predicted, we detected a negative relationship between root-feeding insect abundance and wiregrass cover (which was higher in B stands); however, the effect magnitude of wiregrass was not significant for combined or specific taxa, with the exception of Cebionids (Tables 2-5, 2-6; Appendix D). Low aboveground herbivory of wiregrass has also been documented in studies of grazing in pine-wiregrass savanna (Grelen, 1978), and wiregrass has been shown to be a poor quality food for cattle (Lewis et al., 1975). This may relate to the high C:N ratio, high cellulose content, or low N content of wiregrass fine roots relative to other fine roots which are abundant in the ecosystem (see Jansen, 2007, and Guo et al., 2004). Model output generally suggested that woody plants, forbs, and other grass species were the most influential aspects of vegetative composition for RFI

abundance (Table 2-6). Adult Scarab beetles often feed on broad leaved trees and forbs. Perhaps increases in the cover of hardwoods and shrubs attracted more adults into the vicinity, leading to increased oviposition. Site 6 (B) had the highest combined RFI abundance among the 3 B sites (Figure 2-7), and also had the highest abundance of other grass species (Table 2-3), predominantly toothache grass (*Ctenium aromaticum*, Walt.). Within Site 6 (B), plots with higher cover of toothache grass corresponded to lower relative abundances, explaining the seemingly contradictory negative effect of other grasses on RFI abundance in the model (Table 2-6), given the site-wise abundance patterns. Therefore, the negative effect may reflect avoidance of toothache grass or overlap with another important environmental gradient (e.g. soil moisture).

It could be argued that the observed relationships between understory composition and abundance might be due to the influence of root herbivores on the vegetative composition. Though others have stated that root-herbivores can influence plant community composition and succession (e.g. De Deyn et al., 1994 and Brown and Gange, 1989), we suggest that this was not likely the case in this study system, because the frequent fire disturbance, which is a well known driver of plant species composition, overwhelmed the top-down forces exerted by RFIs (see Chapter 5), which were well below the densities reported in cases where significant damage to crops and turf have been documented. Therefore, correlations were interpreted as a bottom-up influence of vegetative composition on RFI abundance, rather than the reverse.

Soil Properties and RFI Abundance:

We predicted that root-feeding insect abundance would be positively related to soil organic matter, soil available N, and soil moisture (see Stevens et al., 2008; van Herk and Vernon, 2006; Dalthorp et al., 2000b). Evidence to support this was mixed; soil parameters were

not consistently important across taxa in GEE models, but most soil variables were important for at least some taxa (Table 2-5, 2-6, and Appendix D). Soil NH_4^+ (positively related) and CEC (negatively related) appeared to be the two most important soil parameters for combined taxa (Table 2-6); however they were not significant ($p > 0.1$). The positive effect of soil N indicated a possible larval preference for relatively nutrient rich micro-sites. This was in support of findings by Stevens and Jones (2006), who observed increased herbivory in response to soil fertilization. Soil moisture may lead to mortality or deeper migration in the soil (see Stevens et al., 2008). Though soil moisture was not included in GEE models due to lack of resolution of soil water measurements and collinearity with sites, we speculated that it might still be an important variable below some moisture threshold. Turkey woods (B Site 5) was considerably drier than all other sites, and also had the lowest combined RFI abundance. We sampled for RFIs deeper in the soil but did not find evidence that vertical migration was occurring below the 20 cm sample depth. Flooding has also been suggested to be a potential mechanism for mortality of wireworms (van Herk and Vernon, 2006). Intermittent flooding (standing water above the soil surface) was noted in B Site 4 (Baker Woods) and B Site 6 (21-Acre Woods) during the Summer 2009, Fall 2009, and Spring 2010 sampling periods, which could have resulted in larval mortality (van Herk and Vernon, 2006). However, our results (Figure 2-9) do not show any clear indication of below average abundances for B Sites 4 and 6 during these time periods (sites were sampled after water levels receded).

Fine Root Standing Crop and RFI Abundance:

We wanted to know how RFI abundances related to fine root standing crop estimates because this could provide clues about the relative importance of bottom-up influences of fine root standing crop on RFI abundances. Our estimates of fine root standing crop were within the

previously reported range for longleaf pine stands in the vicinity of the study system (Hendricks et al., 2006). Experimental models suggested that fine root standing crop parameters were important covariates for grub, wireworm, Cebionid, and Cicada abundances, but not for weevils, or combined RFIs (Table 2-5, Appendix D). Also, fit statistics for predictive models of weevil, Cebionid, and cicada abundances, were improved by including fine root standing crop parameters (Table 2-5, Appendix D). The positive relationship between fine root standing crop and RFI abundances supported our hypothesis that belowground herbivores may be influenced by belowground ecosystem structure and fine root productivity.

Fire and Individual Biomass:

Though the average individual biomass of most taxa did not differ between fire treatments (UB, B), weevil mass differed substantially. This pattern could indicate that the dominant genera of weevils had strong food preferences (see Chapter 3), or a more specialized diet (e.g. roots of woody plants or understory forbs, which were less abundant in B sites). The pattern could be due to a prevalence for lower growth rates due to decreased food quality in B sites. The wiregrass, which was more dominant in B stands, had a relatively high C:N (see chapter 3), which may have reduced assimilation and/or foraging efficiencies for weevils.

Fire and Taxonomic Diversity:

An increase in taxonomic diversity due to fire exclusion (UB treatment) was apparent in the study. Some taxa of root-feeding insects possibly were more sensitive or better adapted to frequent surface fires during one or more phases of their lifecycle. The higher diversity of the UB treatment suggests that some RFI taxa were unable to thrive in frequently burned areas, perhaps due to the inability to adapt well to the vegetative structure which characterized frequently burned areas.

Spatial and Temporal Dispersion and Inference about the Ecology of Root-Feeding Insects

Root-feeding insect larvae were spatially over-dispersed as predicted; this pattern has been commonly observed for insect count data (see Southwood, 1978). Possible mechanisms for aggregation included vegetative compositional patterns, resource patchiness, patchiness of predators, adult congregation during periods of oviposition, and low rates of larval dispersal. The extent of overdispersion, based on VMR and other indices of dispersion, differed between fire treatments (UB was more overdispersed) and to a lesser extent, also varied among sites. (Table 2-9, a). This suggested that changes to the ecosystem associated with burn exclusion (UB) were somehow linked to the underlying spatial dispersion of RFIs. Given the importance of woody plants in models of RFI abundance, we speculated that RFIs might have congregated around woody plants during oviposition, leading to higher abundances and greater aggregation in UB sites, where woody plants were more abundant.

Initially, we predicted a consistent seasonal trend with peak abundances in the fall, as has been observed in the Northeastern U.S. in turf grass (Fermanian et al., 2002). We observed significant fluctuations due to sampling period after accounting for environmental covariates (Table 2-6); but, we did not observe clear seasonal fluctuations, which suggested that inter-annual variability may be more important than seasonal cycles in abundance. However, we did not sample RFIs during the winter, which reduced intra-annual resolution. The subtropical climate of the region, may have dampened seasonal patterns of abundances. The timing of peak abundances differed substantially for individual taxa (Figure 2-8).

Sampling period effects also varied among individual sites. Sometimes relatively large increases in the abundance of one or more RFI taxa at one or two sites had enough leverage to drive the average for the sampling period (Figure 2-9). This site by sampling period interaction

(Figure 2-9) suggested that RFIs were aggregated at the landscape scale, and that peaks in population density occurred asynchronously across the landscape. Asynchronicity in peak abundances, or in the timing of particular developmental stages, could be an important source of heterogeneity for belowground herbivory at the landscape scale. The lesser extent of temporal variability relative to more northern climates, may be due to warmer, less variable soil temperatures associated with the subtropical climate of the region.

We predicted that RFIs were patchily distributed, and that abundances at specific locations in space would remain stable over time. We found that spatial overdispersion was substantial, but RFIs did not appear to be temporally overdispersed. The variance to mean ratio (VMR) among plots (nested within sites), averaged across time periods (Table 2-9, a), was greater than variability among time periods (nested within plots), averaged across sites (Table 3-9, b). The standardized quantile variance plot (SQV) suggested that within patches where no insects were found (10th percentile), or where the highest abundances were recorded (90th percentile), there was relatively high temporal stability across the six sampling periods. However, plots where abundances tended to be intermediate were more variable (Figure 3-10). It should also be noted that there is a natural tendency for SQV to increase at very high or low quantiles, simply because the expected variance decreases considerably; therefore, the increasing trend for SQV at the 0.1 and 0.9 quantiles, may be exaggerated (Dalthorp et al., 2000, a). Insects from B plots had a slightly higher SQV for the 0.1 quantile than the 0.9 quantile, but UB plots had a similar SQV in the highest and lowest quantiles.

Taken together, observations on the spatial-temporal distributions of larvae suggested that adults were consistently attracted to particular locations (prior to oviposition) and that larvae did not migrate far from the oviposition site, or that larvae migrate to and congregate in

particular micro-sites, or both. Perhaps adults are the primary determinant of larval distribution at scales larger than the migration distance of larvae, but at smaller spatial scales, larvae may orient to specific cues, which could explain the overdispersion that was observed within plots (unpublished data).

Recommendations for Future Research

Linking adult behavior and abundance patterns with larval abundances may be an important next step in the ecological study and management of RFIs. We recommend a study linking adult abundance estimates with below ground densities, which could provide a less labor intensive method for estimating larval abundance. These estimates would be based on estimates of adult densities on above ground vegetation and random net sweep samples. Tandem study of the distributions of RFI larvae, RFI adults, and vegetative composition, using a spatially integrated study design could provide a great deal of meaningful information. In addition to providing a better understand of the role that adults play in the spatial distribution of larvae, this knowledge also has pest management implications. For instance, this could lead to a less labor intensive way to estimate larval abundance and distribution, or improve the precision of pest management. For these and other reasons, it would be of benefit to maintain and publish long-term data sets of both adult and larval abundances in a variety of ecosystems.

Given the apparent influence of environmental variables on RFI abundance, we also recommend follow up manipulative experiments. These experiments would utilize a series of insect removal, addition, and control treatments, to isolate specific environmental effects thought to be important, mainly vegetative composition, soil moisture, and soil N.

In the interest of testing the translational power of the predictive models developed as part of the analysis in this study, a follow up field collection and RFI count in tandem with the

measurement of the variables included in the models is recommended. This ground-truthing could be used to test the effectiveness of these models at predicting RFI abundances across a broader range of long-leaf pine sites. Similar predictive models could then be developed for other ecosystems.

CONCLUSIONS

The indirect effects of fire on vegetative composition was a major driver of RFI abundance and diversity in the longleaf pine-wiregrass study system. Burn excluded stands (UB) had higher RFI abundance and greater species diversity. Vegetative composition was significantly related to RFI abundance (combined and most individual taxa). Effects of fire not attributable to vegetative composition did not appear to be important for most taxa.

The abundance of RFIs in the longleaf pine-wiregrass ecosystem was positively related to additional bottom-up factors, particularly soil NH_4^+ concentration and fine root standing mass, which suggested that adults may prefer more productive locations for oviposition, and/or that larvae migrate into more productive habitat patches, as has been suggested by Stevens and Jones (2006). Top-down effects from herbivores on either fine root standing crop or vegetative composition were not apparent (higher RFI abundance was positively related to woody plant abundance and fine root standing crop rather than the reverse; also see Chapter 4). This pattern reflected both the relatively low population densities in the system, and the overarching influence of frequent fire disturbance on understory composition and fine root dynamics in longleaf pine-wiregrass ecosystems.

Fine root herbivores were spatially aggregated ($\text{VMR}>1$) from scales ranging from less than 0.25 m^2 to greater than 0.25 km^2 , suggesting that multiple mechanisms, operating at

different spatial scales, may influence their spatial distribution. For instance, adult behavior may have driven the observed overdispersion at the landscape scale and within-site scale, while larval behavior may have lead to aggregation within local habitat patches. Spatial dispersion was more aggregated than temporal dispersion; furthermore, spatial locations with the lowest and highest population densities were relatively stable over time. Three possible causes of relatively stable abundances are (1) that adult insects chose oviposition sites in the vicinity of their hatching site, (2) that certain environmental features consistently attracted adults and/or larvae to particular locations, and/or (3) that root-feeding larvae did not migrate far from the site of oviposition. In conclusion, bottom-up factors and disturbance regime may be important determinants of RFI abundance, diversity, and spatial distribution.

LITERATURE CITED

- Arimura, G., R. Ozawa, S. Kugimiya, J. Takabayashi, and J. Bohlmann. 2004. Herbivore-induced defense response in a model legume. Two-spotted spider mites induce emission of (*E*)-*B*-Ocimene and Transcript Accumulation of (*E*)-*B*- Ocimene Synthase in *Lotus japonicus*. *Plant Physiology* **135**:1976-1983.
- Bolker, B.M., M.E. Brooks, C.J. Clark, S.W. Geange, J.R. Poulsen, M.H. Stevens, and J-S.S.White. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology and Evolution* **24**:127-135.
- Boring, L.R., C.A. Wilson, J.J. Hendricks, and R.J. Mitchell. 2004. Season of burn and nutrient losses in a longleaf pine ecosystem. *International Journal of Wildland Fire* **13**:443-453.
- Boyer, W. and J.H. Miller. 1994. Effects of burning and brush treatments on nutrient and soil physical properties in young longleaf pine stands. *Forest Ecology and Management* **70**:311-318.

- Brown, V.K. and A.C. Gange. 1990. Insect herbivory below ground. *Advances in Ecological Research* **20**:1-58.
- Brown, V.K. and A.C. Gange. 1989. Differential effects of above- and belowground insect herbivory during early plant succession. *Oikos* **54**:67-76.
- Burnham, K.P., D.R. Anderson, and K.P. Huyvaert. 2011. AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. *Behavioral Ecology and Sociobiology* **65**:23-35.
- Carter, D.C., J.J. Hendricks, R.J. Mitchell, and S.D. Pecot. 2004. Fine root carbon allocation and fates in longleaf pine forests. *Forest Science* **50**:177-187.
- Combs, S.M. and M.V. Nathan. 1998. Soil organic matter. In Brown, J.R. (ed.). *Recommended Chemical Soil Test Procedures for the North Central Region*. North Central Regional Research Publication Bull. No. 221 (revised). Missouri Agricultural Experiment Station SB 1001, Univ. of Missouri, Columbia, Mo. pp. 53-58.
- Dalthorp, D., J. Nyrop, and M.G. Villani. 2000 a. Foundations of spatial ecology: the reification of patches through quantitative description of patterns and pattern repetition. *Entomologia Experimentalis et Applicata* **96**:119-127.
- Dalthorp, D., J. Nyrop, and M.G. Villani. 2000 b. Spatial Ecology of the Japanese Beetle *Popillia Japonica*. *Entomologia Experimentalis et Applicata* **96**:129-139.
- De Deyn, G.B., C.E. Raaijmakers, and W.H. van der Putten. 2004. Plant community development is affected by nutrients and soil biota. *Journal of Ecology* **92**:824-834.
- do Rosario, M., G. Oliveira, M. van Noordwijk, S.R. Gaze, G. Broouwer, S. Bona, G. Mosca, and K. Hairiah. 2000. Auger Sampling, Ingrowth Cores and Pinboard Methods. In: *Root Methods: A Handbook*. Smit, A.L., A.G. Bengough, C. Engels, M. van Noordwijk, S. Pellerin, S.C. van de Geijn (EDS.). Springer, Berlin.
- Drew, M.B., L.K. Kirkman, and A.K. Gholson, Jr. 1998. The vascular flora of Ichauway, Baker County, Georgia: A remnant longleaf pine/wiregrass ecosystem. *Castanea* **63**:1-24.

- Eissenstat, D. M., and R. D. Yanai. 2002. Root Life Span, Efficiency, and Turnover. Pages 221-238 in Y. Waisel, A. Eshel, and U. Kafkafi, editors. *Plant Roots: The Hidden Half*. Marcel Dekker, Inc., New York.
- Fermanian, T.W., M.C. Shurtleff, R. Randell, H.T. Wilkinson, and P.L. Nixon. 2002. *Controlling Turfgrass Pests* 3rd Ed. Prentice-Hall, New York.
- Fortin, M-J, M.R.T. Dale, and J. ver Hoef. 2002. Spatial analysis in ecology. In: *Encyclopedia of Environmetrics* 4:2051-2058. El-Shaarawi, A.H. and W.W. Piegorsch (EDS.). John Wiley and Sons, Chichester.
- Furlan, L. 2004. The biology of *Agriotes sordidus* Illiger (Col., Elateridae). *Journal of Entomology and Nematology* **128**:696-706.
- Goebel, P.C., B.J. Palik, L.K. Kirkman, and L. West. 1997. *Field Guide: Landscape Ecosystem Types of Ichauway*. Technical Report No. 97-1. Joseph W. Jones Ecological Research Center at Ichauway, Newton, Georgia.
- Grelen, H.E. 1978. Forest grazing in the South. *Journal of Range Management* **31**:244-250.
- Guo, D.L., R.J. Mitchell, and J.J. Hendricks. 2004. Fine root branch orders respond differentially to carbon source-sink manipulations in a longleaf pine forest. *Oecologia* **40**:457-457.
- Hendricks, J.J., R.L. Hendrick, C.A. Wilson, R.J. Mitchell, S.D. Pecot, and D. Guo. 2006. Assessing the patterns and controls of fine root dynamics: an empirical test and methodological review. *Journal of Ecology* **94**:40-57.
- Hunter, M.D. 2001. Out of sight, out of mind: the impacts of root-feeding insects in natural and managed systems. *Agriculture and Forest Entomology* **3**:3-9.
- Hunter, M.D. and P.W. Price. 1992. Playing chutes and ladders: Heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology* **73**:724-732.

- Ismail, N. and A. A. Jemain. 2007. Handling overdispersion with negative binomial and generalized Poisson regression models. *Casualty Actuarial Society Forum* (winter): 103-158.
- Jack, S.B., J.K. Hiers, R.J. Mitchell, and J.L. Gagnon. 2010. Fuel loading and fire intensity - effects on longleaf pine seedling survival. In: *Proceedings of the 14th Biennial Southern Silvicultural Research Conference, General Technical Report SRS-121*. J. A. Stanturf (ed.). USDA, Forest Service, Southern Research Station, Asheville, North Carolina.
- Jansen, N.A. 2007. Influence of root branching order on fine root substrate quality and decomposition in a *Pinus palustris* ecosystem. Master's Thesis: University of Florida.
- Johnson, J.B. and K.S. Omland. 2004. Model selection in ecology and evolution. *Trends in Ecology and Evolution* **19**:101-108
- Jonas, J. L. and A. Joern. 2003. Host plant quality alters grass/forb consumption by a mixed-feeding insect herbivore, *Melanoplus bivittatus* (Orthoptera: Acrididae). *Ecological Entomology* **33**:546-554.
- Jones, O.T., and T.H Coaker. 1977. Oriented responses of carrot fly larvae, *Psila rosae*, to plant odors, carbon-dioxide and carrot root volatiles. *Physiological Entomology* **2**:189-197.
- Kalra, Y.P. 1995. Determination of the pH of soils by different methods: a collaborative study. *Journal of the Association of Analytical Chemistry International* **78**:310-321.
- Knapp A.K., J.M. Briggs, J.M. Blair, and C.L. Turner. 1998. Patterns and controls of aboveground net primary production in tallgrass prairie. In: Knapp A.K., J.M. Briggs, D.C. Hartnett, and S.L. Collins (eds), *Grassland dynamics: longterm ecological research in tallgrass prairie*. Oxford University Press, New York, pp. 193-221.
- Lewis, C.E., R.S. Lowrey, W.G. Monson, and F.E. Knox. 1975. Seasonal trends in nutrients and cattle digestibility of forage on pine-wiregrass range. *Journal of Animal Science* **41**:208-212.

- Lura, C.L. and P.E. Nyren. 1992. Some effects of a white grub infestation on northern mixed-grass prairie. *Journal of Range Management* **45**:352-354.
- Maguire, R.O. and S.E. Heckendorn. 2011. Laboratory Procedures: Virginia Tech Soil Testing Laboratory. Publication #452-881, Communications and Marketing, College of Agriculture and Life Sciences, Virginia Tech. Accessed online (9/14/2012) at <http://pubs.ext.vt.edu/452/452-881/452-881_pdf.pdf>.
- Martin-Fernandez, J.A., C. Barcelo-Vidal, and V. Pawlowsky-Glahn. 2003. Dealing with Zeros and Missing Values in Compositional Data Sets Using Nonparametric Imputation. *Mathematical Geology* **35**: 253-278.
- May, B.M. 1966. Identification of the immature forms of some common soil-dwelling weevils, with notes on their biology. *New Zealand Journal of Agricultural Research* **9**:286-316.
- Maynard, D.G., Y.P. Kalra, and J.A. Crumbaugh. Nitrate and Exchangeable Ammonium Nitrogen. 2008. In: *Soil Sampling and Methods of Analysis* (2nd Ed.). Carter, M.R. and E.G. Gregorich. CRC Press, Boca Raton, FL.
- Mitchell, R.J., L.K. Kirkman, S.D. Pecot, C.A. Wilson, B.J. Palik, and L.R. Boring. 1999. Patterns and controls of ecosystem function in longleaf pine-wiregrass savannas. I. Aboveground net primary productivity. *Canadian Journal of Forest Research* **29**:743-751.
- Pan, W. 2001. Akaike's information criterion in generalized estimating equations. *Biometrics* **57**:120-125.
- Price, P. W., Bouton, C.E., Gross, P., McPheron, V.A., Thompson, J.N., Weis, A.E. 1980. Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annual Review Ecology and Systematics* **11**:41-65.
- Rasmann, S., T. G. Kollner, J. Degenhardt, I. Hiltbold, S. Toepfer, U. Kuhlmann, J. Gershenzon, and T. C. J. Turlings. 2005. Recruitment of entomopathogenic nematodes by insect-damaged maize roots. *Nature* **434**:732-737.

- Ritcher, P.O. 1966. *White Grubs and Their Allies: A Study of North American Scarabaeoid Larvae*. Studies in Entomology Number Four. Oregon State University Press, Corvallis, Oregon.
- Royall, R.M. 1997. *Statistical evidence: a likelihood paradigm*. Chapman and Hall, London.
- Sumner, M.E., and W.P. Miller. 1996. Cation Exchange Capacity and Exchange Coefficients. In D. L. Sparks (ed.). *Methods of Soil Analysis: Part 3, Chemical Methods*. Soil Science Society of America, Book Ser. 5. SSSA and ASA, Madison, Wisconsin, pp. 1221-1222.
- Sileshi, G. 2008. The excess zero problem in soil animal count data and choice of appropriate models for statistical inference. *Pedo-biologia* **52**:1-17.
- Sileshi, G. 2006. Selecting the right statistical model for analysis of insect count data by using information theoretic measures. *Bulletin of Entomological Research* **96**:479-488.
- Sileshi, G. and P.L. Mafongoya. 2006. The short term impact of forest fire on soil invertebrates in the miombo. *Biodiversity and Conservation* **15**:3153-3160.
- Southwood, T.R.E., 1978. *Ecological Methods*, 2nd Ed. Chapman and Hall, London.
- Stehr, F.W. 1991. *Immature Insects* (Vol. 2). Kendall-Hunt Publishing Co., Dubuque, Iowa.
- Stevens, G.N., K.O. Spence, and E.E. Lewis. Root feeders in Heterogeneous Systems: Foraging Responses and Trophic Interactions. 2008. In: *Root Feeders: An Ecosystem Perspective*. CAB, Oxfordshire, UK.
- Stevens, G. N., and R. H. Jones. 2006. Patterns in soil fertility and root herbivory interact to influence fine-root dynamics. *Ecology* **87**:616-624.
- Stevens, G. N. 2005. Trophic dynamics in the fine-root based food web: integrating resource heterogeneity, root herbivores, and root foraging. Dissertation. Virginia Polytechnic Institute and State University, Blacksburg.
- Taylor, L.R. 1961. Aggregation, variance and the mean. *Nature* **189**:732-735.

- Thaler, J. S., M. A. Farag, P. W. Pare, and M. Dicke. 2002. Jasmonate-deficient plants have reduced direct and indirect defences against herbivores. *Ecology Letters* **5**:764-774.
- Topp, G.C., W.D. Zebchuk, J.L. Davis, and W.G. Bailey. 1984. The measurement of soil water content using a portable TDR hand probe. *Canadian Journal of Soil Science* **64**:313-321.
- van Herk and Vernon. 2006. Effect of temperature and soil on the control of a wireworm, *Agriotes obscurus* L. (Coleoptera: Elateridae) by flooding. *Crop Protection* **25**:1057-1061.
- Vela, N.P., Olson, L.K., and Caruso, J.A. 1993. Elemental speciation with plasma mass spectrometry. *Analytical Chemistry* **65**: 585-597.
- White, G.C. and R.E. Bennets. 1996. Analysis of frequency count data using the negative binomial distribution. *Ecology* **77**:2549-2557.
- White, JoA. and C.E. Strehl. 1978. Xylem feeding by periodical cicada nymphs on tree roots. *Ecological Entomology* **3**:323-327.
- Whittingham, M.J., P.A. Stephens, R.B. Bradbury, and R.P. Freckleton. 2006. Why do we still use stepwise modeling in ecology and behaviour? *Journal of Animal Ecology* **75**:1182-1189.
- Wikars, L-O. and J. Schimmel. 2001. Immediate effects of fire severity on soil invertebrates in cut and uncut pine forests. *Forest Ecology and Management* **141**:189-200.
- Willams, K.S., K.G. Smilth, and F.M. Stephen. 1993. Emergence of 13-year periodical cicadas (Cicadidae: Magicicada): phenology, mortality, and predator satiation. *Ecology* **74**:1143-1152.

TABLES

Table 2-1: List of taxonomic groups represented in the study. Italicized taxa represent the genus level, and bold font represents the family level. Number of individuals collected, population density, and average individual mass are reported for each taxa.

<i>TAXONOMIC GROUP</i>	# Individuals			# Individuals/m ²			Avg. Mass/Individual (mg)		
	<i>UB</i>	<i>B</i>	<i>Combined</i>	<i>UB</i>	<i>B</i>	<i>Combined</i>	<i>UB</i>	<i>B</i>	<i>Combined</i>
COMBINED TAXA	696	409	1105	6.5 ± 2.5	3.7 ± 1.8	5.1 ± 1.9	36.1 ± 59.1	34.8 ± 44.8	35.5 ± 53.5
All Grubs	157	140	297	1.7 ± 2.9	1.6 ± 2.1	1.6 ± 1.8	42.2 ± 58.2	54.6 ± 60.0	48.5 ± 58.8
<i>Anomala</i>	5	10	15	29.8 ± 18.4	10.0 ± 5.2	18.8 ± 15.8
<i>Cyclocephala</i>	5	2	7	76.7 ± 57.7	*	N.A.
<i>Diplotaxis</i>	15	0	15	8.0 ± 4.9	N.A.	N.A.
<i>Maladera</i>	39	0	39	18.8 ± 16.2	N.A.	N.A.
<i>Phyllophaga</i>	62	123	185	55.3 ± 70.0	56.5 ± 59.5	56.1 ± 63.3
Unknown	5	1	6	*	*	*
All Wireworms	108	44	152	2.0 ± 2.3	0.8 ± 1.8	1.4 ± 1.8	6.7 ± 8.3	7.5 ± 6.1	7.0 ± 7.6
<i>Agriotes</i>	35	10	45	5.8 ± 7.6	6.1 ± 5.2	5.9 ± 6.9
<i>Ctenicera</i>	2	5	7	†	†	†
<i>Hemirhipis</i>	3	0	3	†	N.A.	N.A.
<i>Limonius</i>	5	0	5	2.9 ± 0.8	N.A.	N.A.
<i>Melanotus</i>	53	27	80	7.2 ± 6.3	8.5 ± 6.6	7.6 ± 6.4
<i>Sericus</i>	10	2	12	8.3 ± 19.0	5.2 ± 3.4	7.4 ± 15.7
Cebrionids	125	99	224	1.2 ± 2.3	1.0 ± 1.9	1.1 ± 1.7	44.1 ± 45.2	41.4 ± 45.1	42.8 ± 40.7
Weevils	297	114	411	2.6 ± 3.5	1.1 ± 1.9	1.8 ± 2.3	40.2 ± 74.7	12.0 ± 16.2	30.1 ± 62.0
Cicadas	9	12	21	0.2 ± 2.5	0.2 ± 1.9	0.2 ± 1.8	32.2 ± 30.3	67.6 ± 62.0	52.5 ± 50.0

* There were not enough specimens available to dry and weigh after preserving sample specimens.

† Specimen was so small that it was not able to be weighed after desiccation.

... Population density estimates were not computed at the genus level, only the family level.

N.A. Calculation not applicable, or no specimens found.

Table 2-2: Summary of results for Chi-square test for independence of frequency distributions between fire treatments (UB and B), sampling periods (1-6, see methods), and sites (1-6).

Effect Source	Insect Taxa	Bin Class	χ^2 (chi-sq.)	d.f.	min. expected	p-value
Fire (UB v B)						
	Combined	A	14.4	5	7.0	0.0130
	Grubs	B	13.4	4	5.5	0.0095
	Wireworms	B	18.2	4	2.5	0.0011
	Weevils	B	3.6	4	3.0	0.4613
	Cebrionids	C	7.4	3	3.0	0.0592
	Cicadas	D	0	2	5.0	1.0000
Sampling Period						
	Combined	A	49.2	25	2.3	0.0027
	Grubs	C	17.6	15	3.8	0.2812
	Wireworms	C	51.1	15	2.8	<0.0001
	Weevils	C	30.7	15	3.2	0.0097
	Cebrionids	C	41.3	15	1.0	0.0003
	Cicadas	D	81.6	10	1.7	<0.0001
Site						
	Combined	A	35.7	25	2.3	0.0763
	Grubs	C	29.5	15	3.8	0.0138
	Wireworms	C	29.9	15	2.4	0.0124
	Weevils	C	17.8	15	3.2	0.2748
	Cebrionids	C	28.3	15	1.0	0.0199
	Cicadas	D	16.9	10	1.7	0.0769

BIN CLASSES:

A: 0, 1, 2-3, 4-5, 6-10, >10

B: 0, 1, 2-3, 4-5, >5

C: 0, 1, 2-3, >3

D: 0, 1-2, >2

Table 2-3: Summary of site vegetative composition (estimated by percent ground cover), fine root standing crop, and root feeding insect population density among sites, which were nested within fire treatment (unburned/burned); N = 9 plots per site.

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Fire Treatment	Unburned	Unburned	Unburned	Burned(2 yr)	Burned(2 yr)	Burned(1 yr)
Ichauway Location	Red Dirt Woods	Baker Woods	Baker Woods	Baker Woods	Turkey Woods	21 Acre Woods
Est. % Cover by Vegetation Type						
Wiregrass	19 ± 8	18 ± 7	36 ± 16	66 ± 14	64 ± 6	54 ± 16
Other grasses	4 ± 3	5 ± 3	5 ± 3	36 ± 16	5 ± 3	7 ± 9
Woody shrubs/hardwoods	39 ± 14	44 ± 16	44 ± 16	5 ± 3	8 ± 8	1 ± 1
Forbs (including legumes)	13 ± 9	13 ± 6	25 ± 13	13 ± 6	18 ± 9	15 ± 10
Pine seedlings and saplings	7 ± 14	2 ± 4	2 ± 4	8 ± 11	7 ± 8	1 ± 2
Est. % Cover of leaf litter	43 ± 13	41 ± 12	43 ± 8	8 ± 6	4 ± 2	6 ± 6
Est. % Bareground	4 ± 3	7 ± 5	4 ± 2	10 ± 4	19 ± 12	32 ± 16
Fine Root Standing Crop						
g/m ²	260.4 ± 46.4	213.6 ± 46.0	218.8 ± 40.8	233.6 ± 53.2	196.0 ± 48.4	186.8 ± 40.4
m/m ²	2904 ± 828	1980 ± 396	2044 ± 580	3020 ± 664	2992 ± 924	1956 ± 480
Combined RFI Pop. Density (#/m²)	4.4 ± 2.2	6.2 ± 2.1	8.8 ± 3.1	3.8 ± 2.3	2.6 ± 1.8	4.7 ± 2.5

Table 2-4: Summary of soil volumetric water content, soil organic matter, and soil chemical properties for the three unburned (UB) and three regularly burned (B) sites used in the study. Values are means \pm standard deviations.

