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A LAB-BASED STUDY OF TEMPERATE FOREST TERMITE IMPACTS ON PLANT AND WOOD-ROT FUNGAL GROWTH

by

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THESIS APPROVAL PAGE

This is to certify that the thesis prepared by Jason Martin entitled "A lab-based study of temperate forest termite impacts on plant and wood-rot fungal growth" has been approved by the thesis committee as satisfactorily completing the thesis requirements for the degree Master of Science in Biology.

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ABSTRACT

The ecological role of temperate subterranean termites, such as *Reticulitermes flavipes* is largely unknown because of their cryptic foraging activities. This is also due to the fact that termites are primarily perceived as a pest, which causes substantial damage to man-made wooden structures that costs the United States over a billion dollars annually in prevention and repair. This has resulted in most resources being directed toward developing an understanding of how to eliminate termite colonies. Yet, temperate subterranean termites have a potentially important ecological role. We investigated the ecological role of *R. flavipes* in the context of plant productivity, soil microbiome interactions, and wood-rot fungal interactions. We demonstrated that the presence of *R. flavipes* in soil increased plant productivity, and that antifungal properties of their saliva and gut can affect the growth of two wood-rot fungi *Gloeophyllum trabeum* and *Phanerochaete chrysosporium*. These laboratory results should motivate future research on how we understand the role of temperate subterranean termites in forest nutrient cycling and decomposer community structure.

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1.

Introduction

Termites are eusocial insects that are found around the world. Most termite species fall into one of three nesting strategies; single site nesters, multi-site nesters, and central site nesters (Shellman-Reeve, 1997). Single site nesting termites will spend their entire colony life in a single piece of wood, which serves as the colony's food source and shelter, multi-site nesting termites will spread the colony to multiple pieces of wood over a given area, and the colony will continuously forage for new food sources moving and possibly splitting the colony over time, and central site nesting termites have the colony in one location but forage for food in another location to bring back to the nest (Shellman-Reeve, 1997). Termite colonies consist of different castes that include reproductive members, workers, and soldiers with a division of labor (Shellman-Reeve, 1997). *Reticulitermes flavipes* is a subterranean multi-site nesting termite species found in temperate ecosystems (Maynard et al., 2015). Colonies of *R. flavipes* can have hundreds, thousands, and potentially millions of individuals mostly consisting of the worker caste (Thorne et al., 1999). Foraging workers actively feed on wood and build foraging galleries in soil that link together the multiple nests of the colony (Bulmer et al., 2002). The cryptic subterranean nature of *R. flavipes* colonies makes them hard to observe and therefor difficult to study. Colony boundaries are difficult to define because their nest structures are concealed. The multi-site nesting and foraging behavior of *R. flavipes* makes their colonies amoebae like, moving from food source to food source across the forest floor (Thorne et al., 1999). The colony organization of *R. flavipes* was not well

understood until recent molecular tools were applied which made genotyping individuals and defining colony boundaries possible (Thorne et al., 1999).

Termites are one of the most abundant macroinvertebrates found in soil and dead wood outside tropical ecosystems (King et al., 2013). Temperate subterranean termites, particularly *Reticulitermes flavipes*, are important economic pests costing an estimated 1.5-2 billion dollars a year in prevention, treatment, and repair of wooden structures in the United States (Grant et al., 2012; Maynard et al., 2015). *R. flavipes* is the most widely distributed termite species in North America (Maynard et al., 2015). The range of *R. flavipes* extends as far north as Ontario, Canada and as far south as Florida, covering the eastern United States (Grant et al., 2012; Neupane et al., 2015). *R. flavipes* has also been introduced and become and invasive species in Austria, the Bahamas, Chile, France, Germany, and Uruguay (Neupane et al., 2015). Most of the research regarding *R. flavipes* has been in the context of controlling them as pests and little research has been directed toward understanding their ecological role and their impacts on temperate ecosystems (Maynard et al., 2015). Research on the ecological roles and ecosystem impacts of termites has been directed at tropical and subtropical systems (Neupane et al., 2015). *R. flavipes* is considered a subterranean multi-site nesting species, which makes their abundance, distribution, and effects on temperate ecosystems hard to quantify (Maynard et al., 2015).