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Fire Treatment	Unburned	Unburned	Unburned	Burned (2 yr)	Burned (2 yr)	Burned (1 yr)
Ichauway Location	Red Dirt Woods	Baker Woods	Baker Woods	Baker Woods	Turkey Woods	21 Acre Woods
Avg. Vol. Soil H₂O Content (%)	6.5	9.0	9.1	11.2	5.4	12.5
<i>18-Jul-10</i>	<i>7.0 \pm 3.0</i>	<i>8.5 \pm 3.0</i>	<i>9.8 \pm 2.0</i>	<i>10.6 \pm 1.4</i>	<i>3.2 \pm 0.8</i>	<i>15.3 \pm 1.9</i>
<i>25-Nov-10</i>	<i>3.6 \pm 1.1</i>	<i>6.4 \pm 1.4</i>	<i>6.1 \pm 1.3</i>	<i>8.4 \pm 2.7</i>	<i>4.0 \pm 1.4</i>	<i>8.3 \pm 1.7</i>
<i>4-Jan-11</i>	<i>9.0 \pm 1.8</i>	<i>12.0 \pm 1.3</i>	<i>11.4 \pm 2.1</i>	<i>14.6 \pm 4.0</i>	<i>9.1 \pm 2.2</i>	<i>13.9 \pm 1.9</i>
Organic Matter (%)	1.3 \pm 0.3	1.4 \pm 0.4	1.6 \pm 0.2	2.1 \pm 0.6	1.3 \pm 0.5	2.0 \pm 0.4
pH	5.37 \pm 0.20	5.36 \pm 0.20	5.35 \pm 0.19	5.34 \pm 0.21	5.69 \pm 0.14	5.30 \pm 0.29
Acidity (%)	59.8 \pm 11.8	58.1 \pm 10.5	61.1 \pm 10.2	46.7 \pm 26.9	37.3 \pm 12.9	65.1 \pm 13.6
Base Saturation (%)	40.2 \pm 11.8	41.9 \pm 10.5	38.9 \pm 10.2	32.5 \pm 19.7	62.7 \pm 12.9	34.9 \pm 13.6
Est. CEC (meq/100g)	2.50 \pm 0.73	2.75 \pm 0.81	2.96 \pm 0.55	3.23 \pm 0.67	1.92 \pm 0.93	3.16 \pm 0.32
NH₄⁺ (mg/kg)	17.06 \pm 5.01	20.51 \pm 5.67	18.79 \pm 3.33	24.36 \pm 4.75	22.66 \pm 6.78	27.19 \pm 6.18
P (mg/kg)	2.44 \pm 0.53	2.33 \pm 0.50	2.67 \pm 0.71	3.78 \pm 2.73	2.56 \pm 0.53	2.67 \pm 0.50
K (mg/kg)	9.78 \pm 2.22	9.56 \pm 1.01	11.33 \pm 2.74	10.11 \pm 4.17	11.89 \pm 3.26	13.78 \pm 2.22
Ca (mg/kg)	157.44 \pm 84.92	183.56 \pm 82.74	173.89 \pm 56.05	159.44 \pm 121.97	176.44 \pm 56.78	154.56 \pm 62.03
Mg (mg/kg)	26.44 \pm 11.62	31.78 \pm 14.33	28.67 \pm 8.3	32.78 \pm 24.41	26.22 \pm 6.91	35.44 \pm 13.65
Zn (mg/kg)	0.44 \pm 0.15	0.54 \pm 0.07	0.70 \pm 0.11	1.2 \pm 1.06	0.70 \pm 0.53	0.68 \pm 0.18
Mn (mg/kg)	30.64 \pm 4.81	13.88 \pm 5.86	18.46 \pm 5.86	10.32 \pm 5.82	13.99 \pm 3.27	18.31 \pm 7.75
Cu (mg/kg)	0.22 \pm 0.04	0.24 \pm 0.13	0.22 \pm 0.04	0.84 \pm 1.22	0.20 \pm 0.05	0.29 \pm 0.03
Fe (mg/kg)	15.99 \pm 3.86	17.08 \pm 6.08	17.48 \pm 2.52	15.64 \pm 12.67	14.58 \pm 3.49	22.44 \pm 9.33
B (mg/kg)	0.12 \pm 0.04	0.12 \pm 0.04	0.11 \pm 0.03	0.78 \pm 1.26	0.11 \pm 0.03	0.18 \pm 0.04

Table 2-5: Summary of two sets of alternative GEE models for combined and taxa specific root-feeding insects (RFIs). "Experimental" models incorporated the sources of variance inherent in the experimental design, while the set of "Predictive" models attempted to base inference only on environmental variables (ignoring spatial and temporal sources of variation) . Inference from analysis was based on the change in QIC, QIC_u and Δ (change in QIC_u from the intercept-only model); lower values indicate better model fit. Bolded values represent selected best fitting models, suggesting that the inclusion of parameters listed in that model produced the best information yield of the model set.

Models	Total RFIs			Grubs		
	QIC	QIC _u	Δ Int. Only	QIC	QIC _u	Δ Int. Only
Intercept-only Model	-516.7	-520	0	368.4	365.1	0
Backwards Selected Model	-1089.6	-1101.1	-581.1	434.2	421.5	56.4
Experimental models:						
Fire Site(Fire) SamplePeriod	-991.3	-1006.7	-486.7	442	426.2	61.1
Fire Site(Fire) SamplePeriod Veg	-1144.6	-1167.6	-647.6	430.2	416.5	51.4
Fire Site(Fire) SamplePeriod Soil	-961	-979.8	-459.8	436.4	415.9	50.8
Fire Site(Fire) SamplePeriod Roots	-970.5	-995.6	-475.6	445.2	423	57.9
Fire Site(Fire) SamplePeriod Veg Soil	-1176.4	-1201.7	-681.7	405.8	393.1	28
Fire Site(Fire) SamplePeriod Veg Roots	-1125.5	-1153.3	-633.3	437.2	421.2	56.1
Fire Site (Fire) SamplePeriod Soil Roots	-957.5	-985.1	-465.1	430.8	406.8	41.7
Fire Site(Fire) SamplePeriod Veg Soil Roots	-1160.3	-1188.4	-668.4	387.8	377.4	12.3
Predictive models:						
Veg	-776	-783	-263	402.7	388.9	23.8
Soil	-483.7	-486.7	33.3	434	421.8	56.7
Roots	-771.2	-785.4	-265.4	381.3	374.9	9.8
Veg Soil	-748.9	-757.1	-237.1	431.9	417.7	52.6
Veg Roots	-869	-883	-363	408.4	395.6	30.5
Soil Roots	-728.9	-748.5	-228.5	428.6	414.7	49.6
Veg Soil Roots	-861.2	-880.6	-360.6	411	397.8	32.7
Fire	-643.6	-649.5	-129.5	358	351.4	-13.7
Fire Veg	-739.7	-746.3	-226.3	395.3	383.9	18.8
Fire Soil	-587.7	-593.6	-73.6	429	415.2	50.1
Fire Roots	-764.9	-778	-258	360.7	353.4	-11.7
Fire Veg Soil	-721	-728.6	-208.6	411.6	398.8	33.7
Fire Veg Roots	-835.1	-848.5	-328.5	401.1	391.2	26.1
Fire Soil Roots	-734	-751.5	-231.5	410.5	396.8	31.7
Fire Veg Soil Roots	-826.5	-844.9	-324.9	396.5	384.9	19.8

Table 2-5 (Continued):

Models	Wireworms			Weevils		
	QIC	QICu	Δ Int. Only	QIC	QICu	Δ Int. Only
Intercept-only Model	321.7	320.8	0	-111.3	-113.8	0
Backwards Selected Model	357.7	365.1	44.3	-516.3	-529	-415.2
Experimental models:						
Fire Site(Fire) SamplePeriod	314.5	313.9	-6.9	-506.5	-519.7	-405.9
Fire Site(Fire) SamplePeriod Veg	343.4	341.4	20.6	-498.7	-517.1	-403.3
Fire Site(Fire) SamplePeriod Soil	316.2	317.7	-3.1	-445.1	-457.8	-344
Fire Site(Fire) SamplePeriod Roots	312.1	310	-10.8	-465.3	-484.6	-370.8
Fire Site(Fire) SamplePeriod Veg Soil	335.9	337.7	16.9	-389.2	-434.3	-320.5
Fire Site(Fire) SamplePeriod Veg Roots	343.2	340.8	20	-472.9	-492.9	-379.1
Fire Site (Fire) SamplePeriod Soil Roots	311.6	313.4	-7.4	-296.9	-326.6	-212.8
Fire Site(Fire) SamplePeriod Veg Soil Roots	336.8	339.8	19	-342.7	-395.2	-281.4
Predictive models:						
Veg	388.2	388.1	67.3	-271.1	-274.8	-161
Soil	338.2	338.3	17.5	-109.2	-109	4.8
Roots	377.6	373.4	52.6	-283.4	-293.3	-179.5
Veg Soil	384.4	385.8	65	-234.4	-237.2	-123.4
Veg Roots	383.2	382.4	61.6	-319.6	-327.7	-213.9
Soil Roots	371.4	366.3	45.5	-114.2	-126.8	-13
Veg Soil Roots	379.4	380	59.2	-82	-96	17.8
Fire	364.5	363.5	42.7	-174.3	-178.6	-64.8
Fire Veg	370.5	371.7	50.9	-281.6	-286.1	-172.3
Fire Soil	352.2	353.1	32.3	-149.6	-151.3	-37.5
Fire Roots	357.9	355.5	34.7	-284.6	-294.1	-180.3
Fire Veg Soil	357	361.4	40.6	-247.5	-250.1	-136.3
Fire Veg Roots	369.3	369.5	48.7	-317.4	-325.5	-211.7
Fire Soil Roots	342	342	21.2	-103.2	-114.2	-0.4
Fire Veg Soil Roots	356.8	359.9	39.1	-83.3	-95.8	18

Table 2-5 (Continued): Please note, experimental models for cicadas did not include sampling period because some sampling periods did not contain any cicadas, which prevented the parameter estimates for sampling period from being calculated with GEEs.

Models	Cebionids			Cicadas		
	QIC	QICu	Δ Int. Only	QIC	QICu	Δ Int. Only
Intercept-only Model	560.5	559.7	0	288.4	289.1	0
Backwards Selected Model	468.5	472.2	-87.5	293.9	306.1	17
Experimental models:						
Fire Site(Fire) SamplePeriod	547.1	542.7	-17	313.1	317.1	28
Fire Site(Fire) SamplePeriod Veg	556.8	550.1	-9.6	311.3	319.3	30.2
Fire Site(Fire) SamplePeriod Soil	519.2	516.5	-43.2	316.6	324.9	35.8
Fire Site(Fire) SamplePeriod Roots	504.6	503.2	-56.5	305	311.6	22.5
Fire Site(Fire) SamplePeriod Veg Soil	516.6	515.5	-44.2	314.9	327.6	38.5
Fire Site(Fire) SamplePeriod Veg Roots	510.2	507.9	-51.8	300.3	310.5	21.4
Fire Site (Fire) SamplePeriod Soil Roots	499	498.6	-61.1	293.9	305.6	16.5
Fire Site(Fire) SamplePeriod Veg Soil Roots	486.1	490.8	-68.9	298.2	313.6	24.5
Predictive models:						
Veg	532.7	531.2	-28.5	294.8	299.1	10
Soil	552.9	550.9	-8.8	311.6	315.9	26.8
Roots	532.8	532.2	-27.5	195.4	198.1	-91
Veg Soil	522.2	521.9	-37.8	313.6	321.9	32.8
Veg Roots	515.4	515.3	-44.4	285.2	291.4	2.3
Soil Roots	534.9	534	-25.7	292.2	299.2	10.1
Veg Soil Roots	512.1	513.9	-45.8	300.4	311.3	22.2
Fire	540	538.5	-21.2	288.9	290.2	1.1
Fire Veg	532.9	531.2	-28.5	295.3	300.5	11.4
Fire Soil	522.3	521.2	-38.5	311.9	316.9	27.8
Fire Roots	520.4	519.2	-40.5	273.7	277.3	-11.8
Fire Veg Soil	518.7	519.1	-40.6	314.6	323.9	34.8
Fire Veg Roots	516.2	515.6	-44.1	285.9	293	3.9
Fire Soil Roots	516	515.6	-44.1	292.4	300.3	11.2
Fire Veg Soil Roots	510.1	512.4	-47.3	299.3	311.2	22.1

Table 2-6: Effect size summary for best fit model of total RFI abundance. Effect size and standard error estimates are natural log transformed and cumulative. Estimates of effects can be used to calculate an expected population density for any given plot. Expected insect count for a given plot (Estimate and Std. Err. of count) = $e^{(\text{intercept} + \text{categorical effect } 1\dots n + [\text{continuous effect } 1\dots n * \text{plot parameter value } 1\dots n])}$. Use values 0-100 (not 0-1) for continuous parameter values reported as percentages. Divide by 0.16 to translate to population density $\#_{\text{RFIs}}/\text{m}^2$.

GEE Fit Criteria: QIC = -1176.4; QIC _u = -1201.7						
Parameter (Source)	Estimate	Standard Error	95% C.I.		Z	P > Z
Intercept	1.5084	0.8485	-0.1546	3.1714	1.78	0.0754
Fire Treatment Effects						
Fire: UB (unburned)	0.1098	0.7979	-1.4541	1.6737	0.14	0.8905
Fire: B (burned)	0.0000	0.0000	0.0000	0.0000
Site Effects (nested in fire treatment)						
Site(Fire): Site 1 (UB)	0.0000	0.0000	0.0000	0.0000
Site(Fire): Site 2 (UB)	0.3445	0.2051	-0.5750	0.7465	1.68	0.0930
Site(Fire): Site 3 (UB)	1.2267	0.7637	-0.2700	2.7235	1.61	0.1082
Site(Fire): Site 4 (B)	0.3266	0.2819	-0.2259	0.8790	1.16	0.2466
Site(Fire): Site 5 (B)	0.0000	0.0000	0.0000	0.0000
Site(Fire): Site 6 (B)	0.4272	0.3273	-0.2142	1.0687	1.31	0.1918
Sampling Period Effects						
Sampling Period: Fall 2008	-0.1484	0.2106	-0.5612	0.2644	-0.70	0.4811
Sampling Period: Summer 2009	0.6727	0.2207	0.2402	1.1053	3.05	0.0023
Sampling Period: Fall 2009	0.7816	0.1917	0.4058	1.1574	4.08	< 0.0001
Sampling Period: Spring 2010	0.8970	0.1567	0.5899	1.2040	5.73	< 0.0001
Sampling Period: Summer 2010	0.0000	0.0000	0.0000	0.0000
Sampling Period: Fall 2010	0.7314	0.2237	0.2930	1.1698	3.27	0.0011
Aboveground Vegetation Effects						
Wiregrass (%)	-0.0108	0.0148	-0.0397	0.0181	-0.73	0.4630
Woody Shrubs/Hardwoods (%)	0.0119	0.0064	-0.0006	0.0245	1.86	0.0630
Herbaceous Forbs (%)	-0.0269	0.0131	-0.0525	-0.0013	-2.06	0.0392
Other Grasses (%)	-0.0531	0.0186	-0.0895	-0.0168	-2.86	0.0042
Surface O.M. (%)	-0.0139	0.0060	-0.0257	-0.0021	-2.32	0.0205
Soil Effects						
Soil NH ₄ (mg/kg)	0.0223	0.0204	-0.0176	0.0623	1.10	0.2735
Soil Organic Matter (%)	0.1264	0.4503	-0.7562	1.0090	0.28	0.7789
CEC (meq/100g)	-0.2806	0.1811	-0.6355	0.0744	-1.55	0.1213
Base Saturation (%)	-0.0010	0.0101	-0.0209	0.0188	-0.10	0.9175

Table 2-7: Effect size summary for predictive models of relative abundance of total root-feeding insects. Effect size and standard error estimates are natural log transformed and cumulative. Estimates of effects can be used to calculate an expected RFI population density for a particular location within mature longleaf pine-wiregrass stands in this study system. Expected insect count for a given plot (Estimate and Std. Err. of plot count) = $e^{(\text{intercept} + \text{categorical effect } 1\dots n + [\text{continuous effect } 1\dots n * \text{plot parameter value } 1\dots n])}$. Use values 0-100 (not 0-1) for continuous parameter values reported as percentages. Divide by 0.16 to translate to population density $\#_{\text{RFIs}}/\text{m}^2$.

GEE Fit Criteria: QIC = -868.9899; QIC _u = -882.9720						
Parameter (Source)	Estimate	Standard Error	95% C.I.		Z	P > Z
Intercept	2.3390	0.7903	0.8097	3.8683	3.00	0.0027
Aboveground Vegetation Effects						
Wiregrass (%)	-0.0071	0.0093	-0.0255	0.0112	-0.77	0.4441
Woody Shrubs/Hardwoods (%)	0.0018	0.0108	-0.0194	0.0230	0.16	0.8695
Herbaceous Forbs (%)	-0.0183	0.0095	-0.0370	0.0003	-1.92	0.0544
Other Grasses (%)	-0.0463	0.0128	-0.0714	-0.0212	-3.62	0.0003
Surface O.M. (%)	-0.0007	0.0063	-0.0130	0.0116	-0.11	0.9126
Root Standing Crop Effects						
Fine root biomass (g/m ²)	0.0583	0.1196	-0.1761	0.2928	0.49	0.6258
Fine root length (m/m ²)	-0.0001	0.0001	-0.0003	0.0001	-0.95	0.3437

Table 2-8: Two indices of spatial autocorrelation, Moran's I and Geary's c, were calculated for combined root-feeding insect relative abundances given GPS coordinate data. Calculations were performed in SAS using the VARIOGRAM procedure. Each sampling period was analyzed separately.

<u>Coefficient</u>	Observed	Z	P > Z
<i>Sampling Period</i>			
<u>Moran's I</u> (Expected = -0.0189; Std. Dev.=0.0639; Mean Obs. = -0.027)			
<i>Fall 2008</i>	0.014	0.510	0.610
<i>Summer 2009</i>	-0.048	-0.455	0.649
<i>Fall 2009</i>	-0.026	-0.111	0.115
<i>Spring 2010</i>	-0.015	0.063	0.950
<i>Summer 2010</i>	0.005	0.369	0.712
<i>Fall 2010</i>	-0.092	-1.140	0.255
<u>Geary's c</u> (Expected = 1.0000; Std. Dev. = 0.0871; Mean Obs. = 0.891)			
<i>Fall 2008</i>	0.777	-2.560	0.011
<i>Summer 2009</i>	1.004	0.050	0.961
<i>Fall 2009</i>	0.863	-1.575	0.115
<i>Spring 2010</i>	0.726	-3.141	0.002
<i>Summer 2010</i>	0.707	-3.365	0.001
<i>Fall 2010</i>	1.271	3.110	0.002

Table 2-9, a: Indices of dispersion based on relative abundance (counts) of combined root-feeding insect taxa within each site, reflecting within-site spatial aggregation. Values represent the geometric means of dispersion indices for the 6 time periods.

SITE	Mean	Min	Max	Range	Variance	Var:Mean	SD	CV	K	1/K
All UB	3.9	0	24.1	24.1	30.2	7.8	5.5	1.4	0.6	1.7
All B	2.1	0	10.5	10.5	6.6	3.2	2.6	1.2	1.1	0.9
UB Site 1	2.6	0	7.2	7.2	5.5	2.1	2.3	0.9	2.9	0.3
UB Site 2	3.5	0.4	10.0	9.6	10.3	2.9	3.2	0.9	2.1	0.5
UB Site 3	5.1	0	22.2	22.2	52.4	10.3	7.2	1.4	0.6	1.7
B Site 4	2.1	0.1	6.0	5.9	4.5	2.2	2.1	1.0	2.0	0.5
B Site 5	1.3	0	3.6	3.6	1.9	1.5	1.4	1.1	1.7	0.6
B Site 6	2.7	0	8.6	8.6	9.1	3.4	3.0	1.1	0.7	1.4

Table 2-9, b: Indices of temporal dispersion based on relative abundance (counts) of combined root-feeding insect taxa within each plot, across six time periods, indicating aggregation across time periods. Values represent the geometric means of dispersion indices for the 9 plots within each site.

SITE	Mean	Min	Max	Range	Variance	Var:Mean	SD	CV	K	1/K
All UB	3.1	0.3	7.8	7.5	7.8	2.6	2.8	0.9	1.8	0.6
All B	1.8	0.2	4.6	4.4	3.2	1.7	1.8	1.0	0.7	1.4
UB Site 1	2.6	0.3	6.8	6.5	5.8	2.2	2.4	0.9	1.6	0.6
UB Site 2	3.6	0.3	9.5	9.2	11.3	3.1	3.4	0.9	1.1	0.9
UB Site 3	3.0	0.2	7.3	7.1	7.2	2.4	2.7	0.9	3.3	0.3
B Site 4	1.9	0.1	4.9	4.8	3.4	1.8	1.8	1.0	0.7	1.4
B Site 5	1.5	0.2	3.4	3.2	1.9	1.2	1.4	0.9	0.4	2.5
B Site 6	2.2	0.2	6.0	5.8	5.3	2.4	2.3	1.0	1.3	0.8

FIGURES

Figure 2-1: Property boundaries of the Joseph W. Jones Ecological Research Center. Eastern boundary follows approximately 21 km of the Flint River. About 24 km of the Ichawaynochaway Creek flows through the property. The majority of the property is dominated by longleaf pine forest. The study area is delineated by an orange boundary. Approximate locations of unburned (UB) and burned (B) sites are shown as blue (UB) and red (B) star-shaped symbols.

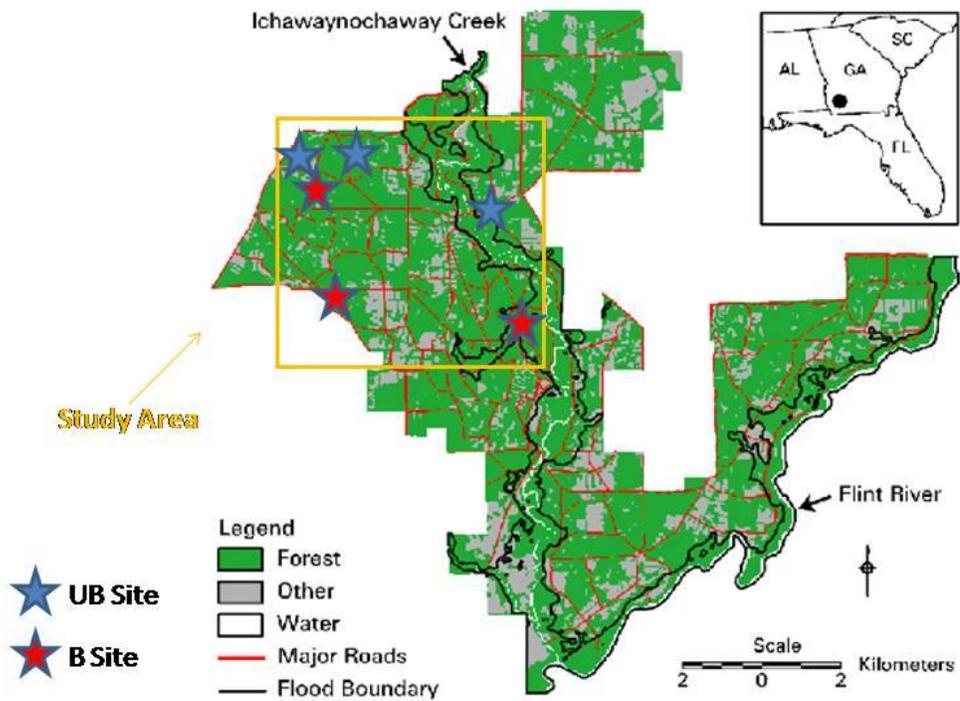


Figure 2-2: Conceptual diagram of sampling scheme across the study sites. Two burn treatments (burn exclusion and fire maintenance) were randomly applied to longleaf pine-wiregrass stands. Three replicates were randomly selected from these stands and within each stand, 9, 4 m x 4 m plots were established. The six sites were not arranged systematically as implied above (see Appendix A for actual coordinates of plots and study sites).

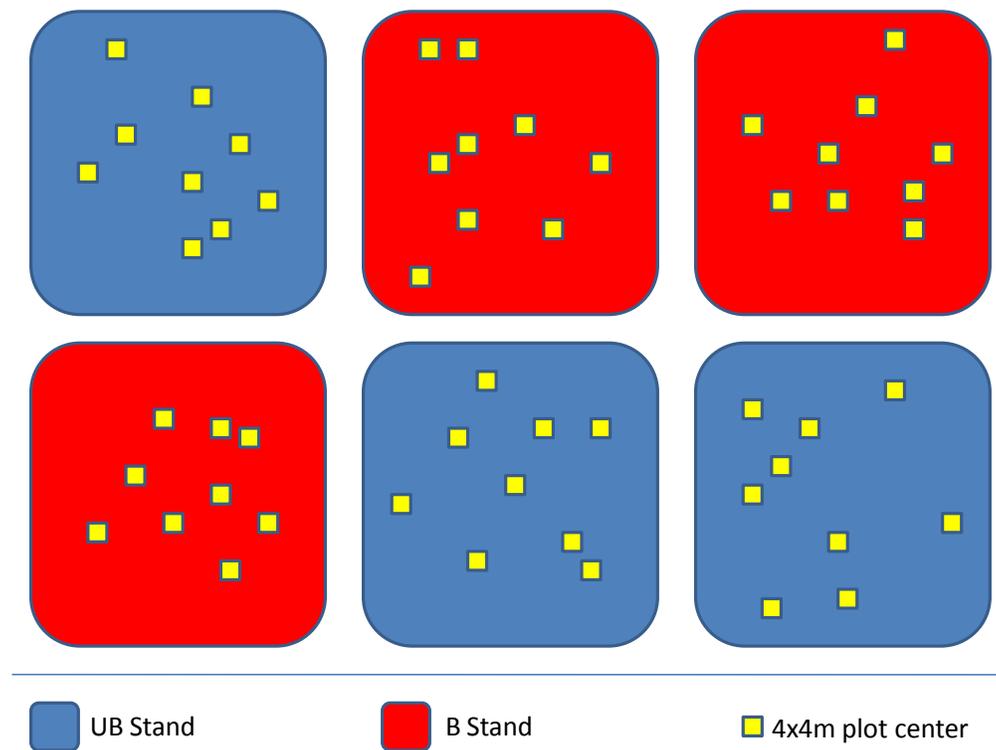


Figure 2-3: Conceptual diagram showing the layout of plots. Plot centers were subdivided into 4 quadrants. Small colored squares represent randomly located insect samples, with different colors denoting different sampling periods. Circles represent fine root standing crop samples, which were collected from peripheral plots (subdivided into halves). Symbols are not drawn to scale.

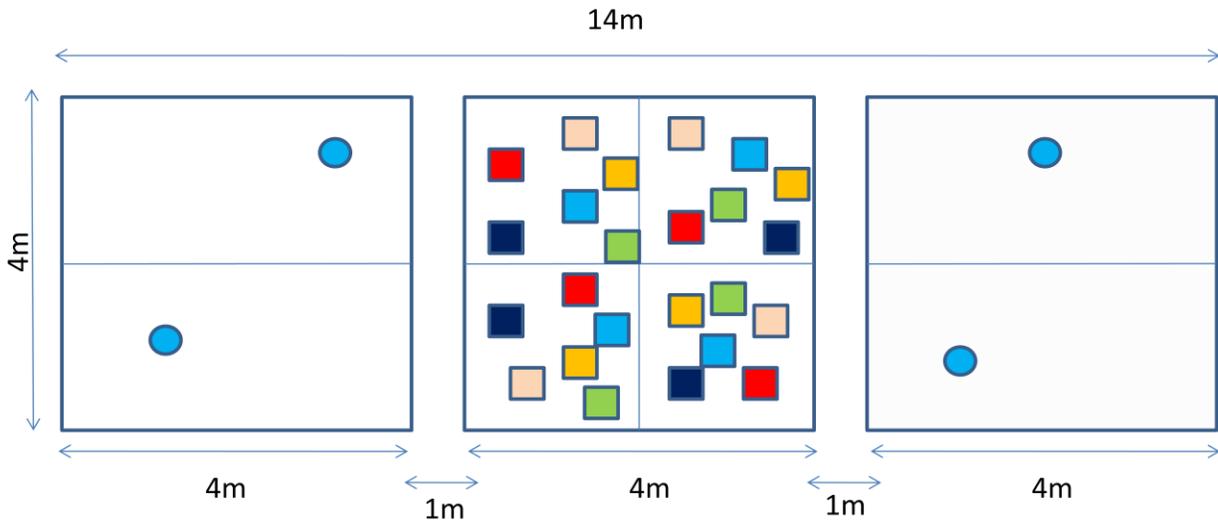


Figure 2-4: Root-feeding insect population density for 27 unburned (UB) and 27 burned (B) plots, sampled during 6 time periods (combined). Box plots of total root-feeding insect abundance (# of individuals/m²) depict median count frequency and the upper and lower quartiles (boxes). Upper and lower bars represent the range (excluding outliers). Outliers are shown as open circles. Results from Chi-squared (χ^2) tests suggested that the frequency distribution of counts differ between fire treatments: $\chi^2 = .4$; d.f. = 5; min. exp. freq. = 7.0; **p = 0.0130**; the six count bins used were 0, 1, 2 to 3, 4 to 5, 6 to 10, and > 10.

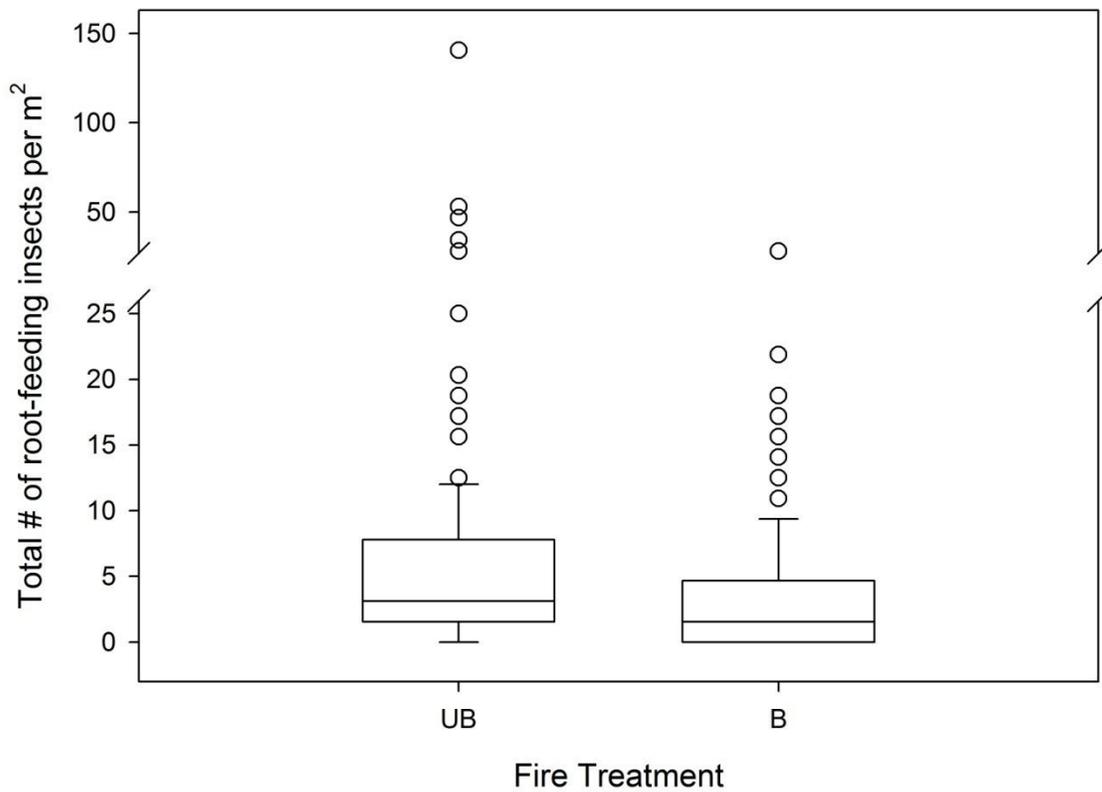


Figure 2-5: Root-feeding insect population density for the unburned (UB) and burned (B) treatment groups. Estimates of mean # of individuals per square meter for each taxonomic group were based on empirical estimates using GEEs as described in the Methods. Error bars represent the empirical estimates of standard error from the GEEs only including fire treatment in the model for each taxa and combined RFI abundance. Wireworms is abbreviated WWs; Ceps is short for Cebrionids. Probability statistics were calculated with GEEs. $P > |Z|$: Grubs = 0.83; WWs < 0.0001; Weevils = 0.14; Ceps = 0.2748; Cicadas = 1.00; Combined = 0.062.

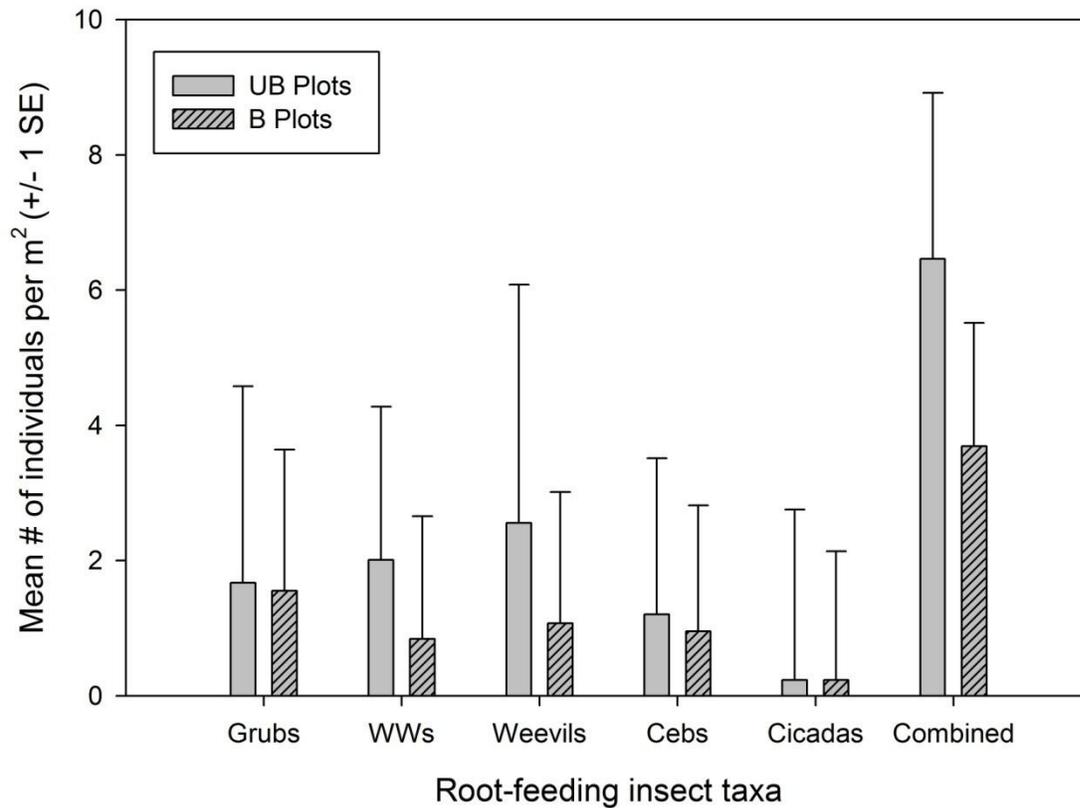


Figure 2-6: Total biomass summary (mass of insects per area sampled) for unburned (UB) and burned (B) treatments. Total biomass was only measured during 4 of 6 sampling periods: May 23 to June 13, 2009, October 8 to November 9, 2009, March 8 to April 2, 2010, and November 12 to 29, 2010. **(a):** Combined taxa; **(b):** by insect families; **(c):** by Scarab (white grub) genera; **(d):** by Elaterid (wireworm) genera.

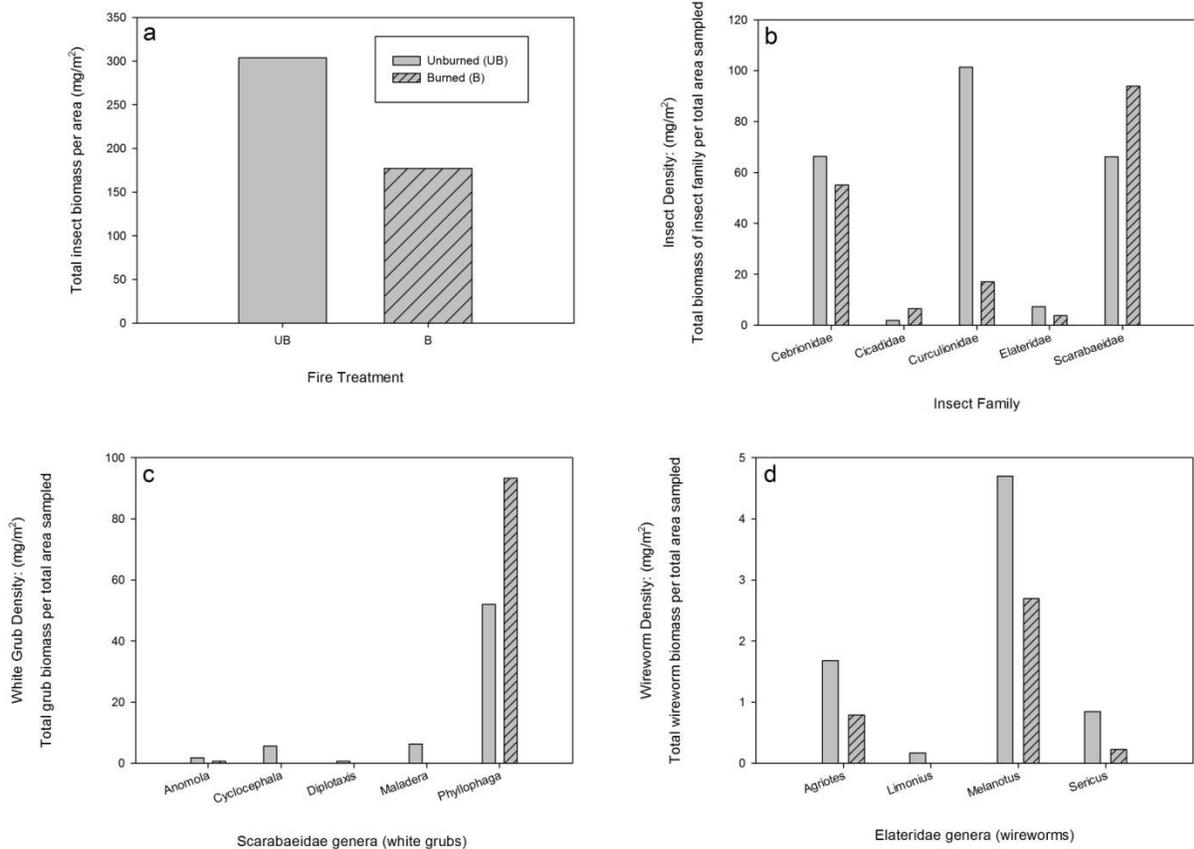


Figure 2-7: Root-feeding insect population density for the 3 unburned (UB) study sites and 3 burned (B) study sites. Estimates of mean # of individuals per square meter for each taxonomic group were based on empirical estimates using GEEs (models) as described in the Methods. Error bars represent the empirical estimates of standard error from the GEEs.

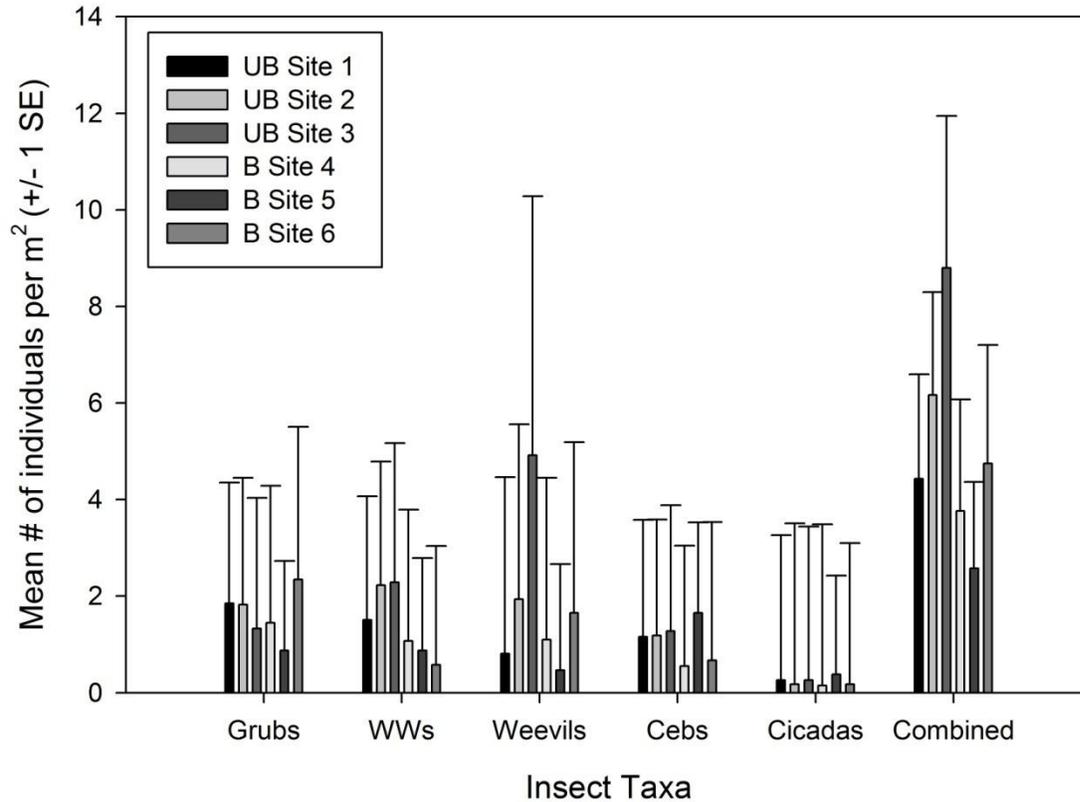


Figure 2-8: Root-feeding insect population density for the 6 sampling periods. Data for both fire treatments and all sites are combined for each time period. Estimates of mean # of individuals per square meter for each taxonomic group were based on empirical estimates using GEEs (models) as described in the Methods. Error bars represent the empirical estimates of standard error from the GEEs.

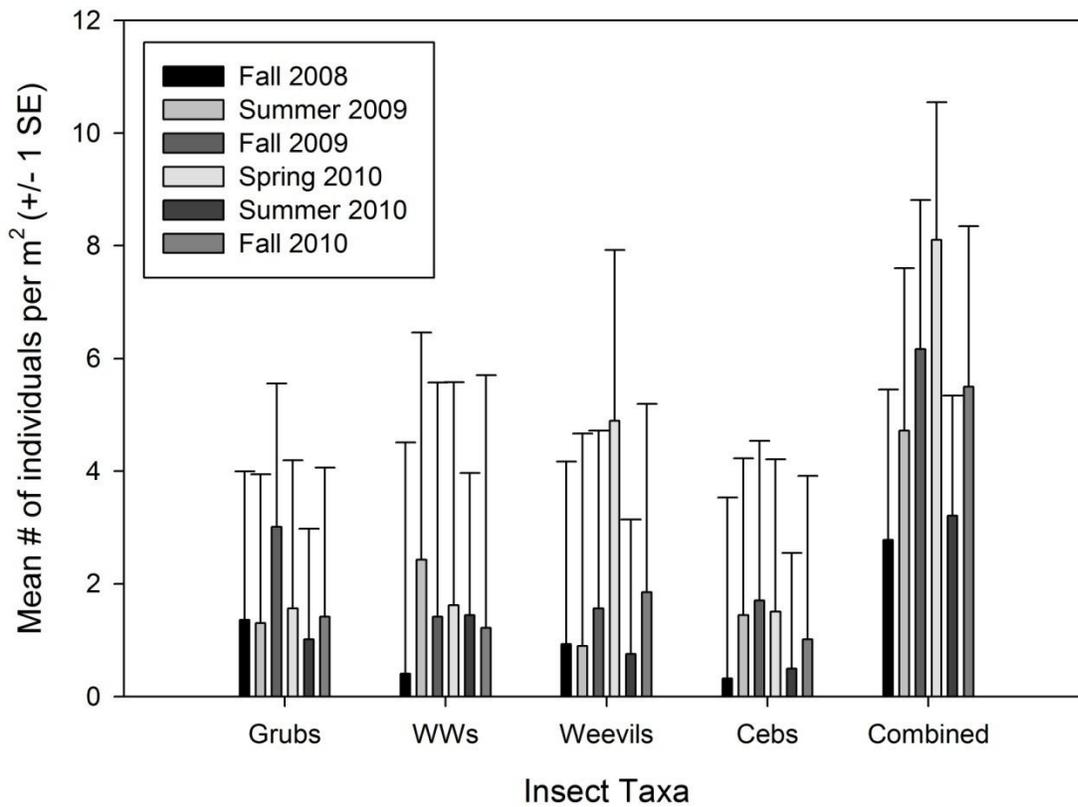


Figure 2-9: Combined root-feeding insect population density for each unburned (UB) or burned (B) study site at each of six time periods (site x time period interaction). Estimates of mean # of individuals per square meter were based on empirical estimates using GEEs (models) as described in the Methods. Error bars represent the empirical estimates of standard error from the GEEs. All taxonomic groups are combined.

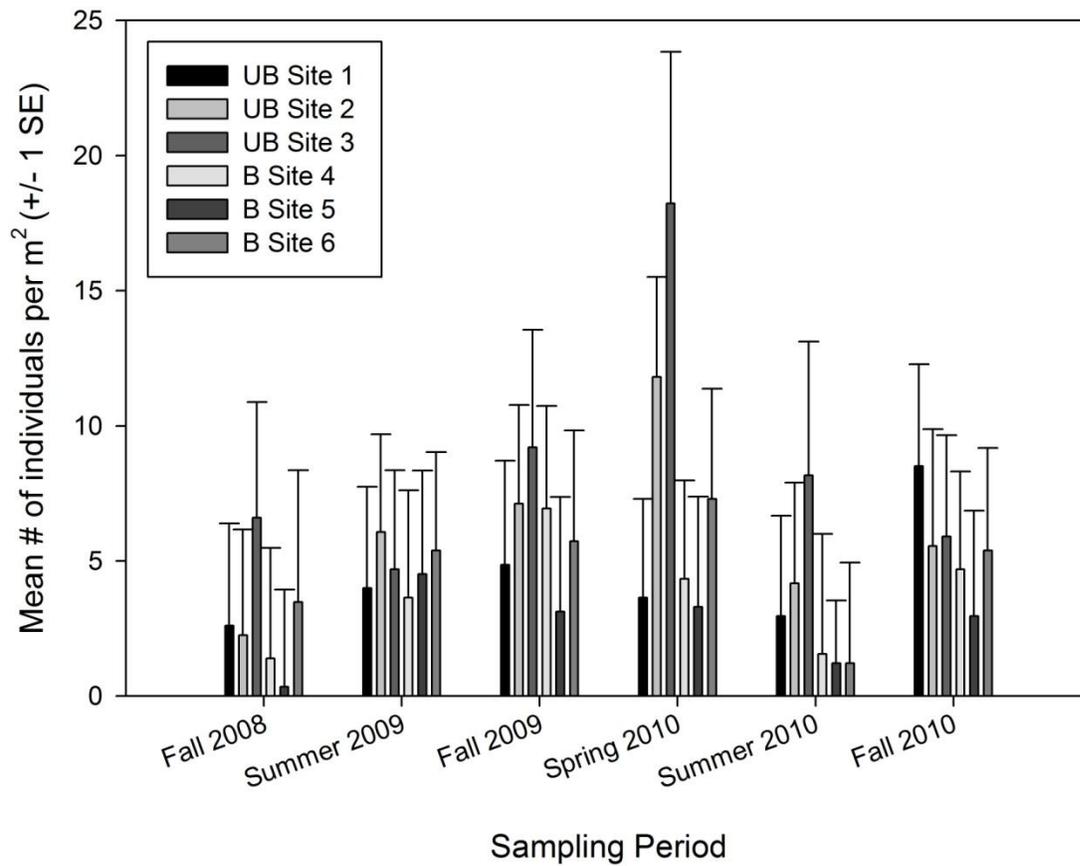
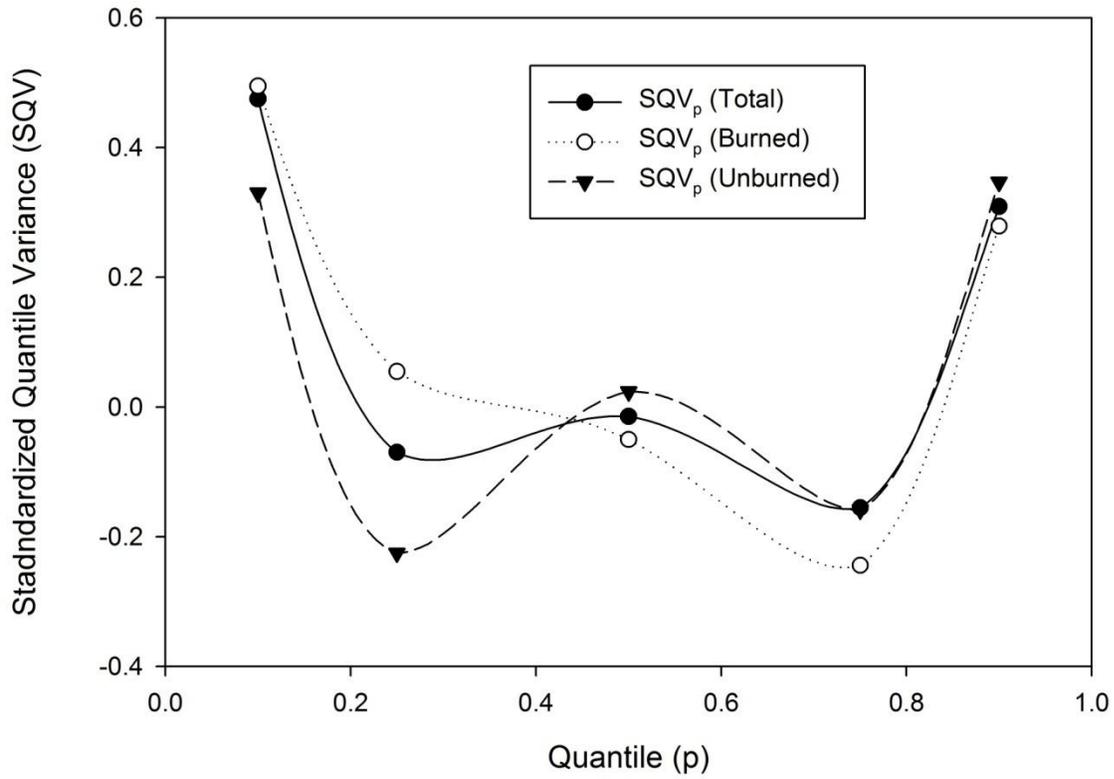


Figure 2-10: Standardized Quantile Variance (SQV) plotted against Quantile (p). SQV was calculated for each of the following quantiles: 0.1, 0.25, 0.5, 0.75, 0.9. SQV was plotted separately for B, UB, and combined plots (see symbol legend).

$$SQV_p = \log \frac{\left(\frac{\text{var}(\# \text{ times in quantile } p)}{\# \text{ time periods}} \right)}{p(1-p)}$$



CHAPTER 3

The Dietary Ecology of Root-Feeding Insect Assemblages in Longleaf Pine-Wiregrass Ecosystems

Matthew Dittler and Robert H. Jones

ABSTRACT

Root feeding insects cause fine root mortality, potentially altering vegetation and ecosystem dynamics. Most studies of root-feeding insects are geared towards pest control and conducted in agricultural settings. Very little is known about their foraging behavior or feeding preferences in forested ecosystems. In this study, stable isotope natural abundance ratios of carbon ($^{12/13}\text{C}$) and nitrogen ($^{14/15}\text{N}$) were used to examine the diets of several root feeding insect taxa across a gradient of fire-manipulated warm season (C_4) grass abundance (i.e. abundance of wiregrass, the dominant C_4 species) in a longleaf pine-wiregrass system. We wanted to know how the C_4 component of the diet of each insect taxa deviated from the proportional composition of C_4 plants. Wiregrass (C_4) dominance was manipulated by burning (B) on a 1 to 2 year interval in 3 study sites, and excluding burning (UB) for about 9 years prior, and throughout the duration of the study in 3 sites with a prior history of frequent burning. The exclusion of burning (UB sites) led to decreased wiregrass and increased woody plant abundance. We found that the diets of belowground insect herbivores were related to local scale vegetative composition. The C_4 component of combined insect diets was significantly lower ($p < 0.00001$) in UB sites, where the C_4 portion of fine root standing crops was also lower. Some insect taxa appeared to exhibit modest levels of dietary selectivity (i.e. some fed more on C_3 vegetation when C_3 plants were

low in abundance, or more on C₄ plants when they were low in abundance). Fine root biomass (g/m² to 20cm soil depth) did not influence the selectivity of herbivore diets across the range of values measured in the system. Nitrogen isotope natural abundance ratios indicated that some root-feeding insects may be omnivorous, possibly amending their diet with decaying forest litter, fungi, or scavenged arthropods. Overall, root-feeding insect diets were predominantly determined by the available composition of fine roots. Therefore, fire frequency may mediate herbivore diet and foraging patterns because it is a strong driver of vegetative composition in the longleaf pine-wiregrass system.

INTRODUCTION

Belowground insect herbivores have several important ecological roles. Through expression of dietary preference, they can potentially influence the structure and dynamics of vegetation (Brown and Gange, 1989; Brown and Gange, 1990), and they directly influence fine root life span, which can have ecosystem scale consequences (Eissenstat and Yanai, 2002). The majority of what is known about root-feeding insect ecology comes from agricultural systems, wherein they are known to lower yields on a variety of food and forage crops by consuming and damaging roots, sometimes killing plants. However, their distribution is not limited to agricultural systems (Andersen et al., 1987; Hunter, 2001). A limited literature suggests that root-feeding insects may have ecosystem-scale impacts in forests as well. For instance, they were linked to altered fine root dynamics in a southern pine forest (Stevens and Jones, 2006). Because of their potential significance to ecosystem functions like net primary production, carbon and nitrogen cycling, and food web dynamics, further study of their ecology in non-agricultural systems is warranted.

One important aspect of root-feeding insect ecology is diet. To better understand interactions among belowground insect herbivores, vegetation dynamics, and ecosystem function, it is necessary to understand the degree to which herbivores exhibit dietary specialization, and if they prefer particular species, or plants with particular characteristics (e.g. low C:N ratio). Herbivores that specialize on a small number of plant types should have very different outcomes for the plant community composition over time than herbivores that feed at random. Studies of aboveground systems show that selective herbivore diets can and do exert changes in vegetative composition (Schowalter, 2011). Presumably, this is the case belowground as well (Brown and Gange, 1989).

Though belowground herbivores are generally described as either specialists or generalists, it is unlikely that all fall to one extreme of the specialist-generalist continuum. Many of the most common and widespread genera of belowground insect herbivores (e.g. white grubs and wireworms) are considered generalists (Stehr, 1991), but it is not known if dietary choices are random or intentional. There are several reasons to suspect they are not random. For one, Root feeding insects are considered pests of cool season (C_3) grasses (Fermanian et al., 2002), and scientific studies of root feeding insects tend to focus on cool season grass species, suggesting a possible food source preference (e.g. Bughrara, 2008; Braman and Ramer, 2006). Additionally, plant tissue quality (e.g. C:N ratio, lignin and cellulose content, and herbivore-inducible defense responses) are variable among plants, and may influence digestibility, toxicity, and the overall dietary benefits incurred by belowground consumers (see Craine et al., 2003; Jonas and Joern, 2003; Thaler et al., 2002; Hunter and Schultz, 1995). Furthermore, root herbivores may use CO_2 concentrations, and other volatile emissions to detect food sources (Jones and Coaker, 1977; Rasmann et al., 2005), which could lead to preferences for particular

species, or for more productive or nutrient rich areas (Brown and Gange, 1990; Stevens, 2005; Stevens and Jones, 2006).

The dietary ecology of root-feeding insects may reflect tradeoffs. Optimal foraging theory (MacArthur and Pianka, 1966) suggests a tradeoff between the nutritional benefits incurred from selective foraging, and the energy or time spent searching for prey. The mobility of root-feeding insects is limited by the structure of the soil; however fine roots (prey) often occur at high densities, with the forage quality dependent on plant community composition. Therefore, root-feeding insect diet may be dynamic in relation to fine root composition and density.

The longleaf pine-wiregrass (*Pinus palustris* Mill.-*Aristida stricta* Mich.) ecosystem is ideal for investigating the dietary ecology of root-feeding insects. Longleaf pine-wiregrass woodlands are endemic to the southeastern portion of the United States and are well known for their dependence on fire regime to maintain high abundances of C₄ grasses and legumes. This natural association with fire provided a relatively easy way of experimentally altering C₄ versus C₃ dominance. Additionally, longleaf pine-wiregrass woodlands tend to occur on sandy soils, allowing soils and insect herbivores to be sampled with relative ease.

We examined the dietary preferences of root-feeding insects across a gradient of fire-manipulated understory vegetation structure (i.e. wiregrass dominance) using stable isotope natural abundance ratios (^{12/13}C and ^{14/15}N) from individual insects and fine root standing biomass samples. Stable isotope natural abundance ratios have been used across many types of ecosystems to determine food sources and feeding preferences of consumers (Fry, 2006). An important reason why this technique is useful, is that δ¹⁵N (isotope ratios, ^{14/15}N, relative to a standard) are usually about 3.4 permil (‰) greater in each higher trophic level, while the isotope

ratio for $^{12/13}\text{C}$ (relative to a standard = $\delta^{13}\text{C}$) does not change due to trophic level (Fry, 2006). Therefore, since the natural abundance ratio of C_3 and C_4 plants differ considerably because of different rates of isotope fractionation during photosynthesis, it is possible to estimate the relative proportions of C_3 and C_4 derived carbon in herbivore diets.

White grubs (*Scarabaeidae*), wireworms (*Elateridae*), weevil larvae (*Curculionidae*), and cicada nymphs (*Cicadidae*), are geographically widespread and are often found in abundance. These were the dominant root-feeding insects sampled in our study of the longleaf pine-wiregrass ecosystem (see Chapter 2), and were the focus of this study. We also frequently encountered larvae of family Cibrionidae, the ecology of which is described as unknown in the literature (Stehr, 1991). These were included in the study because they belong to the same superfamily as wireworms; therefore, we were interested to know if Cibrionid larvae also consumed fine roots.

The insects included in the study spend the majority of their lives as root-feeding larvae, potentially consuming significant fine root biomass; yet, very little is known about the specific diets of most taxa outside of the agricultural systems in which they are considered pests. Most of the sampled taxa had mouthparts consistent with root chewing, except for Cicada nymphs, which are described as xylem feeding herbivores (White and Strehl, 1978). Scarabaeidae larvae (white grubs) are considered to be generalist root feeders (Stehr, 1991), and are most notorious for damaging sod and turf grass, suggesting a possible feeding preference. Wireworms are also described as generalist root feeders; however, omnivorous and carnivorous genera also exist (Stehr, 1991). Root-feeding wireworms are thought to go through intermittent phases of intense feeding during larval development (approximately 25% of development time) during which times they can cause significant damage to crops (Fermanian et al., 2002; Furlan, 2004). Weevil

larvae are regarded as destructive agricultural pests, causing significant above- and below-ground agricultural damage every year. Most weevils are considered strict herbivores, and the adults and some larvae may specialize on a particular species or type of food. Some weevil species are known root feeders as larvae; other species feed on aboveground vegetation prior to entering the soil, though these tend to pupate soon after entering (May, 1966). Very little is known about the larvae of Cebrionidae, other than that they dwell in the soil (Stehr, 1991). For more information on the natural history of these herbivores, see Chapter 1.

The overall purpose of the study was to evaluate the extent of dietary selectivity and foraging specificity exhibited by the insect herbivores sampled in the longleaf pine-wiregrass system, and to what extent these traits varied with taxonomic identity. We proposed two alternative hypotheses that might explain observations of either random or selective foraging for polyphagous root-feeding insect larvae, based on the assumption that the mobility of larvae is constrained by the soil. **H₁**, *Random foraging*: Because soil limits the mobility of root-feeding insects, search time outweighs the benefits of selective foraging, resulting in random foraging behavior. Therefore, composition of insect diets will not deviate significantly from the composition of the existing vegetation, and will closely track the influence of fire on vegetative composition. **H₂**, *Facultative selective foraging*: Because fine roots occur at high density, a diversity of fine roots may be accessible with very little mobility required. Therefore, root herbivores could realize nutritional gains associated with dietary selectivity by varying the time spent feeding on individual plants. As a result, a bias toward favored foods would be apparent, despite manipulation of vegetative composition (i.e., fire-altered vegetative composition does not alter diet). We tested our random and selective feeding predictions by evaluating the relationship between insect diets (taxa-specific) and local-scale fine root composition in response to fire

treatment (B or UB). The high C:N ratio of wiregrass fine roots (dominant C₄ species in the system) relative to most other measured C₃ plants (Appendices E, F; also see Jansen, 2007 and Guo et al., 2004) was of particular interest, because the C₃ and C₄ contributions to the diet can be easily distinguished, and because C:N ratio may negatively relate to tissue quality. Furthermore, wiregrass has been considered a low quality forage for cattle except early in spring and during the period of new growth following fire (see Lewis et al., 1975). We therefore predicted that if non-random feeding occurred (**H**₂), then there would be a bias against wiregrass. Additionally, we tested whether fine root standing biomass (a proxy of prey density) related positively to foraging selectivity, as we predicted in consideration of optimal foraging theory (MacArthur and Pianka, 1966).

METHODS

Study System

This study took place at the Joseph W. Jones Ecological Research Center (Ichauway), located near the town of Newton, in Baker County, Georgia USA (31° N, 84° W), and utilized research sites consisting of mature longleaf forests in the northwestern portion of Ichauway property. Ichauway comprises about 12,000 hectares of property (approximately 7,500 forested hectares) surrounding the confluence of Ichawaynochaway Creek and the Flint River (see Chapter 2, Figure 2-1). The climate of the region is humid subtropical and the precipitation averages 132 cm/year, with average daily temperatures ranging from 5°-17°C in winter to 21°-34°C in summer (Goebel et al. 1997, cited in Hendricks et al. 2005). For further information about the study location, see Hendricks et al. (2006), Mitchell et al. (1999), and Drew et al. (1998).

Overstory vegetation at the research sites consisted primarily of 60-90 year old (Mitchell et al., 1999), second growth *Pinus palustris* Miller (longleaf pine). Understory composition was dominated by the C₄ grass *Aristida stricta* Michaux (wiregrass), and to a lesser extent, also contained regenerating longleaf pine, shrubs, hardwood seedlings and small trees, C₃forbs, legumes, and other predominantly C₄ grasses (see Chapter 2 for estimates of percent cover for these categories of vegetation). Fire maintenance is a regular part of the forest management at Ichauway. Most existing stands are burned on a 1-5 year interval (Hendricks et al., 2005). Periodic fires promote increased dominance of wiregrass and fire-adapted legumes, but fire-sensitive vegetation rapidly encroaches, and surface organic matter accumulates during fire exclusion.

Soils at the study sites were sandy; primarily, Typic Quartzipsamments and Grossarenick Paleudults, lacking developed organic horizons, and exhibiting minimal vertical stratification of the mineral soil (Hendricks et al. 2006). These soil properties allowed efficient sampling of root feeding insects and fine roots, and reduced the potential for problems with sample contamination by fine soil particulates and organic matter, which may cling to the roots during washing. See Chapter 2 for specific data on soil properties within the six sites used for this study.

Study Species

Any genus of larvae belonging to family Scarabaeidae or Elateridae (white grubs and wireworms, respectively) for which at least 4 individuals were found, was included in the analysis (Table 3-1). For Elateridae, three genera were included: *Agriotes*, *Melanotus*, and *Sericus*; for Scarabaeidae, *Anomala*, *Cyclocephala*, *Diplotaxis*, *Maladera*, and *Phyllophaga* were included. Three additional families, Cicadidae (cicadas), Curculionidae (weevils), and Cebrionidae, were also included, because they were encountered commonly during sampling,

and were considered to be potential root feeders. The larvae of weevils were addressed broadly in this study, as family Curculionidae, because they are notoriously difficult to identify (Stehr, 1991), but weevil relative abundance was dominated by a single species, which was believed to be root-feeding based on external morphology and persistence in the soil across sampling periods. Cicadas and Cibrionids were also only identified to the family level.

Experimental Design

Several stands were selected to remain unburned (UB) prior to initiation of this experiment, to study how fire and N control vegetative composition, productivity, and nutrient cycling. A final burning in the fall of 2000 initiated the burn exclusion, which continued past 2012 (beyond the duration of the experiment). Four of these UB stands were considered for use in the experiment because they were being used as controls in an ongoing burn-exclusion experiment (i.e. not receiving irrigation or fertilization treatments). Six frequently burned stands (B) were considered for the experiment prior to stand selection, because each was burned at least every other year, and exhibited dominance of mature longleaf pine in the overstory and dominance of wiregrass in the understory. These stands were numbered and 3 each of B and UB sites (six sites total) were randomly selected. Two of the selected B stands were burned on a 2-year cycle (fall), while one was burned annually (summer). The selected UB stands remained unburned from Fall of 2000 through the completion of the experiment in 2010. Both stand types (B and UB) contained wiregrass in the understory at the initiation of the study; however, B stands were visibly higher in percent cover of wiregrass (see Chapter 2), as is characteristic in this ecosystem when burning occurs regularly.

In each of the 6 selected sites, nine rectangular plots (4 m x 14 m) were established by pacing along randomly drawn compass vectors. Plots were oriented with a compass to the

cardinal directions, with the long dimension of the plot either running North to South, or East to West, depending on a coin toss. Pin flags were used to mark the corners of the plot and to delineate three adjacent 4 m x 4 m areas (separated by 1 m boundaries): one in the center, where insects would be sampled, and one on each end for sampling root standing biomass.

Insects were sampled from plots on four occasions: May 23 to June 13, 2009 (Summer 2009), October 8 to November 9, 2009 (Fall 2009), March 8 to April 2, 2010 (Spring 2010), and November 12 to November 29, 2010 (Fall 2010). Fine root standing biomass was sampled on one occasion during Summer 2009. During Fall 2010, soil-dwelling isopods were collected for comparison with soil dwelling insects from three plots. During Summer 2009 and Fall 2010, fine roots from wiregrass, longleaf pine, legume species, and other C₃ forbs were collected to define isotopic ratios for individual species belonging to different plant functional groups. This provided context for interpreting the isotope ratios of root standing crops and the relative contributions of C₃, C₄, and N-fixing legume species to insect diets (end-points). A full list of end-point species and their corresponding delta values are provided in Appendix E.

Not all larvae and root samples were included in the isotope analysis. Roots were excluded if no larvae were collected from the same plot. In total, 42 plots were used in the analysis (22 from UB and 20 from B plots). To reduce costs of analysis but maintain a diversity of insect larvae and good representation across treatments, a maximum of two larvae from any one taxon in any one plot were drawn blindly for the isotope analysis. Therefore, the work presented in this chapter was not intended to describe patterns in insect relative abundance or density (rather, see Chapter 2). Table 3-1 provides a quantitative summary of the number of individuals from each insect taxa that were used in the study, and the number of sample plots from which each taxa was collected (tallied both by burn treatment and sampling period).

Sampling and Processing of Root-Feeding Insect Larvae

Insects were collected from the plot centers (4 m x 4 m), further sectioned into 4, 1 m x 1 m quadrants. During each of four sampling events, one 40 cm x 40 cm x 20 cm deep volume of soil was excavated from each quadrant (4 samples per plot). To determine sample locations, 6 numbered pin flags were unsystematically placed throughout each quadrant during each sampling event (sample overlap was not permitted), and one flag was selected randomly. A frame, used to consistently delineate the sample dimension during excavation, was centered on the selected pin flag prior to excavation. Insects and soil were excavated using a narrow, rounded shovel (sharpshooter) and transferred to a large plastic bin (to prevent escape of insects). Each soil increment was hand-scattered when added to the sieve surface to aid in spotting larvae before sieving, which could otherwise damage some of the larvae, making identification and washing more difficult. Soil was then dry-sieved (4 mm mesh) in manageable increments over a wheelbarrow. Any material that did not pass through the sieve was sorted by hand for any larvae. Larvae were identified in the field to the extent possible, and then stored on ice inside small, vented plastic jars until the end of each workday, at which time they were frozen. Insects were later thawed, and gently washed with distilled water to remove soil or other debris attached to their outer surface. Each was then re-examined in the lab under a compound microscope, and identified using Stehr (1991) and Ritcher (1966). White grubs and wireworms were identified to the genus level and other larvae were identified to the family level. Larvae were then placed in separate tin weighing dishes, dried at 60°C, and stored temporarily in separate, paper envelopes.

Sampling and Processing of Fine Roots

Fine root standing biomass was sampled from two 4 m x 4 m areas (located on opposing ends of the of the rectangular plot) that were each further subdivided in two halves. One sample

was taken per each 4 m x 2 m half (4 samples total per plot). Location of samples was determined in the same manner as for insect samples. Root standing biomass was sampled by first removing aboveground vegetation and forest floor litter to reveal the mineral soil around an area slightly larger than the core diameter. A 10 cm (interior diameter) coring pipe was then driven in (perpendicular to earth) to approximately 20 cm soil depth with a sledgehammer and then retracted with soil intact. Soil was then removed from the core and sieved in the field (approximately 3 mm mesh) to remove the bulk of soil. Remaining soil and roots were then stored in plastic zip bags and frozen at the end of each work day.

Each root sample was later thawed, rinsed from the sample bag onto a sieve (1 mm mesh), and washed free of soil and organic debris, by gently showering the sample with a garden hose and a nozzle with numerous small output holes, using low to moderate water pressure, overtop a 1 mm mesh sieve. A shallow water trap, located below the sieve, was used to observe, and attempt to recover, any roots that washed through the sieve (root loss did not appear to be a problem). After washing, each root sample was transferred to a paper blotter, folded to contain the roots, sealed inside a small plastic zip bag and frozen for future sorting.

Root samples were later transferred to a shallow plastic tray filled with distilled water to a depth of about 5 cm for sorting. Roots and any small gravels remaining on the blotter were rinsed into the shallow water bath with the aid of a squirt-bottle of distilled water. The bottom of the water bath was transparent and placed respectively over a white and a black surface to provide increased contrast for finding light and dark colored roots during sorting. Roots were freed from any remaining debris by swishing the roots in the water bath, and gently massaging the roots between the fingers and thumb to remove clinging soil particles (if necessary). They were then sorted into two diameter classes; roots < 1mm diameter were considered fine roots.

Roots were snipped with scissors as needed to allow all roots to be placed in the correct diameter class.