Organisms are considered ecosystem engineers if they modulate either directly or indirectly the availability of resources to other organisms, cause changes in abiotic or biotic materials, and/or modify, maintain, and create habitat for other organisms (Jones et al., 1994). Termites can be considered ecosystem and soil engineers because they are

common and numerous members of the soil and dead wood invertebrate fauna that can influence the structure and biological processes of soil and dead wood (King et al., 2013). Termites, especially temperate termites such as *R. flavipes*, accelerate the breakdown of dead wood into soil humus, emit methane, fix nitrogen, and alter forest structure and habitats by reducing the amount of standing and lying dead wood (Grant et al., 2012; Maynard et al., 2015; Neupane et al., 2015). Temperate termites impact the structure and chemistry of soil by tunneling and forming galleries (Maynard et al., 2015). This tunneling activity involves soil manipulation and translocation, deposition of organic matter, alterations to soil drainage, and can influence microbial properties (Jouquet et al., 2006; Maynard et al., 2015; Neupane et al., 2015). These effects on soil influence the availability of resources to other organisms (Jouquet et al., 2006).

Temperate termites, such as *R. flavipes*, play a major role in the decomposition of wood and thus the carbon cycle. Termites and fungi are the dominant players in wood decomposition in temperate forest (Maynard et al., 2015). Termites digest wood mechanically and chemically with the assistance of gut symbionts resulting in an estimated consumption of 50mg of wood per gram of termite per day for *R. flavipes* (Grant et al., 2012; Maynard et al., 2015). This consumption results in an estimated methane emission of 0.02-0.09 $Tgyr^{-1}$ by temperate termites (Maynard et al., 2015). Termites in the tropical forest are also responsible for an estimated 1.9 $Pgyr^{-1}$ of carbon flux in the atmosphere (Cornwell et al., 2009). Termites play a significant role in the carbon cycle because they are the primary macroinvertebrates responsible for the storage and release of the estimated 72 Pg of carbon stored in dead wood (Cornwell et al., 2009; Maynard et al., 2015).

Wood-rot fungi also play a key role in wood decomposition and are considered ecosystem engineers (Lonsdale et al., 2008). Wood-rot fungi directly modulate the availability of resources for several functional groups other than themselves, such as small birds and mammals, by providing habitat and mineralizing organic matter, which enable forest regeneration (Lonsdale et al., 2008). This mineralization of organic matter in the form of storage and release of nutrients and carbon from dead wood makes wood-rot fungi a central player in the functioning of forested ecosystems (Lonsdale et al., 2008; Fukami et al., 2010). Wood-rot fungi are specialized in how they break down dead wood and can degrade all components of dead wood including structural (hemicellulose, cellulose, and lignin) and nonstructural (sugars, lipids, and peptides) components (Cornwell et al., 2009). Wood-rot fungi can fall in to one of two main categories brown or white rot fungi depending on what components of dead wood they degrade (Cornwell et al., 2009). White rot fungi can degrade all components of dead wood including lignin, while brown rot fungi degrade cellulose and hemicellulose but not lignin (Cornwell et al., 2009). Brown rot fungi are more abundant in cool dry regions, while white rot fungi are more abundant in warm moist regions (Maynard et al., 2015).

Wood-rot fungi and termites both inhabit and use dead wood as a food source. This makes interactions between termites and wood-rot fungi inevitable. The environmental conditions that are favored by termites are also favored by wood-rot fungi (Grant et al., 2012). Wood-rot fungi often appear to inhabit dead wood first and then are followed by termites (Grant et al., 2012). Termites are attracted to wood inhabited by some wood-rot fungal species while they are repelled by others (Cornelius et al., 2002). It was originally thought that brown rot fungi attracted and white rot fungi repelled

termites, but it has become clear that this relationship is more complicated (Grant et al., 2012). Termites respond differently to wood decayed by different wood-rot fungi depending on the species of fungi, and there is no positive statistical correlation between the presence of termites and any fungal morphological groups (Grant et al., 2012). Termites do prefer wood decayed by certain wood-rot fungi over non-decayed wood (Cornelius et al., 2002). This may be due to the wood becoming more nutritious for termites after being decayed sufficiently for easy ingestion and digestion (Cornelius et al., 2002). Interestingly, *R. flavipes* has been observed feeding directly on basidiocarps (Zoberi and Grace, 1990).