Fine roots (< 1mm dia.) from standing crop samples were dried in tin weighing dishes for at least 3 days at 65°C, and weighed to the nearest 0.01 g to estimate fine root standing crop (g dry mass/m² to the 20 cm depth). Fine roots were then ground in a Wiley mill (1 mm mesh) and further pulverized and mixed with a mortar and pestle. In between every sample, the Wiley mill was cleaned with compressed air, and the mortar and pestle were cleaned with methanol, using Kimwipes[®]. After pulverizing and mixing, subsamples (approximately 0.5 cm³) from each of the four root samples were composited to create one bulk sample per plot.

Stable Isotope Sample Preparation and Analysis

Previously stored samples of individual insects were processed one by one, by pulverizing and mixing each with a mortar and pestle. Each pulverized fine root and insect sample was weighed in a small tin capsule (approximately 5 mm x 9 mm) to fall within a target weight range (4.0 mg to 5.5 mg for fine root samples, 0.8 mg to 1.5 mg for insects), based on the expected concentration of N and C (%) of the sample and recommendations from the University of California, Davis, Stable Isotopes Facility (SIF). In cases where there was not enough sample material available to meet the target weight range, all of the available material was used. There were three instances in which SIF noted the final weight to be too low, based on total N and total C contained in the sample; therefore, these data were excluded from analysis. Final sample weight was recorded to the nearest 0.01 mg. Tin capsules were crimped to ensure that no sample material could escape. Each capsule was placed in a clean, 96-well plate, covered with a lid to prevent dust contamination. In between processing every sample, all tools and work surfaces used were cleaned with methanol (using Kimwipes[™]), to prevent cross-contamination. Samples

remained inside 96-well plates for shipping, with the lids taped tightly closed prior to transport to prevent sample mix-up. All fine root and insect samples were shipped to U. C. Davis SIF, for analysis of the stable isotope natural abundance ratios of $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$.

Isotope analysis was performed using a PDZ Europa ANCA-GSL elemental analyzer linked with a PDZ Europa 20-20 isotope ratio mass spectrometer (IRMS). Total C, total N, and C:N ratio, along with abundance ratios of $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ were measured simultaneously. Samples were combusted at 1,000°C inside a reactor packed with chromium oxide and silvered copper oxide. After combustion, oxides were removed in a reactor with reduced copper at 650°C. The helium carrier gas then passed through a water trap (with magnesium perchlorate) prior to reaching the GC column. Separation of N_2 and CO_2 occurred on a Carbosieve[®] GC column (65°C, 65 ml/min) prior to entering the IRMS.

Isotope data is presented using delta notation (δ), where samples (x) are compared to a standard (std), and deviation from the standard is reported as permil (‰). Delta values were calculated as $\delta (\text{‰}) = \left(\frac{R_x - R_{std}}{R_{std}} \right) \cdot 1000$, where R is the isotope ratio of interest ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$). SIF used the international standard, Vienna PeeDee Belemnite (V-PDB), for analysis of $\delta^{13}\text{C}$, and air for $\delta^{15}\text{N}$. SIF reports a long-term standard deviation of 0.2‰ for $\delta^{13}\text{C}$ and 0.3‰ for $\delta^{15}\text{N}$. The standard deviation for nylon references used in this assay was 0.07‰ for $\delta^{13}\text{C}$ and 0.05‰ for $\delta^{15}\text{N}$. As a quality control, duplicate samples were analyzed for 9 insects and 6 fine root standing crops. The average difference (\pm 1 standard deviation) between the $\delta^{13}\text{C}$ of duplicates was 0.28‰ \pm 0.21‰ for insects, and 0.38‰ \pm 0.30‰ for fine roots. The $\delta^{15}\text{N}$ of duplicates differed by 0.28‰ \pm 0.17‰ and 0.14‰ \pm 0.16‰, respectively.

Data Analysis

Statistical calculations and analyses were performed using Systat[®] software. The mean $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N ratios of fine roots from the two burn treatments (B, UB) were compared using two-sample t-tests (N = 20 B plot root samples and N = 22 UB plot root samples; separate variances assumed; Bonferroni-adjusted p-values reported). Overall differences in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N ratio of individual insects due to insect type (12 levels), and sampling time (4 levels) were evaluated. Because sample sizes and variability were uneven across sampling times and insect type, we used non-parametric Kruskal-Wallis tests to determine the significance of effects based on rank transformed data. Kolmogorov-Smirnov (K-S) tests, a non-parametric procedure that evaluates the probability of two samples being drawn from the same empirical distribution, were used separately in B and UB plots to make pair-wise comparisons of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of taxonomic groups, independent of the overall effects of fire.

Scatter plots showing sample means and bi-directional error bars for isotope natural abundance delta values, were created with Sigma Plot[®] graphics software. Similar plots showing the $\delta^{13}\text{C}$ values for insects (dependent variable) against the $\delta^{13}\text{C}$ values for root samples collected within the same plot (independent variable) were also created to illustrate insect diets in response to locally available vegetation. Diets (mean values +/- 95% confidence intervals) were compared with a 1:1 line ($\delta^{13}\text{C}$ fine roots: $\delta^{13}\text{C}$ insects), to visually assess dietary randomness for each taxa. The average differences in $\delta^{13}\text{C}$ of individual insects and local-scale fine roots (collected from the same plot as each insect) were used as a proxy to quantify dietary randomness. Positive values indicated consumption of more C₃ vegetation, and negative values indicated consumption of more C₄ vegetation than predicted by the composition of local fine root standing crops, while values approximating zero indicated a more random diet. Fractionation of

C during digestion is minimal relative to the difference between C₃ and C₄ derived C (Fry, 2006). Diets were considered to be approximately random if they differed by less than 1‰ from local (within plot) fine root δ¹³C (‰). If insect diet differed by at least 1‰, and this difference exceeded the 95% confidence interval calculated for δ¹³C of each insect taxa, then a preference for either C₃ or C₄ food sources was considered likely. A C₃ preference was indicated if δ¹³C of insects was less than that of fine roots within the same plot; likewise, a C₄ preference was indicated if δ¹³C of insects was greater than fine roots.

RESULTS

Comparison of Fine Roots from Burned and Unburned plots

Substantial differences in fine root composition between B and UB plots was evident because δ¹³C of fine root standing mass was higher in B plots than in UB plots (Figure 3-1), indicating an increased proportion of wiregrass fine roots in B plots. Summary statistics for δ¹³C, δ¹⁵N, and C:N ratio of fine roots from B and UB plots are provided in Table 3-2. Mean values for B and UB δ¹³C differed significantly by 3.75‰ (t = 5.23, d.f. = 49.77, p < 0.00001). Mean delta values for δ¹⁵N of B and UB fine roots differed by 0.029‰, which was not significant (t = 0.066, d.f. = 43.45, p = 1.00). Mean C:N ratios of fine roots differed by 17.5, which was significant (t = 4.87, d.f. = 31.33, p < 0.0001) and consistent with the decreased proportion of wiregrass, which has relatively high C:N ratio, in UB plots (Appendices E and F; also see Jansen, 2007, and Guo et al., 2004).

Comparison of Insects From Burned and Unburned Plots

Insect assemblages approximately tracked the δ¹³C (proportion of diet derived from C₄ sources) of the fine root standing biomass where they were found. For all taxa combined (Figure

3-2), insects differed by $-0.78 \pm 5.76\text{‰}$ (B) and $-0.61 \pm 5.39\text{‰}$ (UB), relative to fine roots, and average insect $\delta^{15}\text{N}$ was elevated by $4.82 \pm 4.05\text{‰}$ (B) and $3.77 \pm 4.50\text{‰}$ (UB) relative to fine root standing biomass (mean differences \pm an error estimate equal to the combined standard deviations of roots and insects). Mean C:N ratios for insects (6.10 ± 2.17) fell within the expected range for insects and were less variable than the C:N ratios of fine roots. Two-sample t-tests comparing all insects combined from B and UB plots (unequal variances assumed; Bonferroni adjusted p-values presented), were significant for $\delta^{13}\text{C}$ ($t = -10.94$, d.f. = 2.49, $p < 0.00001$) and $\delta^{15}\text{N}$ ($t = -3.54$, d.f. = 3.57, $p = 0.0014$), but non-significant for C:N ratio ($t = 1.08$, d.f. = 3.58, $p = 0.842$). Overall, the UB treatment represented a greater taxonomic diversity and a larger number of individual insects (see Chapter 2).

Dietary Variability ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) Among Insect Taxonomic Groups

The $\delta^{15}\text{N}$ of insects varied with taxonomic identity (Table 3-3), both within and among insect functional groupings (Figure 3-3). Fire treatment was generally not a significant determinant of insect $\delta^{15}\text{N}$; however, weevils were a notable exception, with significantly lower $\delta^{15}\text{N}$ in UB plots (Table 3-4, Figure 3-4). Also, within and among taxa differences were found for $\delta^{13}\text{C}$. For all taxa combined, and for those taxa found in both B and UB plots, lower mean $\delta^{13}\text{C}$ was observed in burn exclusion plots (Figure 3-4). Most taxa (except for cicadas and the grub *Anomala*), had a statistically lower $\delta^{13}\text{C}$ in the UB, compared with the B treatments (see results of Mann-Whitney tests in Table 3-4).

Comparison of Insects Across Sample Dates

Values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of insects were compared across sampling periods (Figure 3-5). The non-parametric, Kruskal-Wallis (KW) tests used to examine the effects of sample date (assuming a χ^2 distribution with 3 degrees of freedom) were significant for $\delta^{13}\text{C}$ (KW = 14.27, p

= 0.0026), but not for $\delta^{15}\text{N}$ (KW = 6.50, $p = 0.09$) or C:N ratio (KW = 5.53, $p = 0.137$). Results of the Kolmogorov-Smirnov tests of pair-wise effects of sample date on $\delta^{13}\text{C}$ revealed that Spring 2010 delta values were significantly lower than in Fall 2010 (max. difference = 0.291, $p = 0.0016$) and Summer 2009 (max difference = 0.206, $p = 0.044$). These differences reflect taxonomic compositional differences (see Table 3-1), and are not likely due to an effect on individual $\delta^{13}\text{C}$ values, since isotope natural abundances integrate the diet of each insect over its lifetime.

Effects of Local Scale Fine Root Composition and Standing Biomass on Insect Diets

Individual insects varied considerably in the extent to which each diet reflected the fine root composition within each plot. Deviation of individual insect diets from in-plot fine roots was most consistently biased towards or against C_4 vegetation, when wiregrass dominance was at the respective low and high ends of the compositional range represented by the experiment (Figure 3-6). The lines fit to both B and UB insects were similar to each other in slope, but both were less positive in slope than the 1:1 line representing a random diet (Figure 3-6).

Several insect taxa $\delta^{13}\text{C}$ tracked local fine root $\delta^{13}\text{C}$ (1:1 line) within one 95% confidence interval for both B and UB treatments (Figure 3-7). Estimates of dietary randomness for several taxa differed by less than one confidence interval from zero (Table 3-5). Exceptions were noted for family Curculionidae (weevils), and the white grub genera *Phyllophaga* and *Diploptaxis*, which exhibited evidence of a C_3 preference, and for the wireworm genus *Sericus*, and family Cibrionidae, which exhibited a C_4 preference (Table 3-5).

There was no statistically detectable relationship between the permil deviation of insects from in-plot fine roots (dietary randomness) and the fine root standing crop estimate (g/m^2 to 20 cm depth) for each plot (Figure 3-8).

DISCUSSION

Generalist Diets of Root-Feeding Insect Assemblages

Assemblages of root-feeding insects functioned as generalist belowground herbivores and all of the root-feeding insects that were studied consumed a mixture of both C₃ and C₄ derived carbon. Root feeding insect assemblages were predominantly Coleopterans -- an insect order that contains many species regarded as generalists. Herbivore taxa present in both B and UB plots usually consumed proportionally less C₄ grass in plots that had less C₄ grass; i.e., where burning was excluded (UB), (Figures 3-3, 3-5). This result suggested that most taxa do not have specialized diets or intentionally seek out a particular proportion of grass roots in their diet; otherwise, the proportion of C₄ roots ($\delta^{13}\text{C}$) in their diet should have been buffered against change in response to fire-altered vegetative composition. Instead, the $\delta^{13}\text{C}$ of insects approximately tracked the $\delta^{13}\text{C}$ of root standing crops, which indicated that RFI diets were not specialized.

Evidence for Preferential Foraging in Some Taxa

When we examined the relationship of individual insect diets relative to the local vegetative composition in the plots (i.e., at a finer spatial scale as opposed to combining data by burn treatment), we detected a possible preference of insects for C₄ fine roots where they were proportionately low in abundance, and for C₃ fine roots when C₄ fine roots were high in abundance (Figure 3-6). This result may suggest that some root-feeding insects exhibit dietary selectivity, possibly as a compensatory response to the low abundance of preferred foods, or as a mechanism by which food source heterogeneity is maintained. Perhaps insects migrate through the soil randomly, but stop to forage for longer times on favored species, or roots that offer a unique or important nutritional contribution. This would promote a more variable diet relative to

the existing composition of vegetation. In this way, diets would be predicted to vary with the composition of roots available, but the observed deviation from the predicted random diet could be explained by increased time spent at plants encountered less frequently -- a dietary strategy that could be considered generalist, but non-random. In our discussion we will refer to this strategy as facultative, selective foraging. Generalist root-feeding insects might regulate their nutrition by such a mechanism, while still operating within our assumption that soil limits mobility, and therefore may constrain the benefits of dietary selectiveness because of increased travel and search time.

The observed pattern of eating preferentially from rarer root types could have been exaggerated because of a sampling artifact. The isotope ratio (reported in delta notation) integrates diet over the lifespan of each insect, during which time it may have fed completely at random but sampled different roots than those represented by the fine root standing biomass. The deviation of insects from the line of prediction may not be due to non-random feeding, but by failure of fine root samples to adequately represent the actual vegetation encountered by the insect during its lifetime. Also, since some taxa appeared to be omnivorous, the carbon signature of heterotrophic prey items could bias estimation of the proportionate consumption of C₄ grasses. Nevertheless, possible increase in error estimates associated with these shortcomings do not explain why individual diets fell consistently above the 1:1 line where C₄ dominance was lowest, and below it where C₄ grass was the most dominant, rather than being distributed on both sides of the 1:1 line (Figure 3-6). This pattern provides support for our interpretation that insects may be acting as facultative selective foragers in order to maintain a mixture of food sources.

A few insect taxa showed evidence of modest dietary preferences (refer to Table 3-5 and Figure 3-7 for remainder of this section); however, no taxa specialized on only C₃ or C₄ species,

because $\delta^{13}\text{C}$ of insects fell between the C_3 and C_4 fine root endpoints (Figure 3-4). Among the white grubs, *Phyllophaga* appeared to exhibit selectivity for C_3 fine roots in the frequently burned plots (B) where the overall proportion of C_3 roots was lower, but not to the same extent in UB plots. Grubs of the genera *Diploptaxis* and *Cyclocephala* were discovered only in the UB plots, so fire treatment comparisons could not be made. *Diploptaxis* species appeared to prefer C_3 roots, and *Cyclocephala* exhibited weak evidence for a C_4 preference; however, sample sizes for both of these genera were considerably limited. *Maladera* larvae tracked the $\delta^{13}\text{C}$ of local roots (implying random feeding); however, these grubs were only found in burn exclusion plots with relatively low wiregrass cover, which may have implied vegetative discrimination by adult females during oviposition, or poor survival of larvae in grass-dominated areas. Grubs of the genus *Anomala* appeared to feed randomly. Two out of four wireworm genera (*Melanotus* and *Limonius*) consumed a representative composition of in-plot fine roots, but *Agriotes* and *Sericus* exhibited a preference for C_4 grass roots. Weevils exhibited a preference for C_3 plants in both fire treatments. Cicadas exhibited a preference for C_3 roots, but low sample size and the high variability of cicada $\delta^{13}\text{C}$ reduces certainty. Larvae of Cebriionidae exhibited differential selectivity in the two burn treatments. They were relatively non-selective in frequently burned plots (B), but in UB plots, where wiregrass abundance was lower, they exhibited selectivity for C_4 plants, which were proportionately less abundant in UB plots. The differential responses of some taxa to local-scale vegetation among B and UB treatment groups implied that the composition of available fine roots may have influenced diet in some genera.

We predicted that the density or availability of fine roots would influence the length of time spent searching, and therefore influence the extent to which individual herbivores would

exhibit dietary selectivity. Contrary to this prediction, dietary selectivity was not related to fine root standing crop (Figure 3-8).

Dietary Variability Among Insect Taxa

Niche separation among some taxa was suggested by our data. Although there was overlap of food sources among taxa, some taxa appeared to have distinguishable dietary differences. When $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were plotted together, taxa separated in the X and Y dimensions (Figure 3-4). Separation along the X-axis ($\delta^{13}\text{C}$) suggested utilization of different proportions of C_3 and C_4 carbon sources, and separation along the Y-axis ($\delta^{15}\text{N}$) suggested variability in the proportions of diet derived from sources other than roots, such as soil or surface organic matter, or mycorrhizal fungi, or possibly from selective consumption of legumes.

Variability in the trophic aspects of diet among taxa was observed, even among genera within the same family (Figure 3-3). Some taxa appeared omnivorous. We observed levels of $\delta^{15}\text{N}$ above the approximately 3‰ increase that would be expected for herbivores, indicating the potential for omnivory among some of the observed taxa. Because we derived isotopic signatures from whole insects, this apparent trophic level elevation could be exaggerated by heterotrophic microbes in the insects' guts; however, undigested food in their guts may have biased $\delta^{15}\text{N}$ in the other direction. The majority of the taxa included in this study were classically considered herbivorous; therefore, if estimates of lifetime root consumption were to be based on the metabolic rate, density, and lifespan (or the lifetime energetic demand of these organisms), then consideration of the omnivorous aspects of their diets could be important. Larvae of the family Cebriionidae had the lowest proportion of their diet derived from roots (high $\delta^{15}\text{N}$), and therefore should be considered independently of the other root-feeding insects in this study. Our data suggested that they are most likely scavengers (similar $\delta^{15}\text{N}$ to isopods),

possibly consuming micro-arthropods or mycorrhizal fungi (see Hobbie et al., 2007). Cicadas appeared to attain their diet predominantly from roots (they were not omnivorous), which was expected given that their mouthparts are adapted for xylem feeding. However, it should be noted that they attained some of their diet from grass roots, which was unexpected, because in the literature they are usually associated with tree root systems (White and Strehl, 1978). The $\delta^{15}\text{N}$ of weevils was overall the lowest, especially in UB plots (Figure 3-3). A portion of their diet may come from partially decomposed forest floor litter, which was found above the uppermost layer of soil (more abundant in UB sites) because the $\delta^{13}\text{C}$ of this litter was lower than that of fine roots (Figures 3-3 and 3-4). It is also possible, that some of the weevils included in our analysis were feeding on aboveground vegetation prior to entering the soil; however, we do not think this was likely given their apparent persistence in the soil as larva (weevil pupa were not encountered during sampling).

The effect of fire treatment on insect $\delta^{15}\text{N}$ was likely an artifact caused by the trophic differences among taxa and the variable species composition between B and UB plots (i.e. more grubs and weevils, which had a lower $\delta^{15}\text{N}$ in UB plots). The lack of change in $\delta^{15}\text{N}$ between B and UB taxa also suggested that the trophic levels of specific taxa do not change significantly due to the changes associated with fire frequency (Table 3-5). Nevertheless, subtle differences were observed in the grub *Anomala*, and the wireworm *Melanotus*, and a significant exception was noted for weevils. It was unexpected that the observed $\delta^{15}\text{N}$ for weevils was lower than other herbivores, and even approaching the $\delta^{15}\text{N}$ values of fine roots (especially in UB plots). The weevils sampled from frequently burned plots (B) were closer to the range we expected for fine root herbivores. It is possible that the weevils fed on forest floor litter (partially decayed leaves, pine needles, and other organic debris) to a greater extent than did the weevils in the

frequently burned plots. The $\delta^{15}\text{N}$ values that we measured for partially decomposed forest floor litter (N=5) were approximately 4‰ lower than for root samples, and litter was considerably more abundant in UB plots (litter coverage differed by approximately 30% based on visual estimates; see Chapter 2).

Influence of Fire Treatment on N-Fixation and the Contribution of Legumes to Insect Diets

Fire significantly influenced $\delta^{13}\text{C}$ of fine roots, but not $\delta^{15}\text{N}$. We initially predicted that a noticeable shift in $\delta^{15}\text{N}$ might be observed in fine roots from B plots, due to the increased abundance of nitrogen-fixing plant species (legumes) that generally occurs with fire; however, $\delta^{15}\text{N}$ of fine root standing biomass samples did not differ significantly between burn treatments (B and UB), though there was variation in $\delta^{15}\text{N}$ among plots (Figure 3-1). Legume fine roots gathered for endpoints appeared to derive less than one third of their N from fixation, which may indicate that sufficiently high soil N availability reduced net N-fixation. Based on our limited data set of legumes collected for endpoints, the mean difference in $\delta^{15}\text{N}$ of legumes and non-legumes was 1.8‰ (though this was variable and sample size of endpoint roots was limited). Therefore, the proportion of legume biomass consumed by root-feeding insects may not be accurately indicated by $\delta^{15}\text{N}$. Estimation of the contribution of legumes to the diet of root-feeding insects was additionally problematic, because insect $\delta^{15}\text{N}$ is also influenced even by proportionately small dietary contributions from higher trophic levels.

Preferential foraging biased towards or against legumes could help to explain the variability of $\delta^{15}\text{N}$ measured among root-feeding insect taxa. Nevertheless, feeding at higher trophic levels was still probably the main reason that some taxa (particularly Cebriionidae) had relatively high $\delta^{15}\text{N}$. This was because $\delta^{15}\text{N}$ of most taxa was similar in both B and UB stands, despite the decreased proportion of the diet made up of C_3 vegetation in B stands. The $\delta^{13}\text{C}$ of

legumes in this system resembled that of other C₃ plants in the system; therefore, if the increased $\delta^{15}\text{N}$ of insects was due to an increase in the proportion of legumes in the diet, it would also have been detected as a C₃ preference, stronger in B stands. This pattern was only observed for one taxa (*Phyllophaga*, see Figure 3-7). The $\delta^{15}\text{N}$ for this taxa was within an expected range for herbivores, and the pattern of having a stronger C₃ feeding bias in B plots could also be explained by facultative, selective foraging. Therefore, the role of legumes in contributing to the variation in insect $\delta^{15}\text{N}$ remains unknown, but more of the variability of insect $\delta^{15}\text{N}$ was probably associated with omnivory than consumption of legumes.

Implications for Facultative Selective Foraging on Vegetation Dynamics

If facultative, selective feeding, as discussed above, is real, then there are implications for vegetation dynamics, as this may further disadvantage plants that are not abundant, possibly creating a positive feedback promoting the establishment of dominance for some plant species. At the generally low herbivore densities measured in this system, we do not expect that root-feeding insects were acting as drivers of vegetation dynamics (see Chapter 2); rather, the effects that low levels of herbivores exerted over vegetation structure were likely overwhelmed by the influence of fire frequency (see Chapter 4). However, in systems with densities of herbivores high enough to cause plant mortality and significant reductions in productivity, the extent to which they exercise selection while they forage would be an important component to be able to understand potential top-down effects on vegetation dynamics.

Recommendations for Future Studies

Knowledge of root-feeding insect foraging behavior and the types of roots that they eat, may help inform strategies that aim to control their abundances. Making direct observations of belowground behavior would greatly strengthen our current understanding of root-feeding insect

foraging, and allow testing of specific hypotheses about foraging behavior. For instance, we could test the mobility-limitation mechanism proposed in our hypothesis by asking how far and how often root-feeding insect taxa move, and whether movements appear directed towards specific targets when foraging. Making such observations is not easy without disturbing the soil, which would likely bias results. Technologies like acoustic imaging and NMRI (nuclear magnetic resonance imaging) are one way that such investigators may be able to make direct observations of belowground behavior. As collaborative efforts continue to span the gaps between scientific disciplines, perhaps such a study could be achieved.

Another approach that could be used to test hypotheses about non-random diets of root-feeding insects are behavioral choice experiments. Such experiments could utilize a series of planted gardens with the composition and proportionate abundances of particular plants manipulated systematically. RFIs would be added to the plot in a central (root-free) zone and then each section of the garden could be harvested separately to infer patterns of herbivore foraging behavior. Choice experiments could also utilize olfactometers, modified for the soil environment to test for selective foraging for particular plant types (e.g. Rasmann et al., 2005).

CONCLUSIONS

The diets of generalist root-feeding insect herbivores in the longleaf pine-wiregrass system were strongly influenced by the composition of local vegetation (their diets responded directly to altered fine root composition). However, some insects may have engaged in facultative selective foraging, generally biased towards fine roots of grasses (C_4) or towards C_3 plants whenever one plant type had relatively low abundance in the plant community. Some taxa differed in magnitude of dietary selectivity between burned (B) and unburned (UB) stands. Fine

root standing biomass (density of prey) did not influence the selectivity of herbivore diets. We also showed that many herbivores may be omnivorous, possibly amending their diet with decaying forest litter, fungi, or scavenged arthropods. Herbivore diets were predominantly determined by the available composition of fine roots, potentially dampening their net influence over plant community composition, especially in consideration of the strong influence of fire regime over vegetative composition in the longleaf pine-wiregrass system.

LITERATURE CITED

- Andersen, D.C. 1987. Below-ground herbivory in natural communities: a review emphasizing fossorial animals. *The Quarterly Review of Biology* **62**:261-286.
- Braman, S.K. and P.L. Raymer. 2006. Impact of Japanese Beetle (Coleoptera: Scarabaeidae) feeding on Seashore Paspalum. *Journal of Economic Entomology* **99**:1699-1704.
- Brown, V.K. and A.C. Gange. 1989. Differential effects of above- and below- ground insect herbivory during early plant succession. *Oikos* **54**:67-76.
- Brown, V.K. and A. C. Gange. 1990. Insect herbivory below ground. *Advances in Ecological Research* **20**:1-58.
- Bughrara, S.S., D.R. Smitley, D. Cappaert, and A.Krauchencko. 2008. Comparison of Tall Fescue (Cyperales: Gramineae) to Other Cool-Season Turfgrasses for Tolerance to European Chafer (Coleoptera: Scarabaeidae). *Journal of Economic Entomology* **96**: 1898-1904.
- Craine, J. W. Bond, W. G. Lee, P. B. Reich, and S. Ollinger. 2003. The resource economics of chemical and structural defenses across nitrogen supply gradients. *Oecologia* **137**:547-556.

- Drew, M.B., L.K. Kirkman, and A.K. Gholson, Jr. 1998. The vascular flora of Ichauway, Baker County, Georgia: A remnant longleaf pine/wiregrass ecosystem. *Castanea* **63**:1-24.
- Grelen, H.E. 1978. Forest grazing in the South. *Journal of Range Management* **31**:244-250.
- Eissenstat, D. M., and R. D. Yanai. 2002. Root Life Span, Efficiency, and Turnover. Pages 221-238 in Y. Waisel, A. Eshel, and U. Kafkafi, editors. *Plant Roots: The Hidden Half*. Marcel Dekker, Inc., New York.
- Fermanian, T.W., M.C. Shurtleff, R. Randell, H.T. Wilkinson, and P.L. Nixon. 2002. *Controlling Turfgrass Pests* 3rd Ed. Prentice-Hall, New York.
- Fry, B. 2006. *Stable Isotope Ecology*. Springer, New York.
- Furlan, L. 2004. The biology of *Agriotes sordidus* Illiger (Col., Elateridae). *JEN* **128**:696-706.
- Goebel, P.C., B.J. Palik, L.K. Kirkman, and L. West. 1997. *Field Guide: Landscape Ecosystem Types of Ichauway*. Technical Report Number 97-1. Joseph W. Jones Ecological Research Center at Ichauway, Newton, Georgia.
- Guo, D.L., R.J. Mitchell, and J.J. Hendricks. 2004. Fine root branch orders respond differentially to carbon source-sink manipulations in a longleaf pine forest. *Oecologia* **40**:457-457.
- Held, D.W. and C.H. Ray. 2009. Asiatic garden beetle *Maladera castanea* (Coleoptera: Scarabaeidae) grubs found in damaged turf in Alabama. *Florida Entomologist* **92**:670-672.
- Hendricks, J.J., R.L. Hendrick, C.A. Wilson, R.J. Mitchell, S.D. Pecot, and D. Guo. 2006. Assessing the patterns and controls of fine root dynamics: an empirical test and methodological review. *Journal of Ecology* **94**:40-57.
- Hobbie, E.A., P.T. Rygielwicz, M.G. Johnson, and A. R. Moldenke. 2007. ¹³C and ¹⁵N in microarthropods reveal little response of Douglas-fir ecosystems to climate change. *Global Change Biology* **13**: 1386-1397.

- Hunter, M. D. 2001. Out of sight, out of mind: the impacts of root-feeding insects in natural and managed systems. *Issues in Agricultural and Forest Entomology* **3**:3-9.
- Hunter, M. D., and J. C. Schultz. 1995. Fertilization mitigates chemical induction and herbivore responses within damaged oak trees. *Ecology* **76**:1226-1232.
- Jansen, N. A. 2007. Influence of root branching order on fine root substrate quality and decomposition in a *Pinus palustris* ecosystem. Master's Thesis: University of Florida.
- Jonas, J. L. and A. Joern. 2003. Host plant quality alters grass/forb consumption by a mixed-feeding insect herbivore, *Melanoplus bivittatus* (Orthoptera: Acrididae). *Ecological Entomology* **33**:546-554.
- Jones, O.T., and T.H. Coaker. 1977. Oriented responses of carrot fly larvae, *Psila rosae*, to plant odors, carbon dioxide and carrot root volatiles. *Physiological Entomology* **2**:189-197.
- Lewis, C.E., R.S. Lowrey, W.G. Monson, and F.E. Knox. 1975. Seasonal trends in nutrients and cattle digestibility of forage on pine-wiregrass range. *Journal of Animal Science* **41**:208-212.
- May, B.M. 1966. Identification of the immature forms of some common soil-dwelling weevils, with notes on their biology. *New Zealand Journal of Agricultural Research* **9**:286-316.
- Michener, W.K. and P.F. Houhoulis. Identification and Assessment of Natural Disturbances in Forested Ecosystems: The Role of GIS and Remote Sensing. Manuscript and Image accessed 3 January 2010, <http://www.ncgia.ucsb.edu/conf/SANTA_FE_CD-ROM/sf_papers/michener_william/michener.html>.
- MacArthur R.H. and E.R. Pianka. 1966. On optimal use of a patchy environment. *American Naturalist* **100**: 603-609.
- Mitchell, R.J., L.K. Kirkman, S.D. Pecot, C.A. Wilson, B.J. Palik, and L.R. Boring. 1999. Patterns and controls of ecosystem function in longleaf pine-wiregrass savannas. I. Aboveground net primary productivity. *Canadian Journal of Forest Research* **29**:743-751.

- Rasmann, S., T. G. Kollner, J. Degenhardt, I. Hiltbold, S. Toepfer, U. Kuhlmann, J. Gershenson, and T. C. J. Turlings. 2005. Recruitment of entomopathogenic nematodes by insect-damaged maize roots. *Nature* **434**:732-737.
- Ritcher, P.O. 1966. *White Grubs and Their Allies: A Study of North American Scarabaeoid Larvae*. Studies in Entomology Number Four. Oregon State University Press, Corvallis, Oregon.
- Schowalter, T.D. 2011. *Insect Ecology: An Ecosystem Approach*, 3rd ed. Academic Press, London, UK.
- Stehr, F.W. 1991. *Immature Insects* Vol. 2. Kendall-Hunt Publishing Co., Dubuque, Iowa.
- Stevens, G. N. 2005. Trophic dynamics in the fine-root based food web: integrating resource heterogeneity, root herbivores, and root foraging. Dissertation. Virginia Polytechnic Institute and State University, Blacksburg.
- Stevens, G. N., and R. H. Jones. 2006. Patterns in soil fertility and root herbivory interact to influence fine-root dynamics. *Ecology* **87**:616-624.
- Thaler, J. S., M. A. Farag, P. W. Pare, and M. Dicke. 2002. Jasmonate-deficient plants have reduced direct and indirect defenses against herbivores. *Ecology Letters* **5**:764-774.
- White, JoA. and C.E. Strehl. 1978. Xylem feeding by periodical cicada nymphs on tree roots. *Ecological Entomology* **3**: 323-327.

TABLES

Table 3-1: Number of individual insects from each taxonomic group included in the study. Numbers in parentheses indicate the number of plots from which the included individuals were collected.

Family / Genus	(B)	(UB)	Sum. 09	Fall 09	Spr. 10	Fall 10	Total
Cebrionidae	30 (12)	33 (13)	10 (8)	19 (14)	19 (15)	15 (13)	63 (25)
Cicadidae	8 (6)	3 (3)	0	6 (5)	3 (3)	2 (2)	11 (9)
Cucurlionidae	34 (14)	45 (13)	15 (9)	20 (10)	25 (15)	19 (15)	79 (27)
Elateridae (4 genera)	30 (13)	51 (21)	19 (14)	21 (15)	31 (24)	10 (7)	81 (34)
<i>Agriotes</i>	8 (7)	15 (9)	9 (8)	6 (5)	6 (5)	2 (1)	23 (16)
<i>Limonius</i>	1 (1)	4 (3)	0	1 (1)	4 (3)	0	5 (4)
<i>Melanotus</i>	19 (8)	24 (12)	10 (8)	13 (9)	12 (9)	8 (6)	43 (20)
<i>Sericus</i>	2 (2)	8 (5)	0	1 (1)	9 (6)	0	10 (7)
Scarabaeidae (5 genera)	41 (14)	76 (22)	28 (18)	38 (24)	25 (17)	26 (18)	117 (36)
<i>Anomala</i>	5 (3)	3 (2)	7 (4)	0	1 (1)	0	8 (5)
<i>Cyclocephala</i>	0	4 (2)	0	4 (2)	0	0	4 (2)
<i>Diplotaxis</i>	0	5 (3)	0	3 (1)	2 (2)	0	5 (3)
<i>Maladera</i>	0	30 (13)	9 (7)	4 (4)	8 (7)	9 (7)	30 (13)
<i>Phyllophaga</i>	36 (11)	34 (13)	12 (10)	27 (20)	14 (10)	17 (12)	70 (24)
All Combined	143 (21)	208 (27)	81 (41)	104 (33)	103 (31)	72 (35)	351 (48)

Table 3-2: Summary of minimum, maximum, and mean $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N ratio for fine root standing crops (<1mm), that were collected from random plots within the burned (B) and unburned (UB) treatments. Number of plots from which fine root standing crops were included is listed on the left (N), and three measures of variability, standard deviation (SD), standard error (SE) and coefficient of variation (CV), are provided on the right.

Fire Treatment	N	Min	Max	Mean	SD	SE	CV
$\delta^{13}\text{C}$							
Burned (B)	20	-25.45	-17.00	-21.25	2.30	0.51	-0.11
Unburned (UB)	22	-29.10	-19.31	-25.01	2.88	0.61	-0.12
$\delta^{15}\text{N}$							
Burned (B)	20	-5.25	-0.16	-2.40	1.78	0.39	-0.74
Unburned (UB)	22	-4.69	-0.28	-2.37	1.40	0.30	-0.59
C:N ratio							
Burned (B)	20	51.39	126.50	80.59	16.25	3.63	0.20
Unburned (UB)	22	46.66	75.00	63.02	7.53	1.61	0.12

Table 3-3: Summary of mean $\delta^{13}\text{C}$ +/- 1 standard deviation (SD), mean $\delta^{15}\text{N}$ +/- 1 SD, and mean C:N ratio +/- SD for each insect taxa included in the study. Insects from burned (B) and unburned (UB) plots, and across all sampling events, are combined.

Family	Genus	N	Mean $\delta^{13}\text{C}$	+/- SD	Mean $\delta^{15}\text{N}$	+/- SD	Mean C:N	+/- SD
Elateridae	<i>Agriotes</i>	25	-22.92	4.34	2.09	2.07	5.38	1.03
Elateridae	<i>Limonius</i>	5	-26.04	4.54	0.78	1.81	5.48	0.72
Elateridae	<i>Melanotus</i>	45	-22.25	2.98	3.15	2.34	4.67	0.79
Elateridae	<i>Sericus</i>	10	-25.10	2.02	2.62	1.67	4.91	0.46
Scarabaeidae	<i>Maladera</i>	31	-26.99	1.17	1.05	1.30	5.65	1.03
Scarabaeidae	<i>Phyllophaga</i>	72	-24.35	3.05	1.99	1.58	6.58	2.25
Scarabaeidae	<i>Anomala</i>	8	-21.91	3.20	1.93	2.16	5.75	0.88
Scarabaeidae	<i>Diplotaxis</i>	5	-25.02	0.49	2.44	1.82	4.83	0.08
Scarabaeidae	<i>Cyclocephala</i>	4	-19.64	4.16	2.62	1.61	7.40	1.46
Cebrionidae	unknown	65	-22.82	2.21	4.72	1.54	4.99	0.84
Cicadidae	unknown	11	-24.21	3.91	1.31	1.59	5.52	1.07
Curculionidae	unknown	79	-25.53	3.68	-1.16	2.90	8.06	2.79
<i>Isopod</i>	unknown	4	-19.43	3.66	5.65	1.10	5.13	0.64
<i>Centipede</i>	unknown	3	-23.38	0.67	3.79	0.99	4.19	0.37

Table 3-4: Summary of Mann-Whitney U tests for significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between burned (B) and unburned (UB) treatments for each insect taxa. All sampling periods are combined.

Insect Taxa	UB Count	B Count	UB Rank Sum	B Rank Sum	Mann-Whitney U	p-value
$\delta^{13}\text{C}$						
Cebrionidae	34	31	7.27E+02	1.42E+03	1.32E+02	<0.000001
Cicada	3	8	1.10E+01	5.50E+01	5.00E+00	0.153041
Weevils	45	34	1.39E+03	1.77E+03	3.55E+02	0.000049
Grubs						
<i>Anomola</i>	3	5	9.00E+00	2.70E+01	3.00E+00	0.179712
<i>Phyllophaga</i>	35	37	8.67E+02	1.76E+03	2.37E+02	0.000003
Wireworms						
<i>Agriotes</i>	16	9	1.51E+02	1.74E+02	1.50E+01	0.001245
<i>Melanotus</i>	25	20	3.85E+02	6.50E+02	6.00E+01	0.000014
<i>Sericus</i>	8	2	4.20E+01	1.30E+01	1.00E+00	0.067577
$\delta^{15}\text{N}$						
Cebrionidae	34	31	1.15E+03	9.92E+02	5.58E+02	0.683867
Cicada	3	8	2.50E+01	4.10E+01	1.90E+01	0.153041
Weevils	45	34	1.25E+03	1.91E+03	2.19E+02	<0.000001
Grubs						
<i>Anomola</i>	3	5	2.10E+01	1.50E+01	1.40E+01	0.025347
<i>Phyllophaga</i>	35	37	1.27E+03	1.35E+03	6.41E+02	0.937136
Wireworms						
<i>Agriotes</i>	16	9	2.05E+02	1.20E+02	6.90E+01	0.865134
<i>Melanotus</i>	25	20	4.70E+02	5.65E+02	1.46E+02	0.016977
<i>Sericus</i>	8	2	4.20E+01	1.30E+01	6.00E+00	0.601508

Table 3-5: Summary of average difference of insect diets (+/- 1 SD) from in-plot fine roots (categorized by taxa, B and UB treatments combined). Dietary preferences were described as *None* if mean deviation from local (within plot) fine root $\delta^{13}\text{C}$ (‰) was not greater than 1‰. The word *Possibly* is was used if mean deviation was at least 1‰, but did not exceed the 95% confidence interval calculated for $\delta^{13}\text{C}$ of each insect taxa. Positive deviation indicates a preference for C_3 vegetation; negative deviation indicates a C_4 preference.

Family	Genus	95% Confidence Interval for $\delta^{13}\text{C}$ of Taxa	Mean Deviation from random (permil)	Dietary Preference
Elateridae	<i>Agriotes</i>	1.70	-1.47	<i>Possibly C₄</i>
Elateridae	<i>Limonius</i>	3.98	-0.86	<i>None</i>
Elateridae	<i>Melanotus</i>	0.88	-0.61	<i>None</i>
Elateridae	<i>Sericus</i>	1.25	-1.34	C_4
Scarabiaedae	<i>Maladera</i>	0.42	0.27	<i>None</i>
Scarabiaedae	<i>Phyllophaga</i>	0.72	1.06	C_3
Scarabiaedae	<i>Anomala</i>	2.22	0.08	<i>None</i>
Scarabiaedae	<i>Diplotaxis</i>	0.43	1.53	C_3
Scarabiaedae	<i>Cyclocephala</i>	4.08	-3.98	<i>Possibly C₄</i>
Cebrionidae	Unknown	0.54	-1.20	C_4
Cicadidae	Unknown	2.31	2.05	<i>Possibly C₃</i>
Curculionidae	Unknown	0.82	1.12	C_3

FIGURES

Figure 3-1: Bivariate plot showing $\delta^{13}\text{C}$ (‰) and $\delta^{15}\text{N}$ (‰) for fine root (< 1 mm dia.) standing crop samples. Samples were composites of four subsamples collected from random plots in fire maintained (B = red symbols) and burn exclusion (UB = black symbols) treatments. Fine roots from specific C_3 and C_4 plant species (see Appendix E), and samples of partially decomposed, forest floor litter, were also collected for use as end points, which are shown as open green symbols (means +/- standard deviations). The decrease in C_4 grass dominance that resulted from approximately 10 years of burn exclusion is illustrated by the higher proportion of UB (black) symbols towards the left side of the plot (decreased $\delta^{13}\text{C}$).

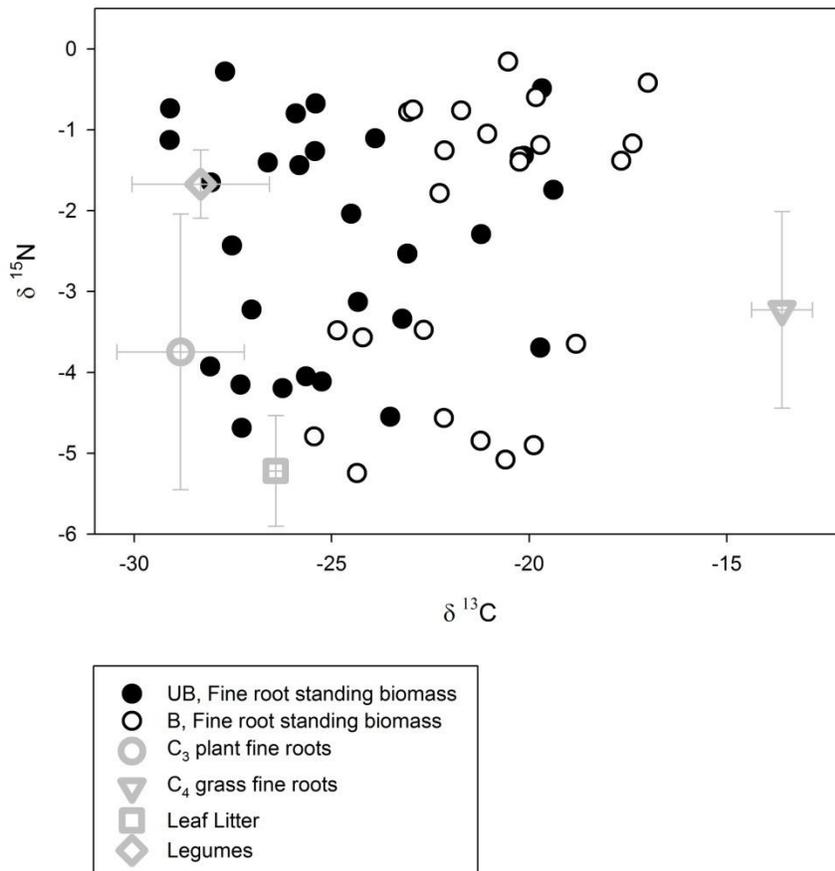


Figure 3-2: The $\delta^{13}\text{C}$ (a), $\delta^{15}\text{N}$ (b), and C:N ratio (c) for insects and roots collected from frequently burned (B) and burn-excluded (UB) plots. Units are permil (‰). Boxes denote median and upper and lower quartiles. Bars denote the range, excluding outliers, which are shown as hollow circles.

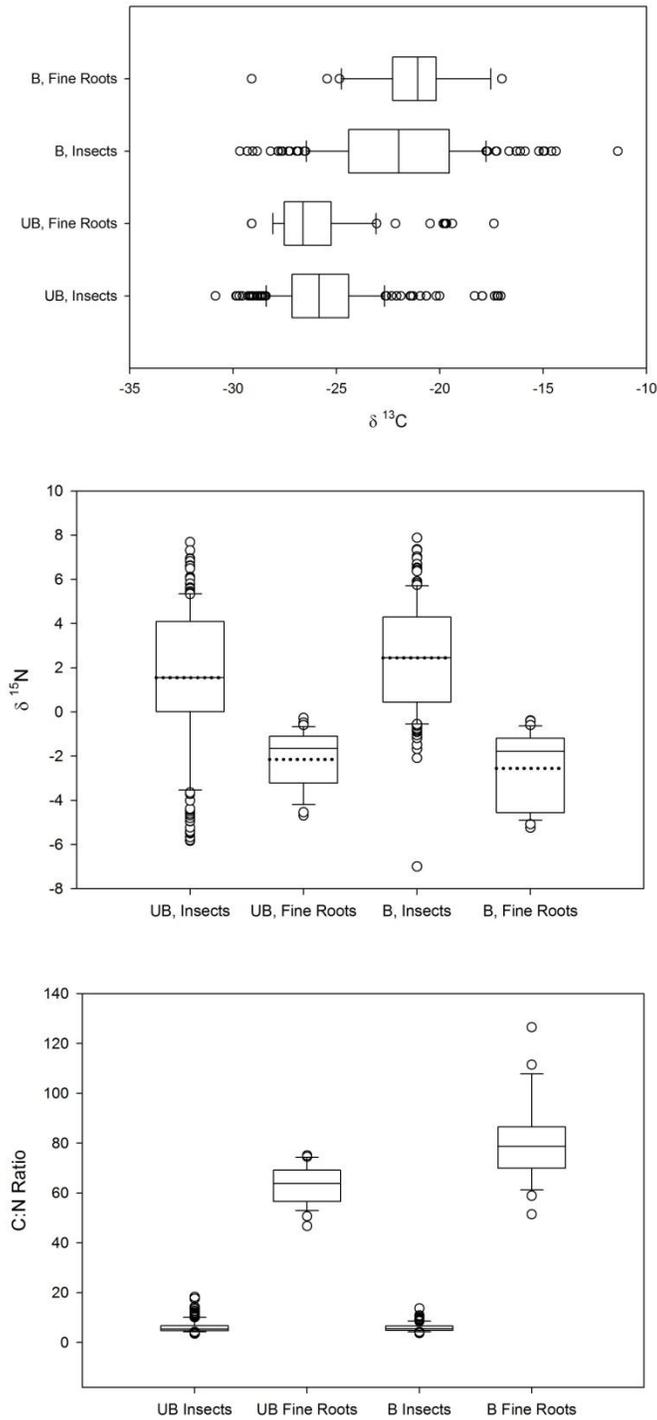


Figure 3-3: Taxonomic variability of $\delta^{15}\text{N}$. Box plots show $\delta^{15}\text{N}$ for insect taxa, isopods, fine root standing crop, and leaf litter; both fire treatments are combined. Higher $\delta^{15}\text{N}$ indicates feeding at higher trophic levels. Boxes denote median and upper and lower quartiles. Bars denote the range, excluding outliers, which are shown as hollow circles.

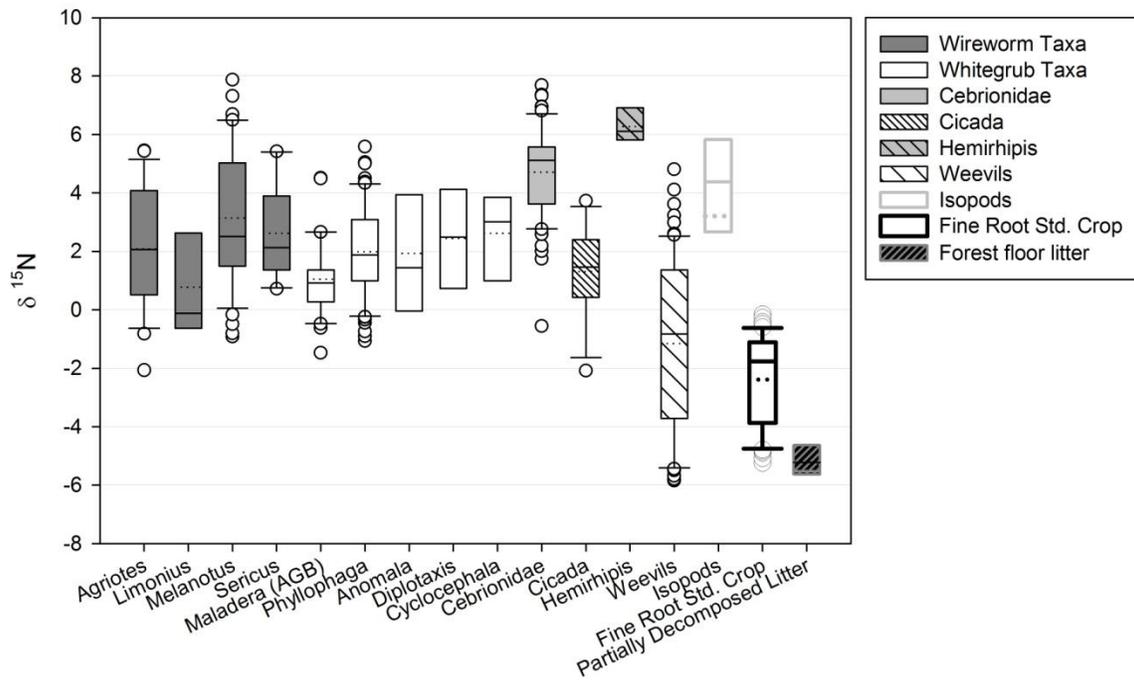


Figure 3-4: Bivariate plot showing $\delta^{13}\text{C}$ (‰) and $\delta^{15}\text{N}$ (‰) of belowground insect taxa (solid symbols) and fine root standing crop samples (< 1 mm dia.) (open hexagonal symbols). Insects collected from fire maintained stands (B) are represented with a variety of red, orange, and pink colors, and insects collected from the burn exclusion stands (UB) are represented by a variety of blue colors. Specific colors refer to the broad insect group (grubs, wireworms, or other, respectively). Symbol shapes correspond to specific genera within each group. Symbols are mean values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, +/- 1 standard error. The proportion of C_4 grasses in the fine root standing crop samples, or in the diet of the insects, increases from left to right. Values for $\delta^{15}\text{N}$ reflect the trophic level of the consumer (a positive shift of approximately 3.4‰ is expected relative to the $\delta^{15}\text{N}$ of the food source). Endpoint values (open symbols, except for red and blue hexagons) were attained by collecting particular species of plants, leaf litter, and isopods (see Appendix E).

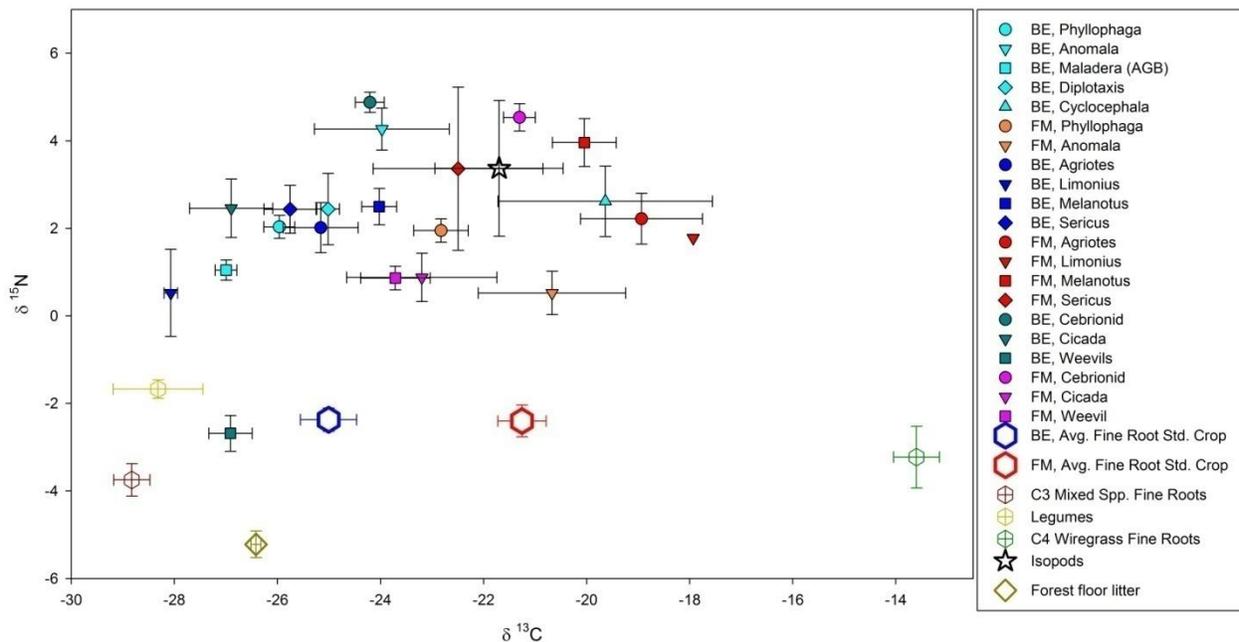


Figure 3-5: The $\delta^{13}\text{C}$ (a) and $\delta^{15}\text{N}$ (b) of insects across four sampling events. Delta values are shown as permil (‰). Boxes denote median and upper and lower quartiles. Bars denote the range, excluding outliers, which are shown as hollow circles.

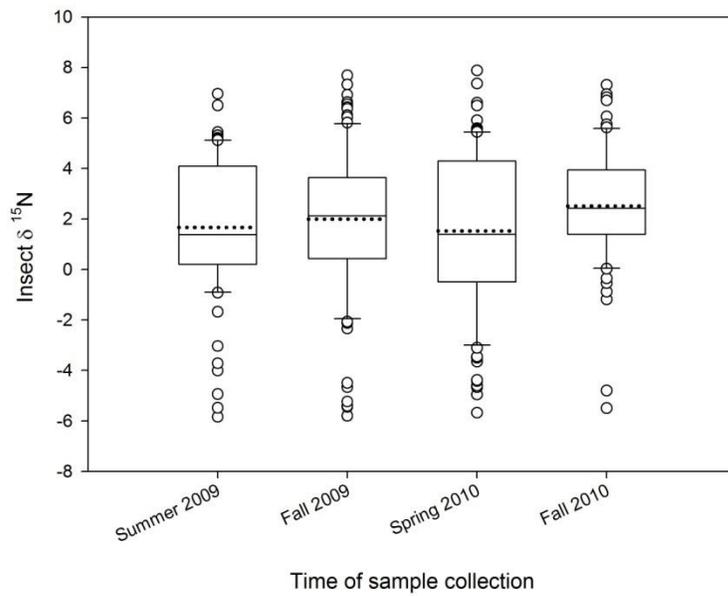
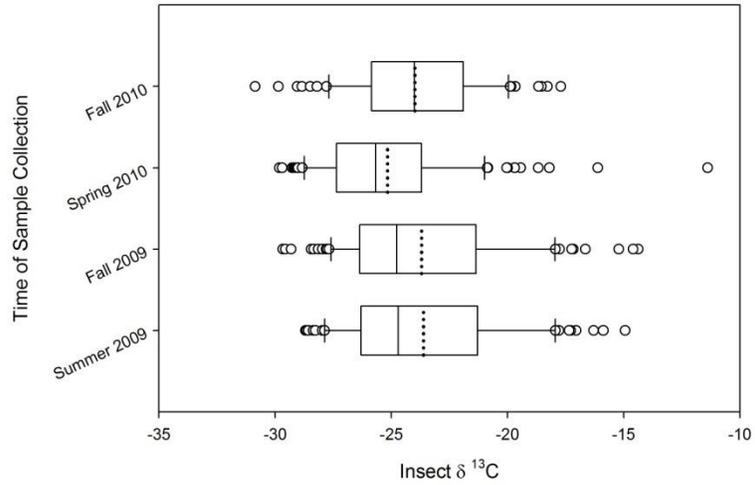


Figure 3-6: Individual insect diet ($\delta^{13}\text{C}$) as a response to local scale (in-plot) fine root $\delta^{13}\text{C}$. Blue and red symbols denote UB and B treatment groups respectively. Symbol shape indicates insect functional type. Blue and red dashed lines represent lines of best fit (linear) for all UB and B individuals, respectively. Black dashed line is the 1:1 line where $\delta^{13}\text{C}$ of insect diet is equal to $\delta^{13}\text{C}$ of local fine roots. Units for $\delta^{13}\text{C}$ are permil.

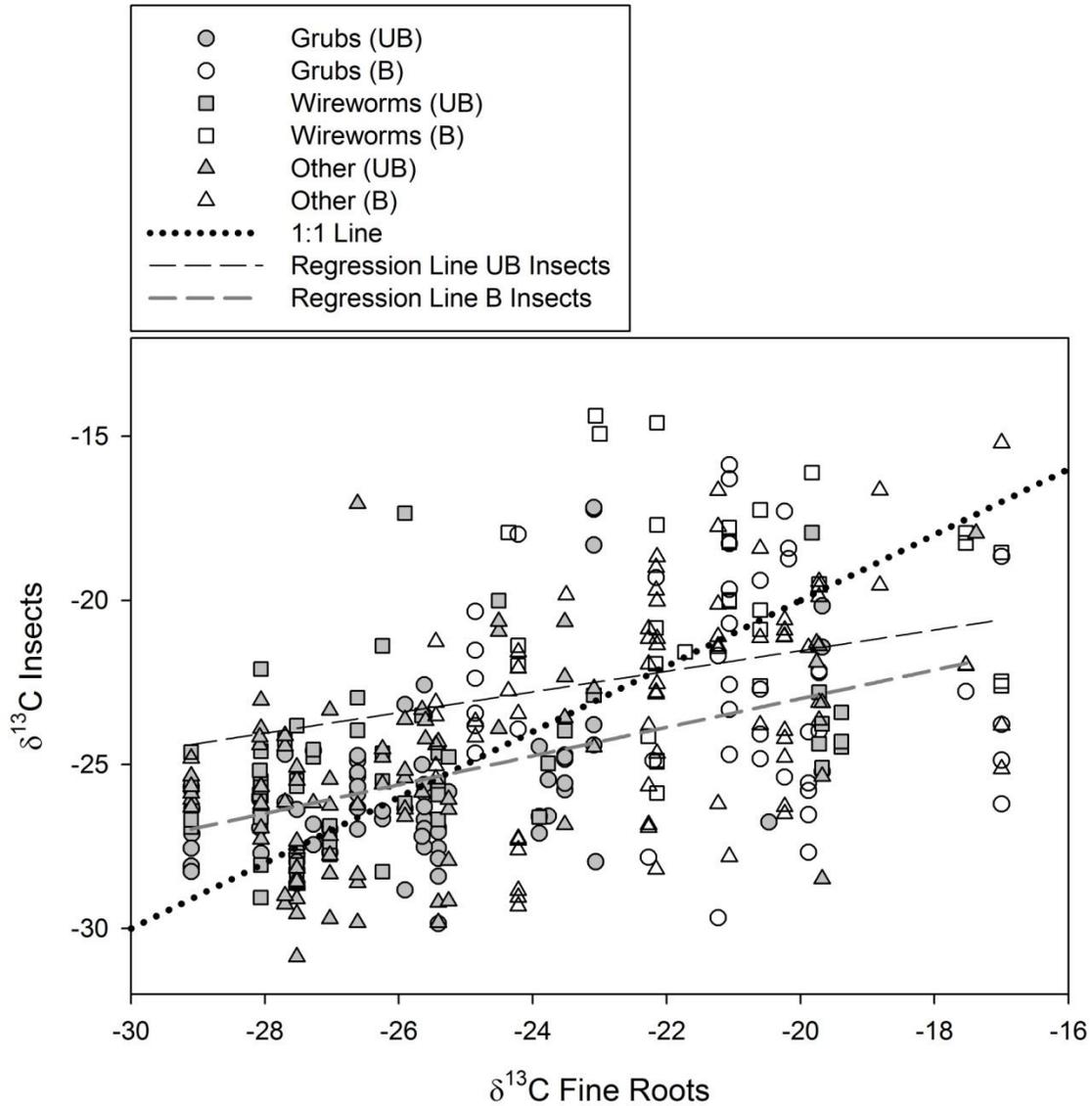


Figure 3-7: Mean $\delta^{13}\text{C}$ for insect taxa +/- the 95% confidence interval. From top to bottom, the graphs show white grub taxa, wireworm taxa, and other taxa. Blue and red symbols denote UB and B plots respectively. Symbol shapes denote specific genera. The black dashed line is the 1:1 line where insect $\delta^{13}\text{C}$ is equal to $\delta^{13}\text{C}$ of local fine roots. Symbols that fall above the 1:1 line indicate a feeding bias towards C_4 species, and those that fall below it suggest a C_3 bias. Units for $\delta^{13}\text{C}$ are shown as permil.

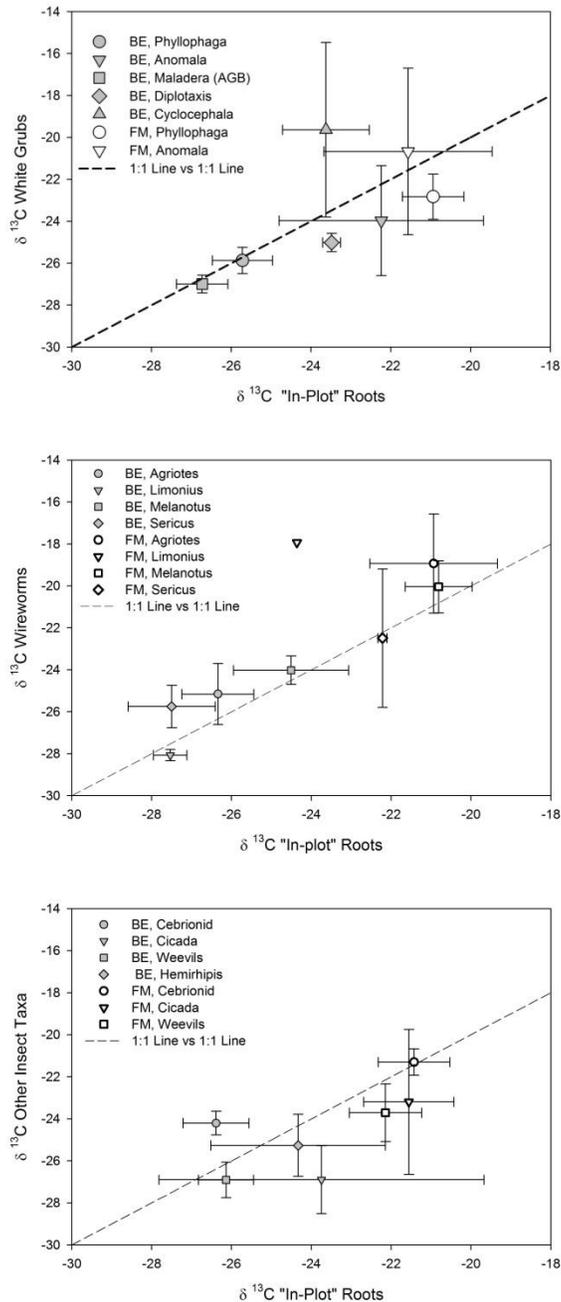
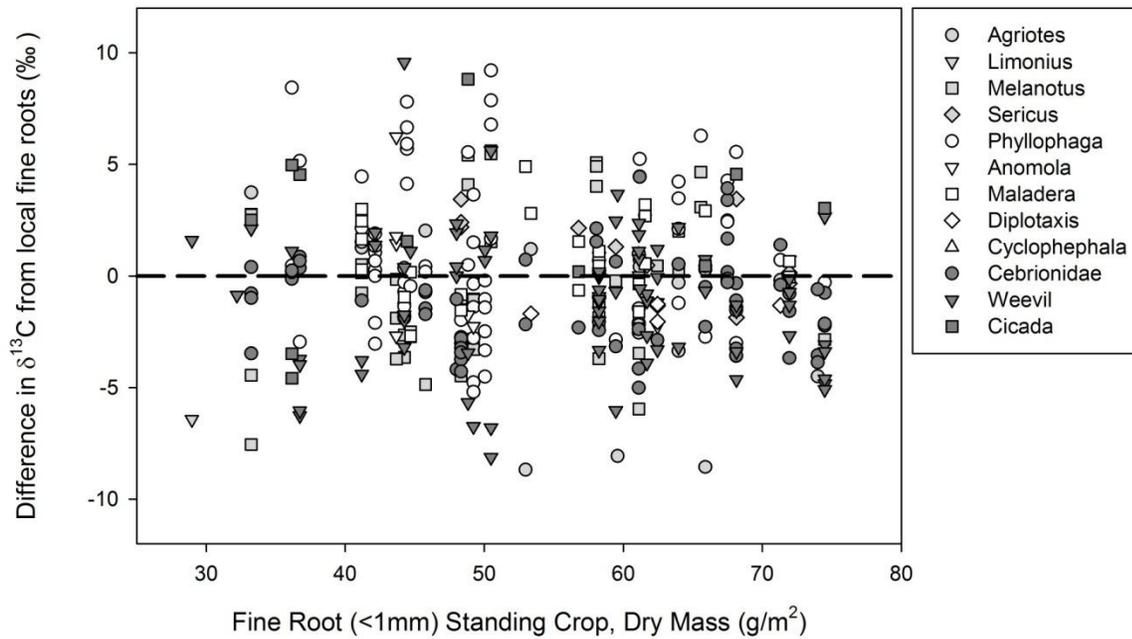


Figure 3-8: Local scale dietary selectivity (permil deviation from local fine root $\delta^{13}\text{C}$) as a response to fine root standing crop (g fine root dry mass per m^2 , to 20 cm depth). Colors indicate insect functional groupings (wireworms, white grubs, and other, from top to bottom of legend), symbol shapes denote specific genera. The dashed line represents a diet that does not deviate in either a positive or negative direction from the theoretically random diet. Positive deviation represents consumption of more C_3 fine roots than predicted, and deviation in a negative direction indicates consumption of more C_4 fine roots than predicted.



CHAPTER 4

Top-Down Influences of Root-Feeding Insects on Fine Root Productivity in Fire-Manipulated Longleaf Pine-Wiregrass Ecosystems

Matthew Dittler, Robert H. Jones, and Robert J. Mitchell

ABSTRACT

Fine root herbivory is a potentially important component of belowground carbon and plant community dynamics in terrestrial ecosystems. Top-down effects of herbivores on fine root dynamics may depend on herbivore density, and may range from little or no visible impact (due to compensatory growth of roots) to significant declines in fine root mass. Root-feeding insects are broadly distributed fine root herbivores that can have negative economic impacts in agricultural systems; however, their effects on ecosystem processes in natural ecosystems are poorly understood. In this study, we asked how root-feeding insects influenced fine root dynamics in fire-manipulated longleaf-pine wiregrass ecosystems of southwest Georgia, USA. Net productivity of grass and non-grass roots (< 0.5 mm and ≥ 0.5 mm diameter classes) in control subplots and those treated with insecticide was measured using ingrowth cores during 3 time intervals over the course of approximately 14 months in 54 plots distributed evenly across frequently burned (B) and burn-excluded stands (UB). Mixed models and univariate statistics were used to quantify the effects of root-feeding insect removal on root ingrowth. Root-feeding insect relative abundance and cumulative biomass were also assessed within plots to determine if they were correlated with fine root ingrowth.

There was a significant increase in non-grass fine root (< 0.5 mm) ingrowth for the control treatment (where root herbivores were not removed), suggesting a possible weak compensatory response of non-grass fine roots to belowground herbivory, commensurate with our estimates of root-feeding insect population densities, which were well below thresholds for damage that have been reported in agricultural and turf grass systems. Root ingrowth was not significantly correlated with relative abundance or total biomass of root-feeding insects, possibly because of low herbivore population densities. The median top-down herbivory effects on fine roots (< 0.5 mm), which were estimated as control minus insecticide-treated ingrowth within each plot, was about $9 \text{ g} \cdot \text{m}^{-2} \cdot \text{y}^{-1}$, respectively (< 10% of annual fine root net primary production). The small net effect suggested that at the ecosystem scale, top down effects from the low population densities of RFIs in this study system were relatively unimportant for fine root dynamics.