The temperate subterranean termite *R. flavipes* is a multi-site nesting termite species that inevitably interacts with the soil microbiome and wood-rot fungi. The nature of these interactions and their ecological consequences are largely unknown. The role of termites as ecosystem and soil engineers has been studied largely using single-site mound building termite species in the subtropical and tropical ecosystems (Neupane et al., 2015). The ecological significance of *R. flavipes* and other temperate termite species is little known and research to-date has focused on their general role as wood decomposers and soil engineers in the most general sense. The competitive interaction between termites, microbes, and fungi in soil and dead wood is predicted to alter those communities and decomposition rates (Ulyshen et al., 2016). This prediction requires substantially more research on the ecology of competition in decomposing wood. The research presented here addresses the following questions: 1) Can foraging *R. flavipes* workers change the composition of soil microbial communities? 2) Does the presence of workers in soil effect plant growth, soil moisture retention, or soil nutrient levels? 3) Does the presence

of *R. flavipes* inhibit the growth of brown and white rot fungi on wood? 4) Do antifungal proteins produced by *R. flavipes* inhibit or possibly promote brown and white rot mycelial growth and sporulation? 5) Do any fungi found in the gut of *R. flavipes* have a role in inhibiting the growth of brown and white rot fungi? Answers to these questions could provide insight into the complex system of termites and their biotic interactions and ecological impact of *R. flavipes*.

2.

A beneficial influence of temperate forest termites on plant growth

Abstract

Termites are considered soil engineers because they modify either directly or indirectly the availability of resources to other organisms and cause changes in abiotic or biotic materials. The eastern subterranean termite *Reticulitermes flavipes* tunnels through soil in search of new food sources. This tunneling activity puts *R. flavipes* in direct contact with the rhizosphere and soil microbiome. *R. flavipes* has been shown previously to increase microbial activity and change nutrient concentrations in soil. Here we demonstrate that the presence of *R. flavipes* in soil significantly increases the concentration of phosphorus and nitrogen in sunflower (*Helianthus annuus*) plant tissue resulting in an increase in plant productivity. The mechanism of this phenomena is still unknown, and will require the use of next generation sequencing technology and more refined soil nutrient testing. *Reticulitermes* are widespread and numerous in temperate forests and this controlled laboratory study suggests that their colonies could impact plant growth within their extensive foraging territories.

Introduction

Termites are one of the most abundant macroinvertebrates found in soil and dead wood outside tropical ecosystems (King et al., 2013). Temperate subterranean termites, particularly *Reticulitermes flavipes*, are important economic pests costing an estimated 1.5-2 billion dollars a year in prevention, treatment, and repair of wooden structures in the United States (Grant et al., 2012; Maynard et al., 2015). Most of the research

regarding *R. flavipes* has been in the context of controlling them as pests and little research has been directed toward understanding their ecological role and their impacts on temperate ecosystems (Maynard et al., 2015). *R. flavipes* is a subterranean multi-site nesting termite species (Maynard et al., 2015). Colonies of *R. flavipes* can have hundreds, thousands, and potentially millions of individuals mostly consisting of the worker caste (Thorne et al., 1999). Foraging workers actively feed on wood and build foraging galleries in soil that link together the multiple nests of the colony (Bulmer et al., 2002). The cryptic subterranean nature of *R. flavipes* colonies makes them hard to observe and therefor difficult to study. Colony boundaries are difficult to define because their nest structures are concealed. The multi-site nesting and foraging behavior of *R. flavipes* makes their colonies amoebae like, moving from food source to food source across the forest floor (Thorne et al., 1999).

Organisms are considered ecosystem engineers if they modulate either directly or indirectly the availability of resources to other organisms, cause changes in abiotic or biotic materials, and/or modify, maintain, and create habitat for other organisms (Jones et al., 1994). Soil organisms can influence a wide range of ecosystem properties and processes such as plant productivity and community composition, soil structure and hydrology, nutrient availability, and organic matter decomposition (Maynard et al., 2015). Given their prevalence in soil, temperate subterranean termites, such as *R. flavipes*, may be important members of the invertebrate fauna that can influence the structure and biological processes of soil (King et al., 2013). Temperate subterranean termites may have a substantial impact on the structure and chemistry of soil when they tunnel and form gallery networks (Maynard et al., 2015). *R. flavipes* workers from

different colonies develop these networks close to the soil surface when expanding their foraging and nesting territories (Thorne et al., 1999) and also migrate to a depth of 1m in soil to escape cold temperatures (Maynard et al., 2015). This tunneling activity involves soil translocation, deposition of organic matter, alterations to soil drainage, and can influence microbial properties (Jouquet et al., 2006; Maynard et al., 2015; Neupane et al., 2015).

The foraging activity of *R. flavipes* puts it in direct contact with the rhizosphere. The rhizosphere is the area between plant roots and soil and is considered one of the most biologically dynamic areas on Earth (Philippot et al., 2013). In the rhizosphere interactions among invertebrates and microorganisms affect plant growth, plant resistance to biotic and abiotic stress, and biogeochemical cycling (Philippot et al., 2013). Soil microbe communities are effected by factors such as soil temperature, moisture, pH, nutrient and oxygen availability, and redox potential (Russo et al., 2012). Soil engineering by termites potentially increases microbial activity and the release of nutrients such as ammonium and nitrate, and enriches the soil in exchangeable Ca, Mg, K, and Na cations (Jouquet et al., 2006). *R. flavipes* increases microbially available carbon, decreases soil moisture content and pH making the soil more acidic, and has been shown to increase microbial biomass in soil (Neupane et al., 2015). Although, this increase in microbial activity was not statistically significant. These effects on soil influence the availability of resources to other organisms (Jouquet et al., 2006).

The genus *Reticulitermes* has a global distribution that includes North America, Eastern and Western Europe, and Eastern and Western Asia (Dedeine et al., 2016). *R. flavipes* is the most widely distributed termite species in North America (Maynard et al.,

2015). The range of *R. flavipes* extends as far north as Ontario, Canada and as far south as Florida, encompassing most of the eastern United States (Grant et al., 2012; Neupane et al., 2015). *R. flavipes* has also been introduced and developed into an invasive species in Austria, the Bahamas, Chile, France, Germany, and Uruguay (Neupane et al., 2015). Given the global distribution of *Reticulitermes* and specifically *R. flavipes* it is important that its ecological impacts are more fully understood. The role of *R. flavipes* as a major decomposer of wood and its effects on the physical and chemical properties of soil have been documented but its influence on soil nutrient and microbial communities is little understood.

Molecular techniques have allowed scientists to investigate changes in microbial communities on a scale never achieved before (Rastogi and Sani, 2011; Philippot et al., 2013). These techniques can be used to investigate the influence termites have on soil microbial communities. In terrestrial ecosystems, soil can contain up to $5x10^{30}$ microbial cells, and up to 1 million species per ton of soil, that play a key role in soil formation and structure, decomposition, and nutrient cycling (Rastogi and Sani, 2011; Russo et al., 2013). Soil ecosystems rely on soil microorganisms to suppress soil born plant disease, promote plant growth and plant community diversity (Rastogi and Sani, 2011; Philippot et al., 2013). Any changes in the soil microbial community can effect nutrient cycling and plant growth. Subterranean termites could have an ecological role outside of wood decomposition and soil engineering that could affect plant growth and thus forest composition.

The beneficial impact of termites on plant growth and community structure through soil engineering that influences aeration and hydration is well known in tropical

and savannah ecosystems (Konate et al., 1999). It is also important to understand the negative or positive impacts of termites on plant growth as a result of manipulating soil microbes and nutrients, especially as subterranean termites are so widespread and numerous in temperate deciduous forests. It is the aim of this research to initiate an investigation of the effects of *R. flavipes* on plant growth with a controlled laboratory study and whether any impacts are the result of changes in soil microbial communities or soil nutrient composition.

Materials and Methods

Termite-soil interaction

Top soil lacking termites, a least 20 meters from foraging termites, and at a depth of 1-25 cm was collected from the forest floor in Greenbelt, Maryland. The soil was passed through a 4mm screen to remove large woody debris and stones. Any macro organisms visible to the naked eye were removed by hand. The soil was well mixed by hand and split evenly into two 6000 cm^3 plastic containers. Termites were divided into one container of soil at the concentration of one termite per cm^3 and one container with no termites. The two containers of soil were covered with a fitted plastic lid and placed in the dark for 3 weeks at 25°C. After 3 weeks the termites were removed from the soil and a sample of each soil type (termite and no termite) was taken and put in a -80°C freezer for later DNA extraction. No dead or diseased termites were found in the soil.