INTRODUCTION

A large proportion of net primary production (NPP) in terrestrial ecosystems, nearly 50%, is allocated to fine root production due to high turnover rates (Eissenstat and Yanai, 2002; Vogt et al., 1986; Nadelhoffer and Raich, 1992). Fine root productivity can vary substantially depending on plant species composition, disturbance frequency, bottom-up factors like site productivity, and top-down factors like herbivory (Ostonen et al., 2005; Eissenstat and Yanai, 2002; Brown and Gange, 1989).

Fine root herbivory is a potentially important, but not well documented fate of fine root production. Fine root herbivores can alter fine root dynamics via direct consumption, or by severing higher order roots. In addition, herbivores can elicit a range of defense responses in plants that potentially alter the allocation of carbon and resources belowground (see Arimura et

al., 2004; Price et al., 1980). Andersen (1987) estimates root consumption in the range of 6% to 30% of fine root production. Stevens and Jones (2006) used ingrowth cores to demonstrate that fine root herbivory and nitrogen (N) availability may interact to influence fine root productivity. Other studies (using mini-rhizotron sampling) demonstrated that the disappearance of fine roots between sampling intervals (possibly from herbivores) was common, and may have accounted for near 40% of fine root mortality (Stevens et al., 2002; Fahey and Hughes, 1994; Hendrick and Pregitzer, 1992). Therefore, if fine root herbivory is not taken into account, then even otherwise careful estimates of fine root NPP may significantly underestimate belowground NPP (Stevens et al., 2002; Hunter et al., 2001). Nevertheless, few studies of fine root dynamics consider top-down effects from fine root herbivores; likewise, few studies of fine root herbivory consider other sources of variability for fine root dynamics that can help define the importance of herbivores in natural ecosystems (see Stevens, 2005; Hunter and Price, 2001).

Top-down forces exerted by root herbivores also have the potential to alter vegetation dynamics and succession (Brown and Gange, 1989). Effects of herbivores are likely density dependent (see Parmesan, 2000; Marquis, 1992). Relatively high population densities of fine root herbivores can result in plant mortality, which could lead to shifts in ecosystem dynamics and species composition, especially if herbivores exert strong feeding preferences (Fermanian et al., 2002; Lura and Nyren, 1992; Brown and Gange, 1990). Low to moderate densities, on the other hand, may have very little influence, or even positive (compensatory) effects on fine root production, such as in patterns of grazing intensity and aboveground productivity observed in grasslands (see McNaughton, 1985).

Root-feeding insects (hereafter RFIs) are economically important fine root herbivores that are distributed across a broad range of latitudes and continents. These insects undergo their root-

feeding phase as larvae or nymphs and then eventually emerge from the soil as adults to feed on aboveground vegetation, breed, and lay eggs back in the soil. Studies in agro-ecosystems have shown that the larvae of the Coleopteran families Scarabaeidae (white grubs), Elateridae (wireworms), and Curculionidae (weevils), and the Nymphs of the Hemipteran family Cicadidae (cicadas) are among the most common and abundant RFIs. Many RFI genera are considered pests of forage crops and turf and are frequently cited for their economic damage to turf-grass and crops (Fermanian et al., 2002; Stehr, 1991). The top-down effects of RFIs in more complex ecosystems are not well known.

Questions and Hypotheses

Our central question was "how much does herbivory by root-feeding insect assemblages influence fine root dynamics," and we tested this by removal of fine root herbivores (using insecticide) and subsequent measurement of fine root production in frequently burned (B) and unburned (UB) longleaf pine-wiregrass ecosystems. Prior to assessing the population density of root-feeding insects, we predicted a negative influence of RFIs on fine root productivity, based on their reputation as pests. However, the low population densities observed after the first insect sampling events -- well below thresholds reported for crop damage (see Chapter 2) -- warranted a revised prediction of either no effect or a compensatory effect on fine root productivity (see McNaughton, 1985). We also asked whether top-down effects from herbivores would differ for grass roots and non-grass roots, or for different root diameter classes (< 0.5 mm and 0.5 to 1 mm). We predicted greater top-down effects on non-grass roots because their lower C:N ratio (see Chapter 3 and Jansen, 2007) might make them a higher quality, more digestible food. This builds upon work investigating cattle grazing in pine-wiregrass savanna (e.g. Grelen, 1978), and studies of the digestibility and nutritional value of wiregrass, which has been considered a low

quality forage for cattle except early in spring (Lewis et al., 1975). Because wiregrass was less abundant in UB sites, we predicted that UB sites might experience higher rates of herbivory, and therefore exhibit stronger top-down effects. Finally, we predicted greater top-down effects on roots < 0.5 mm than on roots 0.5 mm to 1 mm diameter because the former may be easier for RFIs to chew through, and higher in N and cellulose content (Guo et al., 2004).

METHODS

Study Sites

Study sites were located at the Joseph W. Jones Ecological Research Center (Ichauway), which is near the town of Newton, in Baker County, Georgia USA (31° N, 84° W), within the Eastern Gulf Coastal Plain (see Chapter 2, Figure 2-1 for site map). Flat to gently rolling land, which gradually slopes toward the southwest, and elevations range from 25 to 200 meters characterize the region (Drew et al., 1998). The climate is humid subtropical and the precipitation averages 132 cm/year; average daily temperatures range from 5 - 17°C in winter, to 21 - 34°C in summer (Hendricks et al., 2006, Drew et al., 1998; Goebel et al., 1997). Soils at the study sites are mostly Typic Quartzipsamments, but some Grossarenic and Aquic Arenic Paleudults also occur. Soils are underlain by karst Ocala limestone, typically lack developed organic horizons, and exhibit minimal vertical stratification of the mineral soil (Hendricks et al., 2006; Drew et al., 1998). These soil properties allow efficient sampling of fine roots and root feeding insects, and reduce the potential for sample contamination by fine soil particulates and organic matter, which could cling to the roots during washing, biasing mass estimates.

Overstory vegetation consisted primarily of 60-90 year old second growth *Pinus palustris* Miller (longleaf pine), (Mitchell et al., 1999). Understory composition was dominated by the C₄

grass *Aristida stricta* Michaux (wiregrass, or three-awn pine grass), and to a lesser extent, also contained regenerating longleaf pine, shrubs, and hardwoods of varying developmental stage, C₃ forbs, legumes, and other warm-season (C₄) grasses. Fire maintenance had been a regular part of the forest management at Ichauway. Individual stands on the property had been regularly burned on a 1 to 5 year interval (Hendricks et al., 2006). Periodic fires have been shown to promote increased dominance of wiregrass and fire-adapted legumes, and a decreased abundance of fire-sensitive vegetation (e.g. hardwoods).

Experimental Design

Site Selection and Fire Manipulation:

Several forest stands were selected to remain unburned (UB) prior to initiation of this experiment, in order to study how fire and N control vegetative composition, productivity, and nutrient cycling. A final burning in the fall of 2000 initiated the burn exclusion treatment (UB). Four UB stands not receiving irrigation or fertilization treatments were considered for use in the experiment. During an initial survey of the property, six frequently burned stands were considered for the experiment because they met the following *a priori* criteria for inclusion in the experiment: Each had been burned at least every other year, exhibited dominance of mature longleaf pine in the overstory and dominance of wiregrass in the understory, and had no ongoing irrigation or fertilization. Three UB and three B stands were randomly selected from this set of available stands for use in our study of RFI distributions. For two of the selected B stands burning was conducted on a two year cycle (fall), while one was burned annually (summer). Both UB and B stands contained significant wiregrass cover in the understory at the initiation of the study; however, UB stands had less wiregrass and more woody plant abundance than B

stands, which was the expected result of burn exclusion in longleaf pine-wiregrass ecosystems (see Chapter 2).

Plot Establishment and Layout:

In each of the six selected stands, nine rectangular plots (4 m x 14 m) were established by pacing along randomly drawn compass vectors. Coordinates of plot center locations were recorded using a Garmin® 12 handheld GPS (see Chapter 2, Figure 2-2 and Appendix A, for conceptual diagram, and plot coordinates, respectively). Plots were oriented with a compass to the cardinal directions, with the long dimension of the plot either running North to South, or East to West (chosen randomly).

Root-feeding insects were quantitatively sampled during 6 occasions in a stratified random pattern from the 4 m x 4 m centers of plots, which were further divided into quadrants. Fine root standing crops were sampled on one occasion, and fine root ingrowth was assessed during three time periods, within the peripheral portions (4 m x 4 m areas) of each rectangular plot. Either an insect removal (using insecticide) or control treatment was applied to each root sampling subplot (randomly chosen). See Figure 4-1 for a conceptual diagram of the plot layout and sampling scheme. Spatial separation of fine root and insect sample areas was necessary because the high degree of soil disturbance associated with insect sampling could have biased estimates of root productivity, and because insect removal and control treatments needed to be spatially segregated due to the potential for mobility of insecticide within the soil. Insect removal treatments (insecticide treated [T] or Control [C]) were spatially separated within each plot, this was not a split-plot experimental design because there was not a whole plot treatment applied to plots. Each of the two fire treatments were instead applied to randomly selected sites,

within which plots were nested. Therefore, this was a multi-level nested design in which ingrowth samples were clustered within plots (nested experimental units).

Root-Feeding Insects

Plots (4x4m center areas) were sampled for belowground insect larvae on 6 occasions: September 3 to 12, 2008; May 23 to June 13, 2009; October 8 to November 9, 2009; March 8 to April 2, 2010, July 6 to 29, 2010 and November 12 to 29, 2010. Larvae of families Scarabaeidae (white grubs), Elateridae (wireworms), Curculionidae (weevils), Cicadidae (cicadas) were collected, all of which were of order Coleoptera, except for family Cicadidae (order Hemiptera). Insects were identified in the field with a hand lens, placed in collection jars and stored on ice until freezing at the end of each work day. Specimens were later thawed, gently washed to remove soil particles, and re-examined under a dissecting scope to verify identifications using Stehr (1991) and Ritcher (1966). White grubs and wireworms were identified to the genus level and other larvae were identified to the family level. However in this study, only the relative abundances of combined taxa were used in analysis.

Combined RFI dry mass was also estimated for each plot. Following identification, insects that were collected during 4 of the sampling periods (May 23 - June 13, 2009, October 8 to November 9, 2009, March 8 to April 2, 2010, and November 12 to 29, 2010) were dried for at least three days at 60°C and weighed to the nearest 0.1 mg.

Insect Removal Treatment

A fine root herbivore removal treatment was applied to one of the 4 m x 4 m ends of each plot (randomly chosen), while the other end remained untreated as a control (see Figure 4-1). Herbivore removal treatment consisted of the application of a broad spectrum, slow release insecticide, chlorpyrifos (Lorsban™15G, Dow AgroSciences, LTD, Indianapolis), which is

commonly used for root-feeding insect suppression (see Stevens, 2005). Granular chlorpyrifos was applied directly to ingrowth cores by mixing 2.5 ± 0.5 g with root-free soil prior to ingrowth core installation. An additional 25 ± 5 g of insecticide was broadcast over the 4 m x 4 m treated subplot (T). Insecticide was reapplied to the treated subplot during each installation of ingrowth cores (3 times throughout the duration of the study).

Fine Roots

Fine root ingrowth cores, which are root-free columns of soil from which new root growth into the column during some time interval can be harvested, were used to assess the impact of root-feeding insects on productivity. Fine root standing crop samples were also collected during the installation of ingrowth cores as a potential covariate of root ingrowth, and to assess the relationship between fine root herbivory and the standing mass of roots. There were three ingrowth periods. Ingrowth cores were installed initially between May 23, 2009 and June 13, 2009. These cores were harvested in tandem with the installation of the next set of cores, which occurred between October 8 and November 9, 2009. Likewise, the previous set of cores was harvested and the final set of cores was installed, between March 8 and April 2, 2010, and harvested between July 2 and 28, 2010. Standing mass samples collected during the first ingrowth core installation were processed for the first time period for potential use as a covariate in the analysis.

Ingrowth cores ($N = 648$) were installed by driving a steel coring pipe (10 cm inside diameter, 20 cm depth) into the earth with a sledge hammer and extracting the soil volume. The soil from the core was transferred to a large (~ 30 cm x 30 cm) sieve (3 cm mesh). The bulk of the soil was loosened from the roots by gentle shaking and manipulation by hand. Roots were then transferred to plastic bags as fine root standing crop samples. These roots were later

washed with tap water under low pressure over a sieve (1 mm mesh). Washed roots were then frozen until sorting, during which time samples were thawed, placed in a shallow water bath, and sorted into diameter classes (< 0.5 mm, 0.5 to 1 mm, and > 1 mm) using a caliper for verification of diameters. The transparent trays used for sorting were placed respectively over a dark and white surface to illuminate light and dark colored roots. Sorted roots were then dried for 3 or more days at 60°C and weighed to the nearest 0.01 g to determine estimates of fine root standing crop (0 to 20 cm depth). For this study, standing crop estimates were based on combined sampling depths. Soil which was loosened during field sieving was retained in a large wheel barrow. Additional field soil was collected outside of, but in the vicinity of the plot, and added to the wheel barrow after sieving (roots were discarded). Soil was then added back into the excavated cylinder of earth and tamped to approximate the bulk density in the vicinity. Insecticide was added to soil of treated (T) ingrowth cores prior to mixing soil and filling cylinder. The center of each core was marked with a pin flag to facilitate accurate re-sampling of the cylinders during harvest.

Ingrowth cores were harvested much as they were installed, except the coring pipe was a smaller diameter (7 cm dia.) to ensure that only the previously prepared soil would be sampled. Fine roots and soil from ingrowth samples were processed as described above for standing crop samples, except that roots were sorted as grass and non-grass roots, and by diameter class (< 0.5 mm; ≥ 0.5 mm). Determination of grass and non-grass was made by examination of the branching characteristics and coloration of the roots. All roots within ingrowth samples were less than 1.2 mm diameter (almost all were < 1.0 mm), so additional diameter classes were not used.

Data Analysis

Missing Data, Outliers, and Graphics:

There were a few cases in which ingrowth cores were destroyed during harvest due to soil saturation, or in which the sample was otherwise lost. These cases were treated as missing data and degrees of freedom were adjusted accordingly using Systat[®]. Outliers were trimmed at the 0.05 level (upper and lower) prior to mixed model analysis out of concern that leverage from outliers might bias significance estimates. Outliers were not removed for box plots or univariate statistics. All data graphics were generated, and statistics represented in the figures were calculated, using SigmaPlot[®] 10 software.

Univariate Statistics and Fine Root Productivity Calculations:

Univariate statistics for fine root ingrowth, fine root standing crop, and plot-wise top-down effects, were estimated using Systat[®] software. Most ingrowth data were presented on a per square meter basis, with time period specified, or otherwise combined. Approximate estimates of fine root productivity were calculated as the sums of the average and median production from the 3 consecutive sets of ingrowth cores processed during the study. Average ingrowth time intervals were approximately 5, 5, and 4 months for the 3 respective ingrowth periods. Data organization, transformations, unit conversions, and all basic calculations not otherwise specified were performed with MicroSoft[®] Excel[™].

Mixed Models:

Linear mixed models were used to evaluate the fixed effects of the insect removal treatment (T vs. C), fire treatment (UB vs. B), and insect removal by fire treatment interaction, on fine root ingrowth, while also accounting for random effects due to spatial clustering of

ingrowth cores within sites and plots, sampling period, the interactive effect of fire and sampling period, and the nesting of sites within fire treatments. Fixed and random effects were designated following Bennington and Thayne (1994). The average values of T and C ingrowth cores from within each plot were used as experimental units to avoid pseudo-replication. Mixed model analyses were performed using Systat[®] Software. Four individual categories of fine root ingrowth (grass [< 0.5 mm, ≥ 0.5 mm], non-grass [< 0.5 mm, ≥ 0.5 mm]) were individually assigned as dependent variables.

Calculation of Top-Down Effects within Plots:

In addition to evaluating the effect of insect removal with mixed models, the ingrowth difference between control (C) and insecticide-treated (T) cores was estimated within each plot. What will hereafter be referred to as top-down effects were defined as the difference in fine root ingrowth (< 0.5 mm) when ingrowth from T cores was subtracted from the ingrowth of C cores within each plot. Effects were estimated separately for grass and non-grass roots, and for each of 3 ingrowth time periods, and plotted using box and whisker plots.

Correlation Analysis:

Simple Pearson's correlation statistics (r) were calculated for relationships between plot-wise, top-down herbivory effect estimates (control minus insecticide treated ingrowth cores) and three factors that may also vary with this measure: fine root standing crop at the time of ingrowth core installation (< 0.5 mm and < 1 mm), total RFI mass, and RFI relative abundance.

Correlation statistics were also calculated for the relationships between fine root standing crop and RFI abundance and biomass. RFI mass for each plot was defined as the sum of herbivore dry mass within each plot (4 time periods combined). RFI relative abundances were defined as

the square root-transformed sum of the counts (6 sampling periods) within plots. Pearson's r statistics were calculated using Systat[®] software.

RESULTS

Summary of Fine Root Ingrowth in the Study System

Fine root production (< 0.5 mm) averaged across the study system (sum of ingrowth rates from the 3 time intervals) was $113 \text{ g} \cdot \text{m}^{-2} \cdot \text{y}^{-1}$; median production was $88.3 \text{ g} \cdot \text{m}^{-2} \cdot \text{y}^{-1}$ (Table 4-1). Roots < 0.5 mm diameter were approximately 75% of total ingrowth production (all roots < 1 mm), (Table 4-1). Grass roots made up about 25% of the total root ingrowth production (< 1 mm and < 0.5 mm), (Table 4-1); the remaining were roots from hardwoods, forbs, and pine.

Fine root ingrowth (< 0.5 mm) varied among fire treatments (Figure 4-2, a), ingrowth periods (Table 4-2, a; Figure 4-2, b), and insect removal treatments (Table 4-2, b; Figure 4-3, c); furthermore, responses varied between grass and non-grass roots (Table 4-2, b). Mean and median ingrowth were higher in unburned (UB) relative to burned (B) sites (Figure 4-2, a). Across the 3 ingrowth periods, fine root productivity was highest from March to July, 2010, and lowest from October 2009 to March 2010 (Figure 4-2, b). Ingrowth cores receiving the insect removal treatment (T) had lower mean and median ingrowth than control (C) cores (Figure 4-2, c). The compensatory effects of herbivores were larger overall in UB sites. The greater ingrowth in control cores was detectable for non-grass, and combined fine roots, and to a lesser extent, grass roots, in UB sites, but only for non-grass (< 0.5 mm) and combined fine roots (< 0.5 mm) in B sites (Figure 4-3, a-c).

Mixed Effects Models

Sampling period was the most important covariate (random effect) based on the relative effect sizes of all covariates included (Table 4-2, a; also see Figure 4-2, b). After accounting for random effects, the fixed effect of the insect removal treatment was significant for the ingrowth of other roots ($p = 0.07$) and for combined roots ($p = 0.036$) less than 0.5 mm diameter, but not for grass roots or roots greater than 0.5 mm diameter (Table 4-2, b). The fixed effect of fire was not significant in any of the models after accounting for site and sampling period as covariates. The effect from the interaction between fire and insect removal was only significant for combined fine roots < 0.5 mm diameter ($p = 0.047$; Table 4-2, b).

Plot-Wise Estimates of Top-Down Effects

Top-down effects -- measured as the difference between in-growth in control versus insect excluded cores within each sample plot -- were near zero for grass and non-grass fine roots (< 0.5 mm dia.) across the 3 time periods (see Table 4-3; Figure 4-4). The median effect of herbivory on overall fine root (< 0.5 mm dia.) production were slightly positive. Median effect magnitudes for grass and non-grass fine roots (< 0.5 mm dia.) were 1.8 and $11.6 \text{ g} \cdot \text{m}^{-2} \cdot \text{y}^{-1}$, respectively, or $8.9 \text{ g} \cdot \text{m}^{-2} \cdot \text{y}^{-1}$ combined fine roots (Table 5-3) -- possibly representing a net increase of as much as 5 to 10% of median annual fine root net primary production ($104 \text{ g} \cdot \text{m}^{-2} \cdot \text{y}^{-1}$).

Relationships Between Top-Down Effects Estimates and Root-Feeding Insect Relative

Abundances and Biomass

Over the six sample dates, a total of 1,105 RFIs were sampled. Insects were identified from 2 insect orders, and 5 families (see Chapter 2). The mean population density (± 1 standard error) for combined taxa was 5.1 ± 1.9 individuals per square meter; for UB and B treatments, it

was 6.5 ± 2.5 and 3.7 ± 1.8 individuals per square meter, respectively (Table 4-4; also see Chapter 2). The square-root transformed relative abundances, and the total biomass of RFIs, was summarized for UB and B plots (Figure 4-4).

Insect relative abundance and total biomass (each cumulative over the duration of the study), were not correlated with top-down effects within plots, or with fine root standing crop (g/m^2) regardless of diameter class or classification as grass or non-grass (for all, $|r| \leq 0.118$), (Table 4-5). However, there was weak positive correlation between fine root standing crop and total top-down effect on ingrowth, particularly for the finest diameter roots (< 0.5 mm), ($|r| = 0.338$), (Table 4-5). Scatter plots are provided for closer examination of the relationship of root-feeding insect abundance or biomass with total fine root ingrowth (Figure 4-6, a, b).

DISCUSSION

Weak Compensatory Effects of Belowground Insect Herbivores on Fine Root Productivity

Estimates of ingrowth rates were within the range of fine root (< 0.5 mm dia.) ingrowth reported by other investigators in this study system (Hendricks et al., 2006). Results of this study show little in the way of top-down control overall, but some evidence of a weak compensatory growth response. The mixed model analysis (Table 4-2) and our calculation of top-down impacts (Table 4-3; Figure 4-4, a, b) suggested that insecticide application resulted in slightly less fine root ingrowth for non-grass ($p = 0.061$) and combined ($p = 0.036$) fine roots (< 0.5 mm dia.). Very likely, insect density was too low to have larger impacts on root growth (Table 4-3). In studies where insect densities have been greater, significant negative impacts on root production have been observed (e.g. Stevens and Jones, 2006). The less significant response for ingrowth of grass roots (< 0.5 mm dia.), ($p = 0.828$) suggested a possible feeding preference

for non-grass fine roots, or possibly, a difference in physiological responses of grasses to herbivory. This was in line with our hypothesis that wiregrass was a lower quality or less digestible food source (see Guo et al., 2004, and Lewis et al., 1975; also see Chapter 3). There were no significant effects for roots greater than 0.5 mm, which suggests that RFIs prefer finer diameter roots (< 0.5 mm). Because cellulose content and C:N ratio tend to increase with root branching order (Guo et al., 2004), this observation also suggests that tissue quality may influence herbivore feeding patterns.

Fire Treatment and Fine Root Ingrowth

The burn exclusion treatment (UB) appeared to increase fine root (< 0.5 mm dia.) productivity relative to the frequent burning (B), (Figure 4-2, a). Though mixed model analysis suggested that effects on fine root ingrowth due to fire treatment were not significant (Table 4-2, b), these fixed effect sizes may have been underestimated, because including sites and plots (nested within sites) as covariates reduced power to detect the effect of fire treatment. Mixed model analysis revealed a fire by insecticide treatment interaction, but only when grass and non-grass fine roots were combined (Table 4-2, b). This interactive effect is also illustrated by the larger responses of grass and non-grass ingrowth to the insect removal treatment in UB than in B fire treatments (Figure 4-3, a, b). The influence of frequent burning on wiregrass productivity may overwhelm any top-down influences from fine root herbivores when the herbivores occur in low densities.

Spatial and Temporal Variability of Top-Down Effects Dampen Net Influence

Fine root productivity varied substantially with sampling period (Table 4-2, a; Figure 4-2, b), and to a lesser extent with site (Table 4-2, a). Insect removal treatment differences (C vs. T cores) were more apparent in some sites and during some ingrowth periods than others (site and

sampling period effects were an important source of covariance in mixed models; see Table 4-2). Spatial and temporal variation at the within-plot scale was also apparent (see Figure 4-4). The near zero (slightly positive) median of top-down herbivore effects, despite the significant positive effects of herbivores on fine root ingrowth in mixed models, may be due to differences in how spatial heterogeneity was addressed in these two analytical approaches. We attempted to account for spatial clustering of ingrowth samples in mixed models by including plot as a covariate; whereas, the other analytical approach generated a separate estimate of treatment effect within each plot. Therefore, the median top-down effect measured across all of the individual plots (combined time periods) for total fine root ingrowth ($+ 8.9 \text{ g} \cdot \text{m}^{-2} \cdot \text{y}^{-1}$), may be a reasonable estimate of the net impact of RFI assemblages on fine root productivity in longleaf pine-wiregrass ecosystems with low herbivore population densities (< 10 individuals per square meter). These top-down effects were highly variable, and the median effect was small relative to our estimate of median fine root production ($104 \text{ g} \cdot \text{m}^{-2} \cdot \text{y}^{-1}$); therefore the impact of low densities of herbivores were relatively unimportant in relation to annual net primary productivity ($< 10\%$).

Top-Down Effect Magnitude was Not Correlated with Herbivore Abundance or Mass

Contrary to our prediction, we observed no correlation between top-down herbivory effects and RFI relative abundance or total biomass within plots. Other sources of variability, like resource availability and disturbance frequency, and the limited range of abundances measured in the system, may have overwhelmed our ability to detect a relationship. There was a evidence for a very weak positive relationship between RFI relative abundance and herbivory effect in UB, but not B plots (Figure 4-6, b). This fits with observations that the effects of herbivore removal treatment were only significant for UB sites, where herbivore population

densities were approximately 1.8 times greater than in B sites (see Table 4-4 and Figure 4-3, a-c). Perhaps at higher herbivore densities, it would be more likely to detect correlation. Based on observations of crop damage due to root-feeding insect infestations (Fermanian et al., 2002; Dalthope et al., 2000; Lura and Nyren, 1992), there may be some threshold of abundance after which the slope becomes significantly negative; therefore, the relationship between herbivore abundance and herbivory effects may be non-linear across a wider range of RFI population densities.

Potential Pitfalls

The study did not account for possible effects on roots from root-feeding nematodes, entomopathogenic nematodes, or fossorial mammals. The pesticide used was broad spectrum and may have had significant toxicity to both soil insects and nematodes. Therefore, some observed top-down effects could be related to nematode activity. In a study of the fates of fine roots in longleaf pine forests (conducted in nearby sites), the authors suggested that densities of both insect larvae and root-feeding nematodes were very low (Carter et al., 2004). Tri-trophic interactions by entomopathogenic nematodes (natural enemies of some RFIs) were not considered, but if significant, could have dampened top-down effects attributable to RFIs. No evidence of insect mortality due to nematodes was found. No evidence of tunneling or burrowing by mammals or gopher tortoises was observed in the direct vicinity of study plots.

Chlorpyrifos has been shown to be an effective pesticide in another study (Stevens and Jones, 2006) and is commonly used in turf management and agricultural settings. Though we did not test the effectiveness of the insecticide directly, these sorts of direct tests have been done by others (see Stevens et al. 2006). In this study, insecticide was mixed directly to ingrowth core soil at about 10 times the application rate used by Stevens and Jones (2006); furthermore,

additional chlorpyrifos was scattered over the treated subplots at about 5 times the manufacturers recommended application rate; therefore we expected the removal treatment to have caused at least some mortality within treated (T) subplots. During installation of root ingrowth cores, 33 larvae were found in control (C) subplots versus 25 larvae in treatment subplots (T). This tally included the larvae collected during the first installation period, which was prior to insecticide application to the T subplot, but some larvae were found in C and T plots during every ingrowth core installation period. This suggested that the broadcasting of insecticide on soil surface within the area of the T subplots, did not effectively remove larvae from the subplots. Nevertheless, larvae appeared to have been effectively removed within the actual ingrowth cores. Throughout the duration of the study, we found 8 larvae in harvested C cores, and no larvae in harvested T cores. Perhaps the insecticide was more effective within the ingrowth core boundaries because insecticide was applied to cores at a higher rate and mixed directly with ingrowth core soil during installation.

We considered the possibility that fine-scale (within plot) heterogeneity of herbivore distributions may have limited our ability to detect a treatment effect at the sampling intensity used during the study (4 ingrowth cores per 4 m x 4 m plot). Therefore, in 8 additional plots (not included in the main analysis), we increased the number of ingrowth cores by a factor of 5. However, increased sampling intensity did not improve our ability to detect an effect within plots. The intensive sampling reinforced our observations from the rest of the study that top-down effects were highly variable in magnitude and direction. But the intensively sampled plots did not detect a net compensatory effect, possibly due to low replication at the plot level (N=8), or possibly because net effects are near zero.

Recommendations for Future Research

We recommend a similar field experiment or common garden experiment that would use multiple levels of herbivore additions along with herbivore removal and control treatments. This might improve our understanding of the relationship between population density and top-down effects (as in McNaughton, 1985) due to root-feeding insects. Goals of these studies would include identifying population density thresholds at which top-down effects become net negative, and quantifying the relationship with effect magnitude as population densities increase beyond this threshold.

CONCLUSIONS

At the low herbivore densities measured in this system, top-down effects from herbivores were weakly positive but unimportant relative to annual net fine root productivity. The positive effect was only detectable in non-grass roots less than 0.5 mm diameter. This could be due to a feeding bias against wiregrass and roots > 0.5 mm diameter, or due to differing physiological responses of roots to herbivores for wiregrass relative to other C₃ plants in the ecosystem. No significant correlations were detected between RFI biomass or relative abundance and fine root standing crop or ingrowth. Top-down effects of herbivores measured within plots were spatially and temporally variable, both in magnitude and direction. Because median effect sizes approximated zero, top down effects from the low population densities of RFIs in the longleaf pine-wiregrass ecosystems studied were probably unimportant for fine root dynamics at the ecosystem scale.

LITERATURE CITED

- Arimura, G., R. Ozawa, S. Kugimiya, J. Takabayashi, and J. Bohlmann. 2004. Herbivore-induced defense response in a model legume. Two-spotted spider mites induce emission of (*E*)-*B*-Ocimene and Transcript Accumulation of (*E*)-*B*- Ocimene Synthase in *Lotus japonicus*. *Plant Physiology* **135**:1976-1983.
- Andersen, D.C. 1987. Below-ground herbivory in natural communities: a review emphasizing fossorial animals. *The Quarterly Review of Biology* **62**:261-286.
- Bennington, C.C. and W.V. Thyne. 1994. Use and misuse of mixed model analysis of variance in ecological studies. *Ecology* **75**:717-722.
- Brown, V.K. and A.C. Gange. 1990. Insect herbivory below ground. *Advances in Ecological Research* **20**:1-58.
- Brown, V.K. and A.C. Gange. 1989. Differential effects of above- and belowground insect herbivory during early plant succession. *Oikos* **54**:67-76.
- Dalthorp, D., J. Nyrop, and M.G. Villani. 2000. Spatial Ecology of the Japanese Beetle *Popillia Japonica*. *Entomologia Experimentalis et Applicata* **96**:129-139.
- Drew, M.B., L.K. Kirkman, and A.K. Gholson, Jr. 1998. The vascular flora of Ichauway, Baker County, Georgia: A remant longleaf pine/wiregrass ecosystem. *Castanea* **63**:1-24.
- Eissenstat, D. M., and R. D. Yanai. 2002. Root Life Span, Efficiency, and Turnover. Pages 221-238 in Y. Waisel, A. Eshel, and U. Kafkafi, editors. *Plant Roots: The Hidden Half*. Marcel Dekker, Inc., New York.
- Fahey, T. J., and J. W. Hughes. 1994. Fine root dynamics in a northern hardwood forest ecosystem, Hubbard Brook Experimental Forest, NH. *Journal of Ecology* **82**:533-548.
- Fermanian, T.W., M.C. Shurtleff, R. Randell, H.T. Wilkinson, and P.L. Nixon. 2002. *Controlling Turfgrass Pests* 3rd Ed. Prentice-Hall, New York.

- Goebel, P.C., B.J. Palik, L.K. Kirkman, and L. West. 1997. *Field Guide: Landscape Ecosystem Types of Ichauway*. Technical Report No. 97-1. Joseph W. Jones Ecological Research Center at Ichauway, Newton, Georgia.
- Grelen, H.E. 1978. Forest grazing in the South. *Journal of Range Management* **31**:244-250.
- Guo, D.L., R.J. Mitchell, and J.J. Hendricks. 2004. Fine root branch orders respond differentially to carbon source-sink manipulations in a longleaf pine forest. *Oecologia* **40**:457-457.
- Hendrick, R., and K. S. Pregitzer. 1992. The demography of fine roots in a northern hardwood forest. *Ecology* **73**:1094-1104.
- Hendricks, J.J., R.L. Hendrick, C.A. Wilson, R.J. Mitchell, S.D. Pecot, and D. Guo. 2006. Assessing the patterns and controls of fine root dynamics: an empirical test and methodological review. *Journal of Ecology* **94**:40-57.
- Hunter, M.D. 2001. Out of sight, out of mind: the impacts of root-feeding insects in natural and managed systems. *Agriculture and Forest Entomology* **3**:3-9.
- Hunter, M.D. and P.W. Price. 1992. Playing chutes and ladders: Heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology* **73**:724-732.
- Jansen, N.A. 2007. Influence of root branching order on fine root substrate quality and decomposition in a *Pinus palustris* ecosystem. Master's Thesis: University of Florida.
- Lewis, C.E., R.S. Lowrey, W.G. Monson, and F.E. Knox. 1975. Seasonal trends in nutrients and cattle digestibility of forage on pine-wiregrass range. *Journal of Animal Science* **41**:208-212.
- Lura, C.L. and P.E. Nyren. 1992. Some effects of a white grub infestation on northern mixed-grass prairie. *Journal of Range Management* **45**:352-354.
- Marquis, R.J. 1992. Selective impact of herbivores. In: *Plant Resistance to Herbivores and Pathogens: Ecology, Evolution and Genetics*. R.S. Fritz and E.L. Simms (Eds). University of Chicago Press, Chicago, pp. 301-325.