Soil microbe analysis

The DNA was extracted from each soil type (termite and no termite) using a QIAGEN DNeasy Power Soil kit. Partial 18S ribosomal DNA, which included flanking

regions (partial sequence of 18S ribosomal RNA, the internal transcribed spacer 1, 5.8S ribosomal RNA, internal transcribed spacer 2, and partial sequence of 28S ribosomal RNA), was amplified using PCR primers PN3 and PN34 (Viaud et al., 2000: PN3 primer: CCGTTGGTGAACCAGCGGAGGGATC and PN34 primer: TTGCCGCTTCACTCGCCGTT). Partial 16S ribosomal DNA was amplified using PCR primers E8F and E1541R (Baker et al., 2003: E8F primer: AGAGTTTGATCCTGGCTCAG and E1541R primer: AAGGAGGTGATCCANCCRCA). PCR products (single bands) were cleaned (Qiagen PCR purification kit) and restriction digested with HAE III (Promega) using the recommended protocol. The digested DNA was run through an 1.4% agarose gel to detect any differences in band patters between termite and no termite soil for both the eukaryote and prokaryote DNA.

Plant growth

Twenty plastic trays 20cm in diameter were set along a watering line in a greenhouse. Each watering tray was assigned a number 1-20 and the placement of each tray along the watering line was randomized via a random number generator. Even numbers were designated for termite treated soil and odd numbers for the soil with no termites. Four pots (8cm x 6cm x 6cm, height, width, length) were placed in each watering tray for a total of 80 pots. Each pot was filled with equal amounts of soil. Two sunflower (*Helianthus annuus*) seeds were placed 3cm below the soil line in each pot. The pots were evenly watered every two days by filling the watering trays via an automatic watering line. The germination rate was recorded for 7 days after planting and then the seedlings were culled to one per pot. The sunflowers were allowed to grow for

10 weeks while flowering time was recorded. After 10 weeks the plants were cut at the soil line and dried in a dry oven at 60°C until they were fully dehydrated. The dry biomass was weighed for each watering tray and summed for each soil treatment (termite and no termite).

Soil and plant nutrient analysis

A soil sample was taken and dried for each soil type (termite and no termite) after the termites were removed but before the sunflowers were planted, and after the plants were removed. These soil samples along with a tissue sample from each plant was sent to the University of Massachusetts Extension Soil and Plant Nutrient Testing Laboratory for nutrient analysis. The macronutrients and micronutrients were extracted from the soil using the modified Morgan extraction method (McIntosh, 1969) and analyzed using inductively conductive plasma (ICP). The soil nitrate (NO3-) levels were measured using an ion specific electrode. Dried plant tissue samples were mixed for each treatment (termite and no termite), and the total tissue P, K, Ca, Mg, Zn, Cu, Mn, Fe, and B was determined by ICP spectrometry of acid wet digestion using nitric acid, hydrochloric acid, and hydrogen peroxide in a block digester. The total nitrogen (N) of the plant tissue was determined by catalytic combustion.

Soil moisture retention

The moisture retention of each soil type (termite and no termite) was determined by placing an equal amount of each soil type in 14 small pots for a total of 28 pots. The soil in each pot was saturated with sterile H_2O until the maximum reading was achieved on the soil moisture meter. The 28 pots were numbered and placed in a randomized grid

in the dark at 25°C. The soil moisture level in each pot was recorded daily with a soil moisture meter for 5 days.

Statistical analysis

Plant biomass and plant tissue macronutrient concentration data were analyzed with Shapiro-Wilk tests for normality followed by a Mann-Whitney U test.

Results

Soil microbe analysis

PCR produced single bands of expected product size (16S: approximately 1500 bp, 18S: approximately 700 bp). There were no observable differences in the banding patterns between soil treatments with (T) or without termites (NT) for both prokaryote soil DNA (T16 and NT16) eukaryote soil DNA (T18 and NT18).