- McNaughton, S.J. 1985. Ecology of a grazing ecosystem: The Serengeti. *Ecological Monographs* **55**:259-294.
- Mitchell, R.J., L.K. Kirkman, S.D. Pecot, C.A. Wilson, B.J. Palik, and L.R. Boring. 1999. Patterns and controls of ecosystem function in longleaf pine-wiregrass savannas. I. Aboveground net primary productivity. *Canadian Journal of Forest Research* **29**:743-751.
- Nadelhoffer, K. J., and J. W. Raich. 1992. Fine root production estimates and belowground carbon allocation in forest ecosystems. *Ecology* **73**:1139-1147.
- Ostonen, I., K. Lohmus, and Katrin Pajuste. Fine root biomass, production and its proportion of NPP in a fertile, middle-aged Norway spruce forest: Comparison of soil core and ingrowth core methods. *Forest Ecology and Management* **212**:264-277.
- Parmesan, C. 2000. Unexpected density-dependent effects of herbivory in a wild population of the annual *Collinsia torreyi*. *Journal of Ecology* **88**:392-400.
- Price, P. W., Bouton, C.E., Gross, P., McPheron, V.A., Thompson, J.N., Weis, A.E. 1980. Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annual Review Ecology and Systematics* **11**:41-65.
- Ritcher, P.O. 1966. *White Grubs and Their Allies: A Study of North American Scarabaeoid Larvae*. Studies in Entomology Number Four. Oregon State University Press, Corvallis, Oregon.
- Stehr, F.W. 1991. *Immature Insects* (Vol. 2). Kendall-Hunt Publishing Co., Dubuque, Iowa.
- Stevens, G. N. 2005. Trophic dynamics in the fine-root based food web: integrating resource heterogeneity, root herbivores, and root foraging. Dissertation. Virginia Polytechnic Institute and State University, Blacksburg.
- Stevens, G. N., and R. H. Jones. 2006. Patterns in soil fertility and root herbivory interact to influence fine-root dynamics. *Ecology* **87**:616-624.

- Stevens, G. N., R. H. Jones, and R. J. Mitchell. 2002. Rapid fine root disappearance in a pine woodland: a substantial carbon flux. *Canadian Journal of Forest Research* **32**:2225-2230.
- Vogt, K. A., C. C. Grier, and D. J. Vogt. 1986. Production, turnover, and nutrient dynamics of above- and belowground detritus of world forests. *Advances in Ecological Research* **15**:303-377.

TABLES

Table 4-1: Univariate statistical summary of fine root ingrowth production estimates ($\text{g} \cdot \text{m}^{-2} \cdot \text{y}^{-1}$). The average root ingrowth dry mass within each control plot was calculated for each ingrowth time period. The sums of the 3 ingrowth periods within each plot were then used to generate these estimates ($N = 54$).

Root Ingrowth Class	Min	Max	Median	Mean	Std. Dev.	C.V.
Grass <0.5mm	3.23	84.2	23.7	29.7	20.4	0.685
Grass >0.5mm	0	61.2	4.25	7.65	10.3	1.35
Other <0.5mm	14.5	291	61.1	88.2	63.7	0.722
Other >0.5mm	0	137	26.5	35.5	31.7	0.892
Total <0.5mm	38.1	304	104	118	61.6	0.522
Total >0.5mm	0.359	151	34.3	43.2	34.2	0.792

Table 4-2, a: Mixed model estimates of covariance components (random effects). Fine roots are further categorized as grass, other, or combined roots, and by 2 diameter classes (< 0.5 mm and ≥ 0.5 mm). The parameter *Plot(Site(Fire))* was included in the mixed models in order to account for repeated measures within plots and the spatial clustering of ingrowth samples within sites; parentheses indicate nesting.

Output Summary for Linear Mixed Model Analysis						
<i>Estimates of Covariance Components</i>						
Random Effect	Grass Ingrowth (g/m ²)		Other Ingrowth (g/m ²)		Total Ingrowth (g/m ²)	
	<0.5 mm	≥0.5 mm	<0.5 mm	≥0.5 mm	<0.5 mm	≥0.5 mm
Ingrowth Period	7.341	0.3052	138.8	55.45	248.8	79.95
Ingrowth Period*Fire	0.7336	0.1142	64.15	15.30	43.58	7.480
Site (Fire)	5.362	0.4045	62.64	1.815	56.12	3.006
Plot (Site (Fire))	7.007	0.03411	31.23	0.6368	45.01	1.596
Error Variance	32.99	5.999	201.3	65.20	243.3	83.52

Table 4-2, b: Mixed model estimates of Type III Fixed Effects for *Fire*, *Insecticide*, and *Fire*Insecticide* on root ingrowth mass (g/m²). Fine roots are further categorized as grass, other, or combined roots, and by 2 diameter classes (< 0.5 mm and ≥ 0.5 mm).

(b) Output Summary for Linear Mixed Model Analysis				
<i>Type III Tests for Fixed Effects</i>				
Root Class / Fixed Effect	Num df	Denom df	F-ratio	p-value
<u>Grass Ingrowth <0.5 mm</u>				
Fire	1	2	4.648	0.1638
Insecticide	1	236	0.0474	0.8278
Fire*Insecticide	1	236	0.3380	0.5615
<u>Grass Ingrowth ≥0.5 mm</u>				
Fire	1	2	1.504	0.3449
Insecticide	1	236	0.1647	0.6853
Fire*Insecticide	1	236	0.1805	0.6713
<u>Other Ingrowth <0.5 mm</u>				
Fire	1	2	3.155	0.2177
Insecticide	1	236	3.554	*0.0606
Fire*Insecticide	1	236	2.066	0.1520
<u>Other Ingrowth ≥0.5 mm</u>				
Fire	1	2	6.251	0.1296
Insecticide	1	236	2.138	0.1450
Fire*Insecticide	1	236	1.239	0.2669
<u>Total Ingrowth <0.5 mm</u>				
Fire	1	2	1.759	0.3159
Insecticide	1	236	4.441	**0.0361
Fire*Insecticide	1	236	3.998	**0.0467
<u>Total Ingrowth ≥0.5 mm</u>				
Fire	1	2	7.542	0.1110
Insecticide	1	236	1.477	0.2255
Fire*Insecticide	1	236	0.6394	0.4247

Significance: * $\alpha \leq 0.1$; ** $\alpha \leq 0.05$.

Table 4-3: Univariate statistical summary for estimates of top-down effects from root herbivores, based on the average difference (control minus insecticide treated cores) in fine root ingrowth ($\text{g} \cdot \text{m}^{-2} \cdot \text{y}^{-1}$) within each plot (N = 54).

Root Ingrowth Class	Min	Max	Median	Mean	Std. Dev.	C.V.
Grass <0.5mm	-75.0	65.9	1.84	2.79	20.5	7.35
Grass >0.5mm	-19.5	62.7	0.307	2.15	12.1	5.61
Other <0.5mm	-161	197	11.6	15.9	55.0	3.46
Other >0.5mm	-326	119	-1.04	-1.28	57.4	-44.8
Total <0.5mm	-95.0	194	8.89	18.7	51.3	2.75
Total >0.5mm	-263	8.89	-0.491	0.871	52.2	59.9

Table 4-4: Summary of root-feeding insect counts totaled for unburned (UB), burned (B), and combined fire frequency treatments. Insects were sampled from plots during 6 events.

TAXONOMIC GROUP	# Individuals		
	<i>UB</i>	<i>B</i>	<i>Combined</i>
COMBINED TAXA	696	409	1105
White Grubs	157	140	297
Wireworms	108	44	152
Weevils	297	114	411
Cicadas	9	12	21
TAXONOMIC GROUP	# Individuals/m²		
	<i>UB</i>	<i>B</i>	<i>Combined</i>
COMBINED TAXA	6.5 ± 2.5	3.7 ± 1.8	5.1 ± 1.9
White Grubs	1.7 ± 2.9	1.6 ± 2.1	1.6 ± 1.8
Wireworms	2.0 ± 2.3	0.8 ± 1.8	1.4 ± 1.8
Weevils	2.6 ± 3.5	1.1 ± 1.9	1.8 ± 2.3
Cicadas	0.2 ± 2.5	0.2 ± 1.9	0.2 ± 1.8
TAXONOMIC GROUP	Avg. Mass/Individual		
	<i>UB</i>	<i>B</i>	<i>Combined</i>
COMBINED TAXA	36.1 ± 59.1	34.8 ± 44.8	35.5 ± 53.5
White Grubs	42.2 ± 58.2	54.6 ± 60.0	48.5 ± 58.8
Wireworms	6.7 ± 8.3	7.5 ± 6.1	7.0 ± 7.6
Weevils	40.2 ± 74.7	12.0 ± 16.2	30.1 ± 62.0
Cicadas	32.2 ± 30.3	67.6 ± 62.0	52.5 ± 50.0

Table 4-5: Summary of Pearson correlation coefficients for one-way relationships among the fine root ingrowth response to herbivory (C - T ingrowth cores), total RFI biomass, RFI relative abundance (square root of cumulative plot count), and fine root standing crop.

Fine Root Ingrowth Response (g/m²)	RFI Mass (mg)	Sq. Rt. RFI Count	Std. Crop <0.5mm	Std. Crop <1.0mm
	<i>r</i>	<i>r</i>	<i>r</i>	<i>r</i>
Grass				
<0.5mm	0.0479	0.0704	0.235	0.157
≥0.5mm	-0.00260	0.00784	0.246	0.240
Total	0.0326	0.0535	0.282	0.224
Non-grass				
<0.5mm	0.0787	0.0398	-0.273	-0.214
≥0.5mm	0.0734	-0.00873	-0.286	-0.220
Total	0.0907	0.0114	-0.338	-0.262
Combined				
<0.5mm	0.118	0.0114	-0.338	-0.262
≥0.5mm	0.0731	-0.00676	-0.224	-0.159
Total	0.108	0.0347	-0.234	-0.179

Fine Root Standing Crop (g/m²)	RFI Mass (mg)	Sq. Rt. RFI Count
	<i>r</i>	<i>r</i>
Combined		
<0.5mm	-0.149	-0.0376
<1.0mm	-0.133	-0.0681

FIGURES

Figure 4-1: Conceptual diagram showing the layout of plots. Plot centers were subdivided into 4 quadrants. Small squares represent haphazardly located insect samples, with different colors denoting different sampling periods. Plot peripheries were divided into 2 halves. Circles represent fine root ingrowth cores, with color denoting ingrowth harvest periods. See Methods for specific sampling date ranges. Symbols are not drawn to scale. To remove fine root herbivores, one side of each plot (shaded area) was treated with insecticide (granular chlorpyrifos) during each ingrowth core installation period in order to remove fine root herbivores (shaded area).

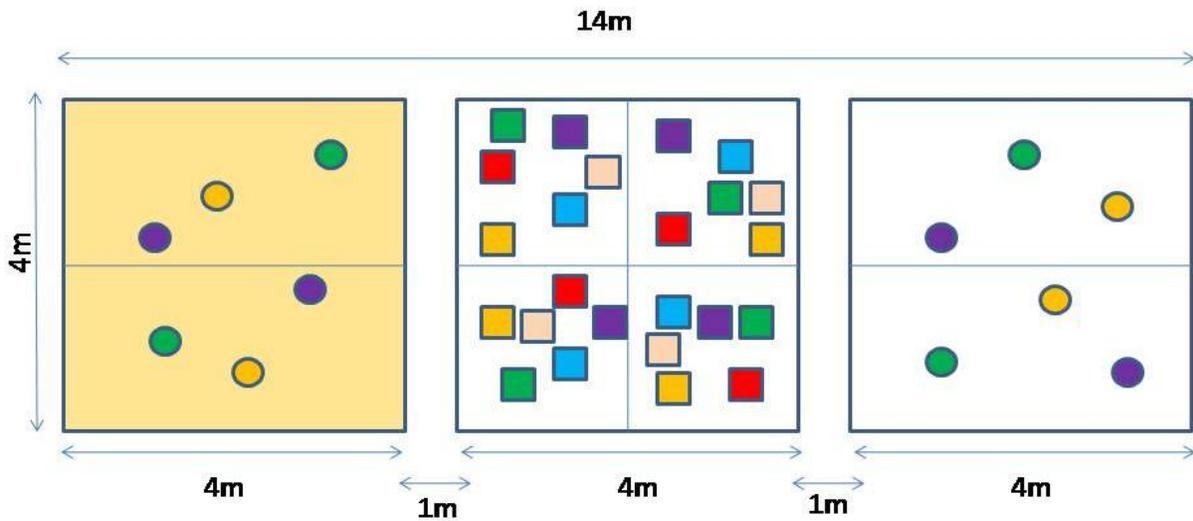


Figure 5-2, a-c: Box and whisker plots of total fine root (<0.5mm) ingrowth. Boxes represent median and inner-quartile range; whiskers represent the range with outliers trimmed; symbols represent outliers. **a:** Unburned (UB) and burned (B) sites. **b:** Three consecutive ingrowth time frames. **c:** Control (C) and insecticide treated (T) ingrowth cores.

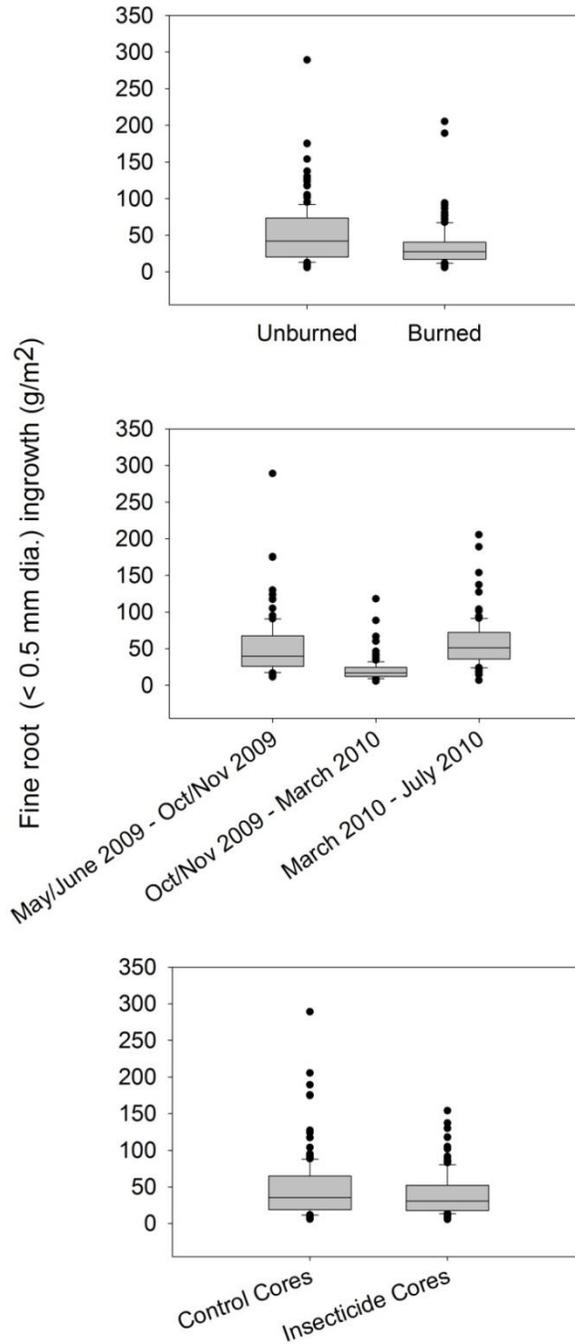


Figure 5-3, a-c: Mean fine root (<0.5mm) ingrowth, + 2 standard errors. Ingrowth (g/m^2) was averaged across three ingrowth periods spanning May 2009 to October 2010 (average ingrowth time interval was about 20 weeks). Two fire treatments, unburned (UB) and burned (B) were applied at the site level, while two herbivore treatments, control (C) and insecticide treated (T) were applied within plots. Significant differences between C and T treated cores within UB or B sites are denoted by asterisks placed between the bars that differ: *: $p \leq 0.10$, **: $p \leq 0.05$.
a: Grass roots; **b:** other roots; **c:** combined roots.

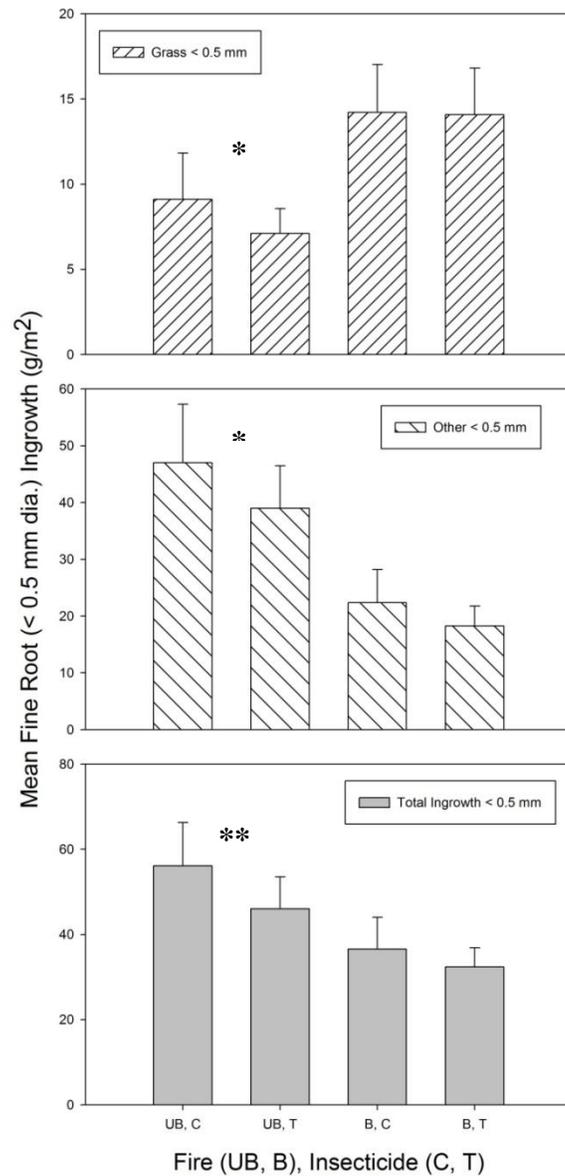


Figure 5-4, a, b: Box and whisker plots of the difference of control (C) and insecticide treated (T) ingrowth cores within plots. Boxes represent the median and quartile distribution of top down effects of herbivores within plots (C minus T ingrowth). Symbols represent outliers. Negative effects represent increased productivity in insecticide treated (T) ingrowth cores; positive effects represent decreased productivity in insecticide treated (T) ingrowth cores. For each consecutive ingrowth period, unburned (UB) and burned (B) plots are shown side by side. Ingrowth periods were May/June 2009 to Oct./Nov. 2009 (~5mo.), to March 2010 (~5mo.), to July 2010 (~4mo.). **a:** Grass roots; **b:** other (non-grass) roots.

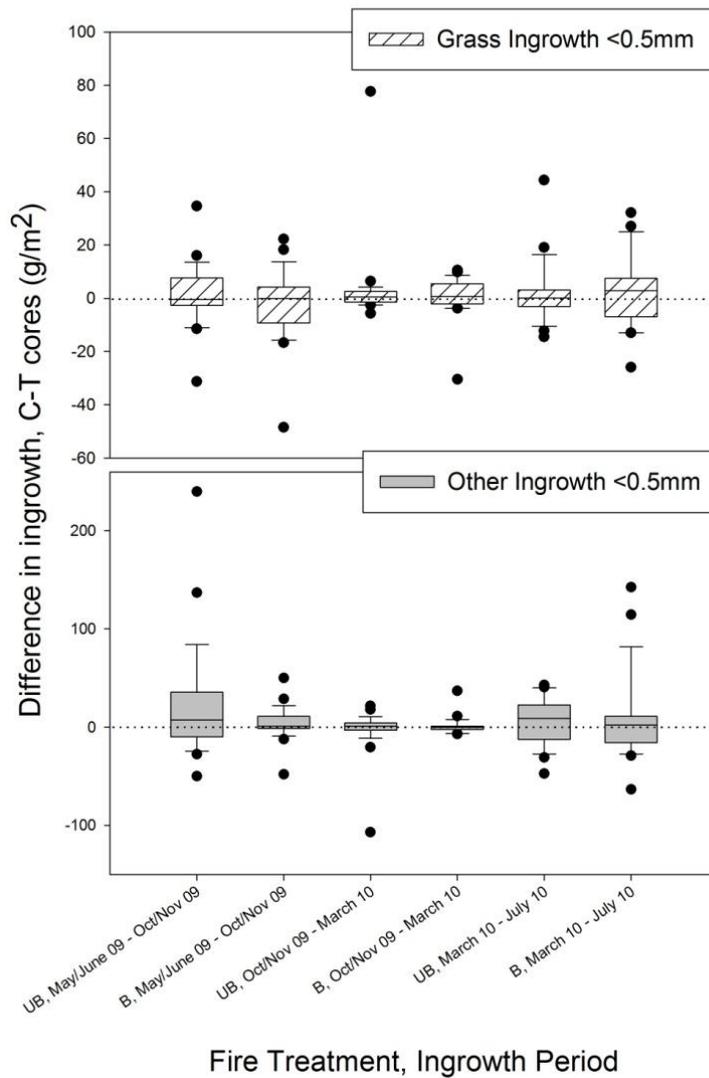


Figure 5-5: Root-feeding insect relative abundance and total biomass for unburned (UB) and frequently burned (B) fire treatments. **a:** Square-root-transformed insect count totals within plots (summed across 6 sampling periods). Boxes represent the median and inter-quartile range; symbols represent outliers. **b:** Total root-feeding insect biomass (mg) collected during 3 sampling periods.

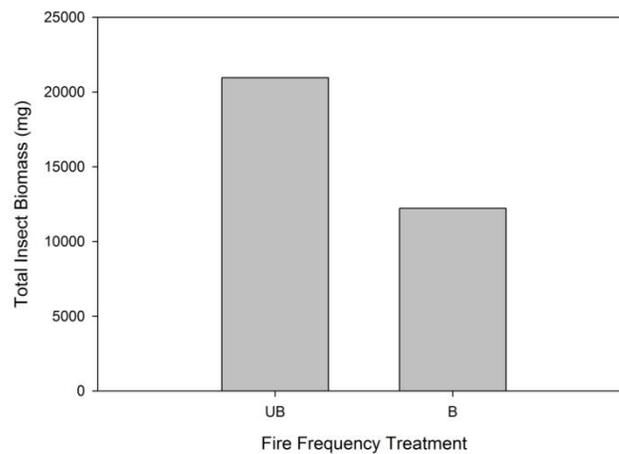
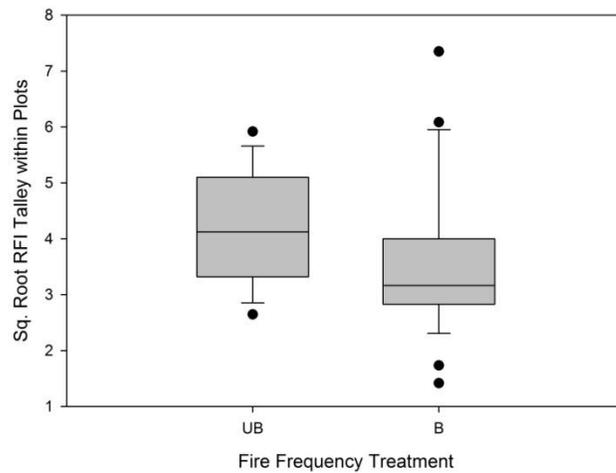
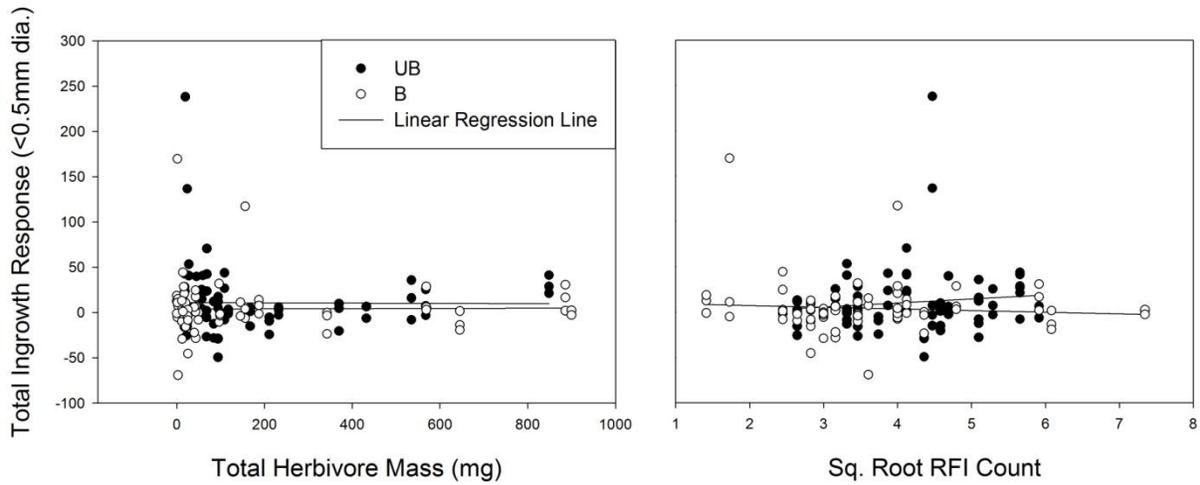


Figure 4-6, a, b: Scatter plots showing total fine root (< 0.5 mm) ingrowth response to herbivore removal (C minus T ingrowth cores) as a response to (a) cumulative herbivore biomass or (b) cumulative herbivore count. Scaling on vertical axes are identical. Unburned (UB) and burned (B) plots are shown as black and white circles, respectively. Linear regression lines (all non-significant at the 0.1 level) are shown separately for UB and B data.



CHAPTER 5

Synthesis

Matthew Dittler

SUMMARY

I used a series of natural and manipulative experiments to answer 3 overarching questions: **(1)** How much do fire and bottom-up factors (vegetative composition, soil properties, and fine root productivity) influence the structure, abundance, distribution, and population dynamics of root-feeding insect assemblages? **(2)** What types of roots do root-feeding insects eat, and do they forage selectively? **(3)** In what way, and by how much, do root-feeding insects influence ecosystem processes (i.e. fine root productivity)? Collectively, my study utilized repeated sampling of fine roots and root-feeding insects, across a set of 54 plots, which were evenly distributed across 3 frequently burned and 3 burn excluded (9 years) sites, along with one time measurements of understory plant composition, soil properties, and fine root standing crops within plots.

To determine the relationship among fire frequency, bottom-up factors, and the structure of insect assemblages (Question 1), we examined spatial-temporal, and taxonomic patterns of root-feeding insect abundances. Abundance, patchiness, and diversity responded positively to decreased burn frequency. The abundance of RFIs in the longleaf pine-wiregrass ecosystem was significantly influenced by additional bottom-up factors, particularly the fine root standing crop and soil NH_4^+ concentration, which suggested that adults may prefer more productive locations for oviposition, and/or that larvae migrate into more productive habitat patches, as has been

observed by Stevens and Jones (2006). Fine root herbivores were spatially aggregated from scales ranging from less than 0.25 m² to greater than 0.25 km², suggesting that mechanisms operating at different spatial scales may influence their spatial distribution. Finally, assemblages of the highest and lowest population densities tended to persist at similar densities over time, which could imply low rates of larval migration or consistent attraction of adults to particular locations prior to oviposition. Overall, my findings support the finding from other authors, that root herbivores may respond to nutrient rich, or more productive microsites (Stevens and Jones, 2006; De Deyn, et al., 2004; Brown and Gange, 1990), possibly by cuing in on CO₂ from root respiration or volatile emissions (van der Putten, 2003; Whittaker, 2003; Jones and Coaker, 1977), or possibly because of congregative behavior during oviposition.

I quantified the diet of root-feeding insects (Question 2) by measuring stable isotope (carbon and nitrogen) natural abundances of the insects. Using carbon isotope abundance, I examined the contribution of warm season grass roots to insect diet, relative to the proportion of warm season grass roots (< 1 mm) within root standing crop samples, collected from plots along with insects, to infer whether, and to what extent, insect diets deviated from the composition of available vegetation. Nitrogen isotope abundance was used to estimate the extent to which root-feeding insects were omnivorous. The diets of generalist root-feeding insect herbivores in the longleaf pine-wiregrass system were strongly influenced by the composition of local vegetation (their diets responded directly to altered fine root composition). There was considerable taxonomic variability, but overall, insects exhibited some evidence of facultative selective foraging, whereby they appeared to make subtle adjustments to maintain a mixed diet (i.e. reducing or increasing warm season grass consumption when its abundance was high or low, respectively). Some taxa differed in magnitude of dietary selectivity between frequently burned

(B) and burn excluded (UB) stands; however, fine root standing crop did not influence the selectivity of herbivore diets. I also showed that many herbivores are omnivorous, possibly amending their diet with decaying forest litter, fungi, or scavenged arthropods. Because herbivore diets were strongly influenced by the available composition of fine roots, their per capita top-down influences over vegetative composition may be unimportant.

I estimated the top-down influence of root-feeding insects on fine root productivity (Question 3) by measuring fine root productivity in response to an insect removal, paired with a control treatment within the study plots. In non-grass roots less than 0.5 mm diameter, I detected a positive (compensatory growth) effect of root-feeding insects on fine root productivity, though there was considerable variability; however, median effect sizes were near neutral, commensurate with the low population densities of fine root herbivores in the ecosystem (well below cited thresholds for crop damage). There was no significant correlation between top-down effect magnitude and herbivore abundance within plots.

IMPLICATIONS FOR ECOSYSTEM DYNAMICS

Because the effect of root-feeding insects on fine root productivity was near zero, the root herbivores I studied were probably unimportant for belowground processes at the ecosystem scale when compared to the effects of fire disturbance frequency, and resource availability. The weak effect of herbivores may reflect both their relatively low population densities in the system, and the very strong influence of frequent fires on understory composition and fine root dynamics in longleaf pine-wiregrass ecosystems. In fire maintained woodlands such as the longleaf pine-wiregrass savanna, the positive responses of root-feeding insects to reduced fire frequency may be an important consideration in the ecology of savannas and other woodland ecosystems,

particularly in light of increasing rates of woody encroachment within grassland-forest eco-tones in many parts of the world (Ratajczak et al., 2012; Knapp et al., 2008; Breshears, 2006). The frequency of fire regulates vegetative composition and affects the abundance of root-feeding insects. Therefore, long durations between disturbance events might lead to higher herbivore abundance and diversity, which at some threshold of abundance could lead to a net negative impact of root-feeding insects on belowground carbon sequestration.

In the longleaf pine-wiregrass savanna, there are several interacting ecosystem processes that may be important to consider in studies of belowground herbivory and ecosystem dynamics. Figure 5-1 is an attempt to conceptualize principal findings of my research with regards to an ecosystem context. I considered several ecological interactions that are potentially important for the ecology of root-feeding insects in fire-driven woodlands. The weighting of the arrows corresponds to the hypothetical importance of each interaction. For interactions addressed by our study, hypothetical weights were inferred from the information-yield analysis and from significance levels of "best fit" models for the population density of root-feeding insect assemblages (see Chapter 2). Not all of the interactions included in Figure 5-1 were addressed by our study, in these cases, weights of arrows are purely speculative, but are based on reasonable assumptions. Fire is portrayed as a major driver of vegetative composition. My research suggested that vegetative composition is a driver of root-feeding insect abundances. Though not a component of my study, vegetative composition across forest-grassland continua are likely to influence fine root productivity and standing mass, as has been shown by Reich et al. (2001). My study suggested that soil N availability might have a positive effect on root-feeding insect abundance. Effects of soil N on fine root dynamics was not assessed in this study, but was inferred based on other work relating to soil fertility and fine root ecology (e.g.

Hendricks et al., 2005). Because important interactions may involve complex pathways, future research in this field might benefit from experiments and analytical approaches that incorporate the use of path analysis. This would allow conceptual models such as Figure 5-1 to be refined and tested.

SIGNIFICANCE OF THIS RESEARCH

The knowledge gained from this work about bottom-up influences on root-feeding insect assemblages, their dietary ecology, and their weak top-down influences on ecosystems, contributes to improved understanding of root-feeding insect ecology and belowground ecosystem processes within the grassland-forest continuum (see Breshears, 2006). My research on the effects of fire, vegetative composition, soil fertility, and fine root standing stock on root-feeding insect abundances suggested that the vegetative changes associated with decreased fire frequency in the longleaf pine-wiregrass ecosystem (decreased wiregrass and increased woody plant cover), have a significant positive effect on the population density and diversity of root-feeding insect assemblages. My research on the diets and foraging behavior suggested that root-feeding insects have non-specialized diets, but that they may disproportionately favor less abundant plant types. Finally, my research on the top-down effects of root-feeding insects suggested that at low densities they do not have a net negative effect on fine root net productivity, and may even have a small net positive effect.

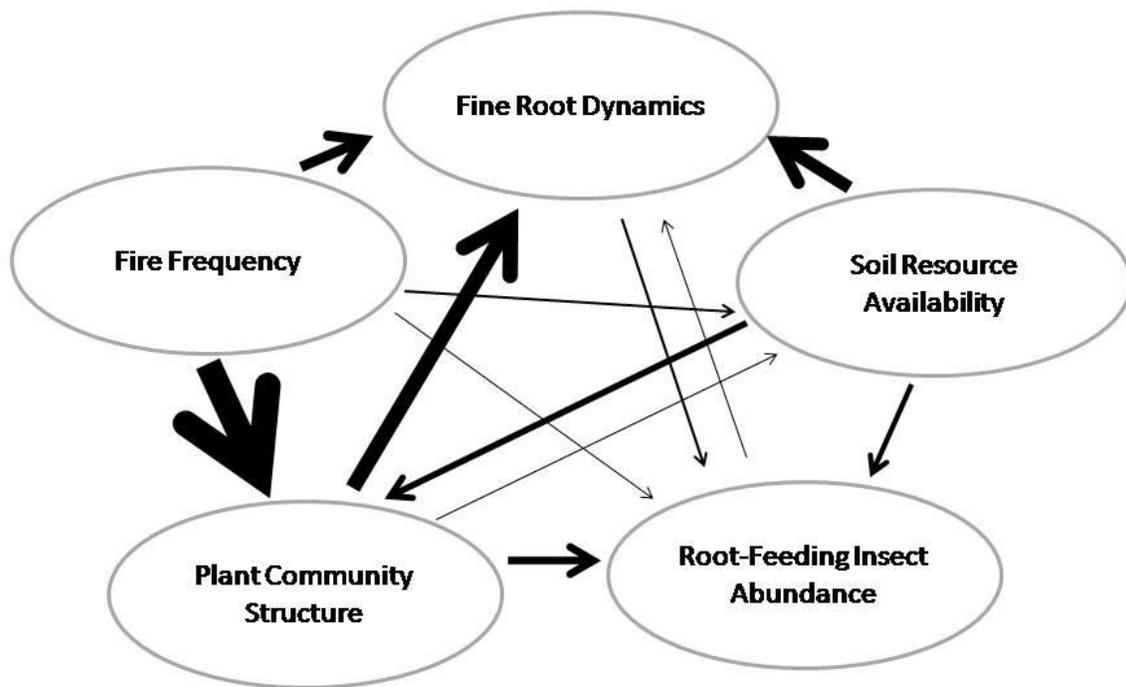
LITERATURE CITED

Breshears, D.D. 2006. The grassland-forest continuum: Trends in ecosystem properties for woody plant mosaics? *Frontiers in Ecology and the Environment* 4:96-104.