Ladder T16 NT16 T18 NT18

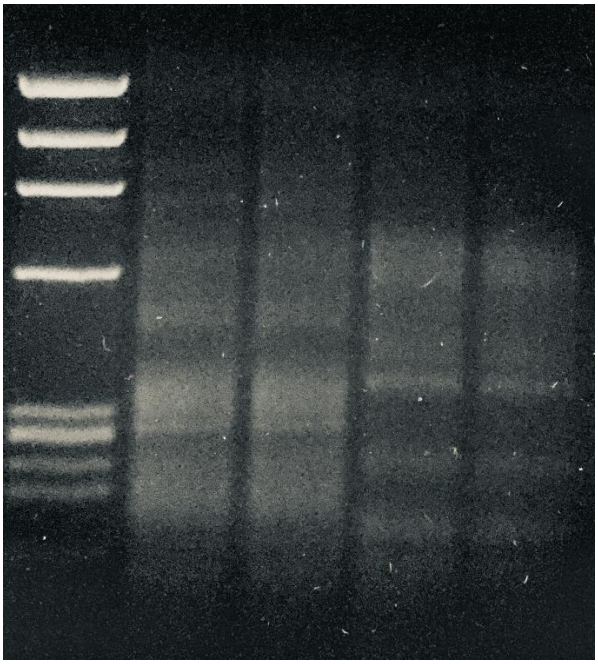

Figure 2.1 Restriction digest of termite and no termite 16S soil DNA (T16 and NT16) and termite and no termite 18S soil DNA (T18 and NT18). Ladder base pair sizes from top to bottom (1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72).

Plant growth

A significant difference was detected between the dry biomass of plants grown in termite soil and plants grown in no termite soil ($Z = -3.06186$, $p = 0.002$) (Figure 2.2). No difference in germination or flowering times were detected (data not shown).

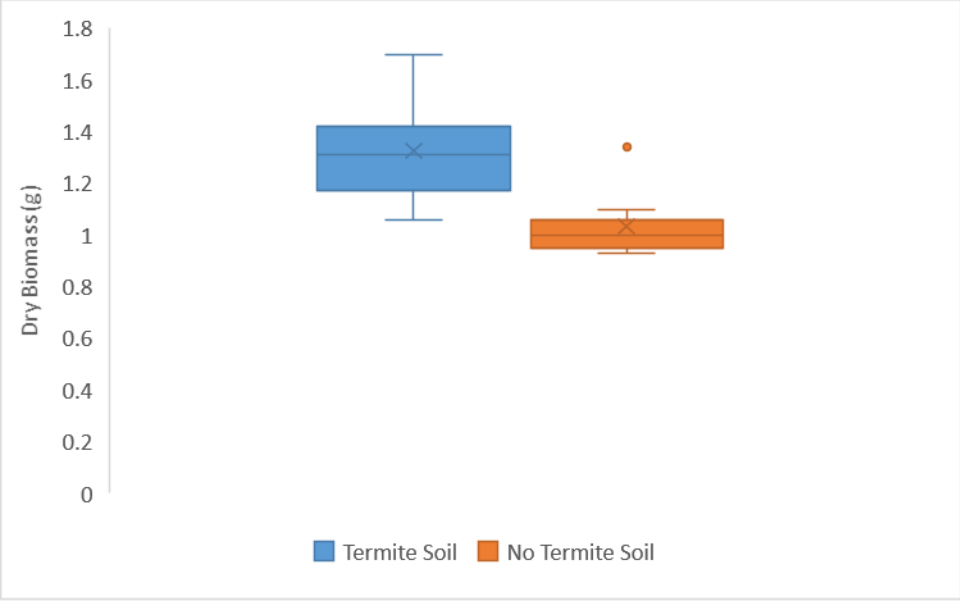

Figure 2.2 Difference in dry plant biomass for termite and no termite soils ($Z = -3.06186$, $p = 0.002$).

Soil and plant nutrient analysis

No significant difference was detected between the K or Ca concentrations in the tissue of plants grown in termite and no termite soil (K: $Z = -1.67382$, $p > 0.05$; Ca: $Z = -1.51052$, $p > 0.05$). A significant difference was detected between the N and P

concentrations in the tissue of plants grown in termite and no termite soil (N: Z = -3.06186, p = 0.002; P: Z= -2.24537, p = 0.024) (Table 2.1 and 2.2).

	Soil Before Planting	*Soil After Harvest*	*Plant Tissue*
Macronutrients	ppm	ppm	%
Phosphorus (P)	12	10.2	0.25
Potassium (K)	187	48	1.97
Calcium (Ca)	2022	1873	5.16
Magnesium (Mg)	188	244	0.91
Sulfur (S)	19.6	59.1	N/A
Nitrate (NO3-N)	42	8	0.76 (N)
Micronutrients			ppm
Boron (B)	0.7	0.1	67.5
Manganese (Mn)	12.9	13.1	175.5
Zinc (Zn)	25.2	12.7	179.9
Copper (Cu)	0.8	0.4	15.9
Iron (Fe)	23.1	18.9	80
Aluminum (Al)	21	17	N/A
Lead (Pb)	5.5	3.8	N/A
pH	5.9	5.5	N/A

Table 2.1 Nutrient concentrations in ppm for no termite soil before planting and after harvest. Plant tissue macronutrients are shown as percent and micronutrients are shown ppm.