- Brown, V.K. and A.C. Gange. 1990. Insect herbivory below ground. *Advances in Ecological Research* **20**:1-58.
- De Deyn, G.B., C.E. Raaijmakers, and W.H. van der Putten. 2004. Plant community development is affected by nutrients and soil biota. *Journal of Ecology* **92**:824-834.
- Hendricks, J.J., R.L. Hendrick, C.A. Wilson, R.J. Mitchell, S.D. Pecot, and D. Guo. 2006. Assessing the patterns and controls of fine root dynamics: an empirical test and methodological review. *Journal of Ecology* **94**:40-57.
- Knapp, A.K., J.M. Briggs, S.L. Collins, S.R. Archer, M.S. Bret-Harte, B.E. Ewers, D.P. Peters, D.R. Young, G.R. Shaver, E. Pendall, and M.B. Cleary. 2008. Shrub encroachment in North American grasslands: Shifts in growth form dominance rapidly alters control of ecosystem carbon inputs. *Global Change Biology* **14**:615-623.
- Ratajczak, Z., J.B. Nippert, and S.L. Collins. 2012. Woody encroachment decreases diversity across North American grasslands and savannas. *Ecology* **93**:697-703.
- Reich, P.B., D.W. Peterson, D.A. Wedin, and K. Wragge. 2001. Fire and vegetation effects on productivity and nutrient cycling across a forest-grassland continuum. *Ecology* **82**:1703-1719.
- Stevens, G. N., and R. H. Jones. 2006. Patterns in soil fertility and root herbivory interact to influence fine-root dynamics. *Ecology* **87**:616-624.
- van der Putten, W. H. 2003. Plant defense belowground and spatiotemporal processes in natural vegetation. *Ecology* **84**:2269-2280.
- Whittaker, J. B. 2003. Root-animal interactions. Pages 363-385 *in* H. de Kroon and E. J. W. Visser, editors. *Root Ecology*. Springer, New York.

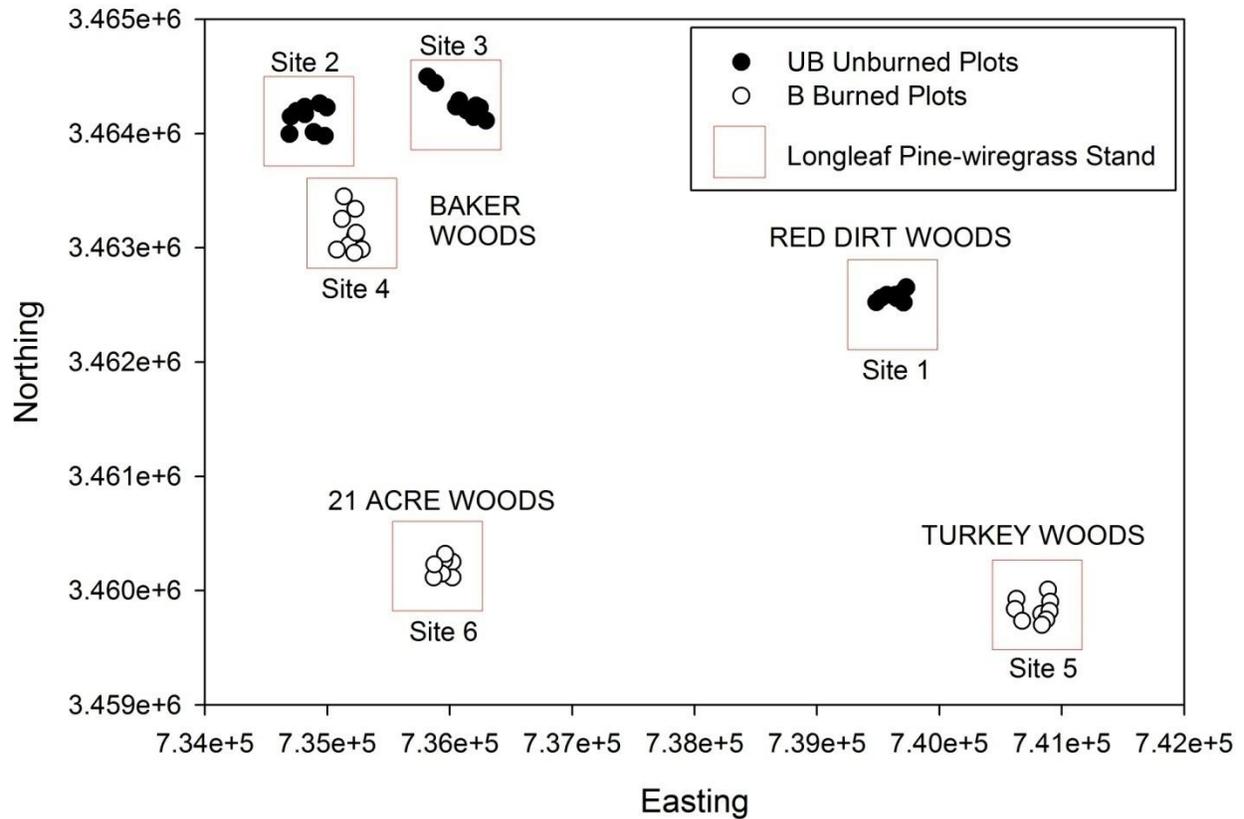
FIGURES

Figure 5-1: Conceptual diagram showing potential interactions between root-feeding insect abundance and other components related to root-feeding insects and fine root productivity in fire-manipulated woodland ecosystems. Arrow weighting reflects hypothetical importance in an ecosystem dynamics context, based on speculation, inference from this research, and other studies.



APPENDIX A

This figure shows site and plot locations; GPS coordinates (Easting and Northing) were recorded from plot centers. A burn exclusion treatment was randomly applied to sites; the spatial pattern is coincidental. Symbols for plots and sites are not drawn to scale.



APPENDIX B

This appendix provides additional justification for the data analyses used in Chapter 2.

USE OF THE NEGATIVE BINOMIAL DISTRIBUTION TO ADDRESS OVERDISPERSION OF COUNT DATA

There are several challenges associated with the analysis of count data (Sileshi, 2008, 2006). There inherently are a large number of zeros and distributions tend to be skewed towards lower counts (Sileshi, 2008). Experimental variances are often compared with a theoretical distribution to assess the rate of dispersion. An excess of zeros tends to lead to overdispersion, resulting in greater observed variance than the theoretical distribution (Sileshi, 2008). Also, heterogeneity of variances among experimental groups and populations are common (Sileshi, 2008, Taylor, 1961). Entomological studies have often relied on statistics based on assumptions of normality to assess treatment affects on count-derived response variables; however, doing so does not account for the heterogeneity of variance and overdispersion often associated with count data, and can lead to inaccurate estimates of effect sizes and associated error (Sileshi, 2008). One of the most reliable and appropriate ways to address overdispersion of data is to incorporate an extra parameter to account for heterogeneity of variances among experimental groups; therefore, the negative binomial distribution was well-suited for this type of data analysis, as has been demonstrated in other studies (Ismail and Jemain, 2007; Sileshi, 2006; White and Bennets, 1996). The use of Marginal Longitudinal Generalized Linear Models via Generalized Estimating Equations (GEEs), provided a statistically sound method of accounting for the lack of independence between repeated measures. Furthermore, using the negative binomial distribution in the GEEs accounted for the overdispersion of RFI count data.

MODEL SELECTION USING AN INFORMATION THEORETIC APPROACH

Researchers in ecology and evolutionary biology have begun to change the way they draw inference from models by adopting a model selection approach in which a set of competing hypotheses are evaluated given the data (Johnson and Omland, 2004). This Information Theoretic (IT) approach can be used to identify a "best" model, or to produce weighted estimates based on the relative amount of model support (Burnham et al., 2011; Johnson and Omland, 2004). Common practice of the past has been to test an alternative hypothesis against a null hypothesis and then to base dichotomous inference off of test statistics and p-values (Burnham et al., 2011; Bolker et al., 2008). In classical hypothesis testing, the p-value is the probability of obtaining a test statistic at least as large as the computed (observed) statistic, assuming that the null hypothesis is true. It is therefore not direct evidence in support of the alternate hypothesis (Burnham et al., 2011; Royall, 1997). Researchers often redefine p-values to be the probability of the null given the data; however, this is incorrect and can lead to poor inference (Burnham et al., 2001).

Model selection is a critically important phase of any model-based analysis, so care should be taken to assure that it is done in a thoughtful way. Often data are simply run through an automatic selection procedure (e.g. backwards selection) resulting in a subset of variables with significant p-values (Burnham et al., 2011). However, it is possible to find significant relationships with unimportant variables, or to miss important relationships as a consequence of the following an automatic procedure, particularly in datasets with large numbers of (Whittingham et al., 2006). IT model selection, in contrast, is based on weighting a set of competing hypotheses, in terms of the amount of additional information that variables add to the model, relative to the additional complexity. Therefore, model selection should begin with the

formation of a set of meaningful, competing statistical hypotheses. Ideally, these would be hypotheses that are formed based on critical thinking and available understanding of the variables thought to be important to the response of interest (Johnson and Omland, 2004). Various *Information Criteria* (IC), such as *Akaike Information Criterion* (AIC), provide a measure of model fit based on deviance that penalizes for higher complexity (number of parameters). Quasi-likelihood-based IC (QIC) are suitable when data are overdispersed (Burnham et al., 2011). Comparison of QIC and QIC_u values across the set of models allowed a comparison of the strength of evidence for the competing models given the data (Burnham et al., 2011). The IT approach to model selection allowed the evaluation of competing alternative hypotheses, and the relative contributions of environmental parameter sets, given the data.

USE OF THE GEOMETRIC MEAN FOR INDICES OF SPATIAL AND TEMPORAL DISPERSION

The geometric mean was used because it is less influenced by the largeness of values, but rather weights the average independently of the size of values, leading to more conservative estimates of the mean. It is generally considered to be more appropriate for determining the means of data derived from non-log transformed proportions (e.g. VMR). Furthermore, measures of variance often range from small to relatively large values; therefore use of the geometric mean reducing the bias towards larger variance that could be problematic using the arithmetic mean. In a relatively few instances of our calculations of k (dispersion factor for negative binomial distribution), we calculated a negative or zero value for k , which cannot be included in the calculation of the geometric mean. In these instances, we substituted a 0.1 for the value (one significant digit below the smallest positive values), which is a reasonable

approximation given the range of values and the frequency of occurrence in this data set (see Martin-Fernandez et al., 2003).

ANALYSIS OF SPATIAL AUTOCORRELATION:

Though our study was not designed to efficiently utilize geostatistical analytical tools, strong spatial autocorrelation could influence interpretation of results from our IT analysis, as this could challenge the assumption of spatial independence within sites (Fortin et al, 2002). However, it should be noted that sample size within site is limited for calculations of both Moran's I and Geary's C (Fortin et al, 2002). Furthermore, within-site spatial autocorrelation is potentially the result of co-varying environmental parameters, which we did account for in our analysis, and environmental parameters were of more central importance to our underlying questions than spatial autocorrelation in general. We did not detect evidence for spatial autocorrelation using Moran's I, which is generally considered better suited for detection of large scale patterns. We did detect evidence of spatial autocorrelation using Geary's C, which is more appropriate for localized patterns of spatial autocorrelation. Significant values reported for Geary's C may be due to a tendency for plots which are closer together to be more similar in herbivore population density. The apparent clustering could be biased by the fact that plots were clustered within distinct study sites, which could naturally inflate the estimate of autocorrelation; therefore, we recommend that inference from our estimates of Geary's C statistics be drawn with caution. The spatial correlation detected is also difficult to separate from underlying biological mechanisms without sophisticated geostatistical analyses which simultaneously account for spatial locations of insect population density and for multiple independent variables. Nevertheless, this does not undermine our analysis of the distribution of herbivores with regards

to correlations with environmental variables, because we accounted for site and sampling period differences in our models (GEEs). Geostatistical tools (e.g., corelelograms, variograms, semi-variograms, and kriging), which could address spatial autocorrelation across a set of response and predictor variables, are available; however, this approach to analysis would not work well with this data set because samples are not distributed sufficiently across the spatial extent of the study area (see Rossi et al., 1992).

LITERATURE CITED

- Bolker, B.M., M.E. Brooks, C.J. Clark, S.W. Geange, J.R. Poulsen, M.H. Stevens, and J-S.S.White. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology and Evolution* **24**:127-135.
- Burnham, K.P., D.R. Anderson, and K.P. Huyvaert. 2011. AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. *Behavioral Ecology and Sociobiology* **65**:23-35.
- Fortin, M-J, M.R.T. Dale, and J. ver Hoef. 2002. Spatial analysis in ecology. In: *Encyclopedia of Environmetrics* **4**:2051-2058. El-Shaarawi, A.H. and W.W. Piegorsch (EDS.). John Wiley and Sons, Chichester.
- Johnson, J.B. and K.S. Omland. 2004. Model selection in ecology and evolution. *Trends in Ecology and Evolution* **19**:101-108
- Martin-Fernandez, J.A., C. Barcelo-Vidal, and V. Pawlowsky-Glahn. 2003. Dealing with Zeros and Missing Values in Compositional Data Sets Using Nonparametric Imputation. *Mathematical Geology* **35**: 253-278.
- Ismail, N. and A. A. Jemain. 2007. Handling overdispersion with negative binomial and generalized Poisson regression models. *Casualty Actuarial Society Forum* (winter): 103-158.

- Rossi, R.E., D.J. Mulla, A.G. Journel, and E.H. Franz. 1992. Geostatistical tools for modeling and interpreting ecological spatial dependence. *Ecological Monographs* **62**:277-314.
- Royall, R.M. 1997. *Statistical evidence: a likelihood paradigm*. Chapman and Hall, London.
- Sileshi, G. 2008. The excess zero problem in soil animal count data and choice of appropriate models for statistical inference. *Pedo-biologia* **52**:1-17.
- Sileshi, G. 2006. Selecting the right statistical model for analysis of insect count data by using information theoretic measures. *Bulletin of Entomological Research* **96**:479-488.
- Taylor, L.R. 1961. Aggregation, variance and the mean. *Nature* **189**:732-735.
- White, G.C. and R.E. Bennets. 1996. Analysis of frequency count data using the negative binomial distribution. *Ecology* **77**:2549-2557.
- Whittingham, M.J., P.A. Stephens, R.B. Bradbury, and R.P. Freckleton. 2006. Why do we still use stepwise modeling in ecology and behaviour? *Journal of Animal Ecology* **75**:1182-1189.

APPENDIX C:

These tables summarize count tallies for root-feeding insects collected from unburned (UB), burned (B), and combined sites. Specific genera are italicized and broader taxonomic groups are in bold font. Grub and wireworm specimens from Fall, 2008 were not identified below the family level.

TAXONOMIC GROUP	# Individuals		
	B	UB	Combined
COMBINED TAXA			
Fall 08	33	74	107
Summer 09	84	114	198
Fall 09	112	131	243
Spring 10	102	213	315
Summer 10	23	86	109
Fall 10	75	89	164
COMBINED TOTAL	409	696	1105
All Grubs			
Fall 08	18	29	47
Summer 09	24	19	43
Fall 09	49	35	84
Spring 10	29	27	56
Summer 10	13	22	35
Fall 10	21	28	49
COMBINED TOTAL	140	157	297
<i>Anomala</i>			
Fall 08	<i>Unavailable</i>	<i>Unavailable</i>	<i>Unavailable</i>
Summer 09	7	2	9
Fall 09	0	0	0
Spring 10	0	2	2
Summer 10	3	1	4
Fall 10	0	0	0
COMBINED TOTAL	10	5	15

<i>Cyclocephala</i>			
Fall 08	<i>Unavailable</i>	<i>Unavailable</i>	<i>Unavailable</i>
Summer 09	0	0	0
Fall 09	0	5	5
Spring 10	0	0	0
Summer 10	2	0	2
Fall 10	0	0	0
COMBINED TOTAL	2	5	7
<i>Diplotaxis</i>			
Fall 08	<i>Unavailable</i>	<i>Unavailable</i>	<i>Unavailable</i>
Summer 09	0	0	0
Fall 09	0	6	6
Spring 10	0	2	2
Summer 10	0	6	6
Fall 10	0	1	1
COMBINED TOTAL	0	15	15
<i>Maladera</i>			
Fall 08	<i>Unavailable</i>	<i>Unavailable</i>	<i>Unavailable</i>
Summer 09	0	11	11
Fall 09	0	6	6
Spring 10	0	9	9
Summer 10	0	3	3
Fall 10	0	10	10
COMBINED TOTAL	0	39	39
<i>Phyllophaga</i>			
Fall 08	<i>Unavailable</i>	<i>Unavailable</i>	<i>Unavailable</i>
Summer 09	16	6	22
Fall 09	49	17	66
Spring 10	29	12	41
Summer 10	8	11	19
Fall 10	21	16	37
COMBINED TOTAL	123	62	185
Unknown			
Fall 08	<i>Unavailable</i>	<i>Unavailable</i>	<i>Unavailable</i>
Summer 09	1	0	1
Fall 09	0	1	1
Spring 10	0	2	2
Summer 10	0	1	1
Fall 10	0	1	1
COMBINED TOTAL	1	5	6

All Wireworms			
Fall 08	6	8	14
Summer 09	15	17	32
Fall 09	15	25	40
Spring 10	5	31	36
Summer 10	2	29	31
Fall 10	7	6	13
COMBINED TOTAL	44	108	152
<i>Agriotes</i>			
Fall 08	<i>Unavailable</i>	<i>Unavailable</i>	<i>Unavailable</i>
Summer 09	6	9	15
Fall 09	4	4	8
Spring 10	0	6	6
Summer 10	0	14	14
Fall 10	0	2	2
COMBINED TOTAL	10	35	45
<i>Ctenicera</i>			
Fall 08	<i>Unavailable</i>	<i>Unavailable</i>	<i>Unavailable</i>
Summer 09	4	2	6
Fall 09	1	0	1
Spring 10	0	0	0
Summer 10	0	0	0
Fall 10	0	0	0
COMBINED TOTAL	5	2	7
<i>Hemirhipis</i>			
Fall 08	<i>Unavailable</i>	<i>Unavailable</i>	<i>Unavailable</i>
Summer 09	0	0	0
Fall 09	0	3	3
Spring 10	0	0	0
Summer 10	0	0	0
Fall 10	0	0	0
COMBINED TOTAL	0	3	3
<i>Limonius</i>			
Fall 08	<i>Unavailable</i>	<i>Unavailable</i>	<i>Unavailable</i>
Summer 09	0	0	0
Fall 09	0	1	1
Spring 10	0	4	4
Summer 10	0	0	0
Fall 10	0	0	0
COMBINED TOTAL	0	5	5

<i>Melanotus</i>			
Fall 08	<i>Unavailable</i>	<i>Unavailable</i>	<i>Unavailable</i>
Summer 09	5	6	11
Fall 09	9	16	25
Spring 10	4	16	20
Summer 10	2	11	13
Fall 10	7	4	11
COMBINED TOTAL	27	53	80
<i>Sericus</i>			
Fall 08	<i>Unavailable</i>	<i>Unavailable</i>	<i>Unavailable</i>
Summer 09	0	0	0
Fall 09	1	1	2
Spring 10	1	5	6
Summer 10	0	4	4
Fall 10	0	0	0
COMBINED TOTAL	2	10	12
Cebrionids			
Fall 08	3	8	11
Summer 09	21	29	50
Fall 09	27	32	59
Spring 10	27	25	52
Summer 10	3	14	17
Fall 10	18	17	35
COMBINED TOTAL	99	125	224
Weevils			
Fall 08	5	27	32
Summer 09	23	45	68
Fall 09	16	37	53
Spring 10	38	130	168
Summer 10	5	21	26
Fall 10	27	37	64
COMBINED TOTAL	114	297	411
Cicadas			
Fall 08	1	2	3
Summer 09	1	4	5
Fall 09	5	2	7
Spring 10	3	0	3
Summer 10	0	0	0
Fall 10	2	1	3
COMBINED TOTAL	12	9	21

APPENDIX D

The tables below summarize effect sizes of model parameters and significance values for the best fit experimental and predictive models (based on QIC_u) for each individual root-feeding insect taxa included in the study. In cases where ΔQIC_u for the intercept only model indicated that none of the alternative experimental or predictive models were better than the intercept only model, then the intercept only model was presented. Effect size and standard error estimates are natural log transformed and cumulative. Estimates of effects can be used to calculate an expected population density for any given plot. For an expected insect count for a given plot (Estimate and Std. Err. of count) = $e^{(\text{intercept} + \text{categorical effect } 1\dots n + [\text{continuous effect } 1\dots n * \text{plot parameter value } 1\dots n])}$. Use values 0-100 (not 0-1) for continuous parameter values reported as percentages. Divide by 0.16 to translate to population density $\#_{RFS}/m^2$.

White Grubs (Scarabaeidae):

Parameter (Source)	Estimate	Std. Err.	95% C.I.		Z	P > Z
<i>Intercept Model, GEE Fit Criteria: $QIC = 401.6$; $QIC_u = 397.9$; $\Delta = 0$</i>						
Intercept	0.0304	0.1634	-0.2898	0.3506	0.19	0.8524
<i>Predictive Model, GEE Fit Criteria: $QIC = 358.0$; $QIC_u = 351.4$; $\Delta = -13.7$</i>						
Intercept	-0.0990	0.2972	-0.6815	0.4836	-0.33	0.7392
Fire Treatment Effects						
Fire: UB (unburned)	0.1401	0.3430	-0.5321	0.8123	0.41	0.6829
Fire: B (burned)	0.0000	0.0000	0.0000	0.0000

Wireworms (Elateridae):

Parameter (Source)	Estimate	Std. Err.	95% C.I.		Z	P > Z
<i>Intercept Model, GEE Fit Criteria: QIC = 327.6; QICu = 326.7; Δ = 0</i>						
Intercept	-0.0938	0.1316	-0.3605	0.1729	-0.69	0.4908
<i>Experimental Model, GEE Fit Criteria: QIC = 358.0; QICu = 351.4; Δ = -13.7</i>						
Intercept	-1.1396	0.7213	-2.5534	0.2741	-1.58	0.1141
Fire Treatment Effects						
Fire: UB (unburned)	0.6007	0.4312	-2.4440	1.4458	1.39	0.1636
Fire: B (burned)	0.0000	0.0000	0.0000	0.0000
Site Effects (nested in fire treatment)						
Site(Fire): Site 1 (UB)	0.0000	0.0000	0.0000	0.0000
Site(Fire): Site 2 (UB)	0.4599	0.3388	-0.2042	1.1241	1.36	0.1747
Site(Fire): Site 3 (UB)	0.5160	0.3745	-0.2181	1.2501	1.38	0.1683
Site(Fire): Site 4 (B)	0.2896	0.2896	-0.5857	1.1648	0.65	0.5167
Site(Fire): Site 5 (B)	0.0000	0.0000	0.0000	0.0000
Site(Fire): Site 6 (B)	-0.3040	0.3999	-1.0878	0.4798	-0.76	0.4471
Sampling Period Effects						
Sampling Period: Fall 2008	-1.1121	0.4847	-2.0620	-0.1621	-2.29	0.0218
Sampling Period: Summer 2009	0.7597	0.4492	-0.1208	1.6402	1.69	0.0908
Sampling Period: Fall 2009	0.1282	0.4746	-0.8020	1.0584	0.27	0.7871
Sampling Period: Spring 2010	0.1738	0.4252	-0.6595	1.0072	0.41	0.6827
Sampling Period: Summer 2010	0.0000	0.0000	0.0000	0.0000
Sampling Period: Fall 2010	-0.0256	0.5520	-1.1076	1.0563	-0.05	0.9629
Root Effects						
Fine Root Std. Crop (g)	0.0301	0.1338	-0.2322	0.2932	0.22	0.8221
Fine Root Std. Crop (cm)	0.00003	0.0001	-0.0002	0.0002	0.18	0.8592

Weevils (Curculionidae):

Parameter (Source)	Estimate	Std. Err.	95% C.I.		Z	P > Z
<i>Experimental Model, GEE Fit Criteria: QIC = -506.5; QICu = -519.7; Δ = -405.9</i>						
Intercept	-2.2956	0.3542	-2.9898	-1.6013	-6.48	< 0.0001
Fire Treatment Effects						
Fire: UB (unburned)	0.704	0.4776	-0.2321	1.64	1.47	0.1405
Fire: B (burned)	0	0	0	0
Site Effects (nested in fire treatment)						
Site(Fire): Site 1 (UB)	0	0	0	0
Site(Fire): Site 2 (UB)	0.9061	0.4873	0.4878	-0.0489	1.86	0.0629
Site(Fire): Site 3 (UB)	1.8945	0.8308	0.2662	3.5228	2.28	0.0226
Site(Fire): Site 4 (B)	1.0148	0.4158	0.1999	1.8297	2.44	0.0147
Site(Fire): Site 5 (B)	0	0	0	0
Site(Fire): Site 6 (B)	1.3879	0.3941	0.6156	2.1603	3.52	0.0004
Sampling Period Effects						
Sampling Period: Fall 2008	-0.0774	0.3578	-0.7786	0.6238	-0.22	0.8288
Sampling Period: Summer 2009	0.5235	0.3761	-0.2137	1.2608	1.39	0.1640
Sampling Period: Fall 2009	0.7267	0.3331	0.0739	1.3795	2.18	0.0291
Sampling Period: Spring 2010	1.8273	0.2746	1.2891	2.3654	6.65	< 0.0001
Sampling Period: Summer 2010	0	0	0	0
Sampling Period: Fall 2010	1.2308	0.3348	0.5746	1.887	3.68	0.0002
<i>Predictive Model, GEE Fit Criteria: QIC = 358.0; QICu = 351.4; Δ = -13.7</i>						
Intercept	2.9466	1.16	0.673	5.2202	2.54	0.0111
Aboveground Vegetation Effects						
Wiregrass (%)	-0.0272	0.013	-0.0527	-0.0017	-2.09	0.0367
Woody Plants (%)	-0.0153	0.0187	-0.0519	0.0214	-0.82	0.4147
Herbaceous Forbs (%)	-0.033	0.0197	-0.0717	0.0057	-1.67	0.0946
Other Grasses (%)	-0.0748	0.0183	-0.1105	-0.039	-4.1	< 0.0001
Forest Floor Litter (%)	-0.0099	0.0127	-0.0347	0.015	-0.78	0.4364
Root Effects						
Fine Root Std. Crop (g)	0.2768	0.2253	-0.1649	0.7184	1.23	0.2193
Fine Root Std. Crop (cm)	-0.0003	0.0002	-0.0007	0.00005	-1.68	0.0933

Cebrionids (Cebrionidae):

Parameter (Source)	Estimate	Std. Err.	95% C.I.		Z	P > Z
<i>Experimental Model, GEE Fit Criteria: QIC = 486.1; QICu = 490.8; Δ = -68.9</i>						
Intercept	-4.9534	1.2358	-7.3755	-2.5312	-4.01	< 0.0001
Fire Treatment Effects						
Fire: UB (unburned)	-0.0714	0.6824	-1.4088	1.2661	-0.10	0.9167
Fire: B (burned)	0.0000	0.0000	0.0000	0.0000
Site Effects (nested in fire treatment)						
Site(Fire): Site 1 (UB)	0.0000	0.0000	0.0000	0.0000
Site(Fire): Site 2 (UB)	0.4372	0.1963	0.0525	0.8218	2.23	0.0259
Site(Fire): Site 3 (UB)	0.0413	0.2959	-0.5387	0.6212	0.14	0.8891
Site(Fire): Site 4 (B)	-0.8718	0.3677	-1.5926	-0.1511	-2.37	0.0177
Site(Fire): Site 5 (B)	0.0000	0.0000	0.0000	0.0000
Site(Fire): Site 6 (B)	0.0333	0.4808	-0.9091	0.9756	0.07	0.9449
Sampling Period Effects						
Sampling Period: Fall 2008	-0.4238	0.4484	-1.3027	0.4552	-0.94	0.3447
Sampling Period: Summer 2009	1.0935	0.3055	0.4947	1.6923	3.58	0.0003
Sampling Period: Fall 2009	1.2560	0.3208	0.6274	1.8847	3.92	< 0.0001
Sampling Period: Spring 2010	1.1045	0.2764	0.5627	1.6463	4.00	< 0.0001
Sampling Period: Summer 2010	0.0000	0.0000	0.0000	0.0000
Sampling Period: Fall 2010	0.7217	0.3476	0.0404	1.4030	2.08	0.0379
Aboveground Vegetation Effects						
Wiregrass (%)	0.0153	0.0079	-0.0003	0.0308	1.92	0.0548
Woody Plants (%)	0.0008	0.0077	-0.0143	0.0159	0.11	0.9156
Herbaceous Forbs (%)	0.0074	0.0073	-0.0069	0.0218	1.01	0.3116
Other Grasses (%)	0.0245	0.0155	-0.0060	0.0549	1.58	0.1150
Forest Floor Litter (%)	0.0157	0.0063	0.0034	0.0281	2.49	0.0126
Soil Effects						
Soil NH ₄ (mg/kg)	-0.0491	0.0177	-0.0837	-0.0145	-2.78	0.0054
Soil Organic Matter (%)	0.5391	0.4702	-0.3824	1.4607	1.15	0.2516
CEC (meq/100g)	0.0002	0.1978	-0.3875	0.3879	0.00	0.9993
Base Saturation (%)	0.0289	0.0089	0.0115	0.0464	3.25	0.0012
Root Effects						
Fine Root Std. Crop (g)	0.6098	0.2565	0.1071	1.1125	2.38	0.0174
Fine Root Std. Crop (cm)	0.00005	0.00005	-0.00008	0.00002	0.67	0.5056

Cebrionids (Cebrionidae), Continued:

Parameter (Source)	Estimate	Std. Err.	95% C.I.		Z	P > Z
<i>Predictive Model, GEE Fit Criteria: QIC = 510.1; QICu = 512.4; Δ = -47.3</i>						
Intercept	-3.7399	1.0891	-5.8745	-1.6052	-3.43	0.0006
Fire Treatment Effects						
Fire: UB (unburned)	0.3834	0.4857	-0.5684	1.3353	0.79	0.4298
Fire: B (burned)	0	0	0	0
Aboveground Vegetation Effects						
Wiregrass (%)	0.0073	0.0074	-0.0071	0.0218	0.99	0.3210
Woody Plants (%)	0.0032	0.0070	-0.1060	0.0170	0.45	0.6501
Herbaceous Forbs (%)	0.0099	0.0064	-0.0025	0.0224	1.56	0.1182
Other Grasses (%)	0.0205	0.0189	-0.0166	0.0576	1.08	0.2785
Forest Floor Litter (%)	0.0084	0.0085	-0.0083	0.0251	0.98	0.3257
Soil Effects						
Soil NH ₄ (mg/kg)	-0.0396	0.0185	-0.0758	-0.0034	-2.15	0.0319
Soil Organic Matter (%)	0.7843	0.4802	-0.1569	1.7255	1.63	0.1024
CEC (meq/100g)	-0.2298	0.1825	-0.5874	0.1278	-1.26	0.2078
Base Saturation (%)	0.0339	0.0075	0.0191	0.0486	4.51	< 0.0001
Root Effects						
Fine Root Std. Crop (g)	0.0985	0.0641	-0.0272	0.2241	1.54	0.1246
Fine Root Standing Crop (cm)	0.00003	0.00005	-0.00008	0.0001	0.68	0.4982

Cicadas (Cicadidae):

Parameter (Source)	Estimate	Std. Err.	95% C.I.		Z	P > Z
<i>Intercept Model, GEE Fit Criteria: QIC = 288.4 ; QICu = 289.1 ; Δ = 0</i>						
Intercept	0.1488	0.4000	-0.6352	0.9329	0.37	0.7098
<i>Predictive Model, GEE Fit Criteria: QIC = 195.4; QICu = 198.1; Δ = -91.0</i>						
Intercept	-3.0013	0.6474	-4.2702	-1.7324	-4.64	< 0.0001
Root Effects						
Fine Root Std. Crop (g)	0.1871	0.1149	-0.0038	0.4122	1.63	0.1033
Fine Root Standing Crop (cm)	-0.00003	0.00005	-0.0002	0.00008	-0.47	0.6394

APPENDIX E

The table below lists the sources for isotopic endpoints used in analysis and provides the corresponding values estimated for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

End Point Type	Species	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	C:N	N
C ₃ Plant	<i>Crotalana rotundifolia</i>	-29.36	-1.59	23.06412	1
C ₃ Plant	<i>Gaylussacia dumosa</i>	-30.1	-6.14	157.9077	1
C ₃ Plant	<i>Liquidambar styraciflua</i>	-29.5	-5.34	51.16467	1
C ₃ Plant	<i>Pinus palustris</i>	-28.76	-3.37	130.7119	3
C ₃ Plant	<i>Pityopsis graminifolia</i>	-30.62	-4.82	61.45818	1
C ₃ Plant	<i>Pteridium equilinum</i>	-28.83	-2.36	60.13455	1
C ₃ Plant	<i>Quercus virginiana</i>	-29.46	-2.57	60.76415	1
C ₃ Plant	<i>Quercus falcata</i>	-29.29	-0.61	117.9176	1
C ₃ Plant	<i>Rubus cuneifolius</i>	-30.59	-3.6	61.7	2
C ₃ Plant	<i>Solidago odora</i>	-28.74	-4.14	68.8062	1
C ₃ Plant	AVERAGE	-29.525	-3.454	79.3629	10
C ₄ Plant	<i>Aristida stricta</i>	-13.6	-3.23	134.0083	3
Legume	<i>Albizia julibrissen</i>	-29.48	-2.07	18.08762	1
Legume	<i>Chamaecrista nictitans</i>	-28.3	-1.58	24.80656	1
Legume	<i>Clitoria mariana</i>	-29.62	-1.91	28.07721	1
Legume	<i>Tephrosia virginiana</i>	-25.86	-1.12	19.96138	1
Legume	AVERAGE	-28.315	-1.67	22.73319	4
Forest Floor Litter		-26.41	-5.22	64.80889	5
Soil dwelling centipedes		-23.38	3.79	4.193978	3
Soil dwelling isopods		-19.42	5.65	5.134451	4

APPENDIX F

The figure below shows $\delta^{13}\text{C}$ (independent variable) versus C:N ratio (dependant variable) of fine root standing crops. $\delta^{13}\text{C}$ is positively related to the proportion of wiregrass in the each root sample taken. The C:N ratio positively relates to $\delta^{13}\text{C}$ ($p = 0.00018$; $r^2 = 0.247$), reflecting the relatively high C:N of wiregrass.