	Soil Before Planting	*Soil After Harvest*	*Plant Tissue*
Macronutrients	ppm	ppm	%
Phosphorus (P)	14	8.4	0.23
Potassium (K)	197	44	1.73
Calcium (Ca)	2225	1811	4.79
Magnesium (Mg)	208	224	0.95
Sulfur (S)	21.1	54.4	N/A
Nitrate (NO3-N)	43	5	0.75 (N)
Micronutrients			ppm
Boron (B)	0.8	0.1	66.9
Manganese (Mn)	13.3	10.3	167.8
Zinc (Zn)	21.4	12.4	180.6
Copper (Cu)	0.7	0.4	16.2
Iron (Fe)	18.3	17.1	43
Aluminum (Al)	18	17	N/A
Lead (Pb)	5	3.6	N/A
pH	5.9	5.5	N/A

Table 2.2 Nutrient concentrations in ppm for termite soil before planting and after harvest. Plant tissue macronutrients are shown as percent and micronutrients are shown ppm.

Soil moisture retention

There was no detectable difference between the moisture retention of termite and no termite soil (Figure 2.3).

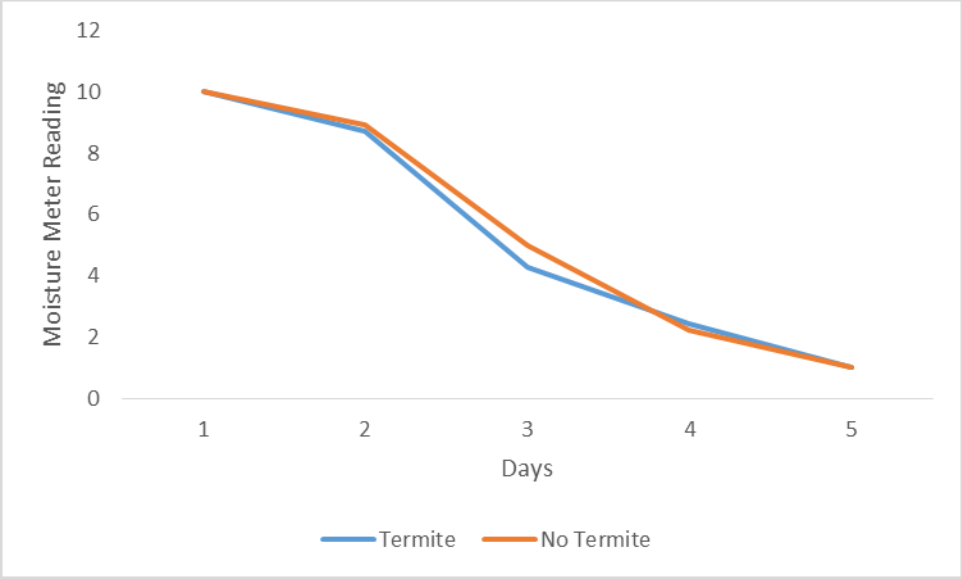

Figure 2.3 Moisture retention of termite and no termite soil measured with a soil moisture meter for 5 days.

Discussion

The subterranean termite *R. flavipes* has a significant impact on plant growth. This was not caused by a physical change in the soil due to tunneling improving drainage or aeration of the soil or moisture retention. Instead, a mechanism that allowed the sunflower plants to absorb and assimilate more nitrogen and phosphorus in termite treated soil appeared to stimulate growth. Because the soil was well mixed before the seeds were planted, tunnels were destroyed and any chemical or biological changes made to the soil in close proximity to those tunnels were evenly distributed. The soil microbial analysis did not show any differences between the two treatments. Any subtle impacts that *R. flavipes* workers have on the soil microbiome requires next generation sequencing analysis, especially of the of the diverse prokaryote community structure associated with soils.

An alternative mechanism for the increase in nitrogen in the tissue of plants grown in termite soil could be mediated by nitrogen fixing bacteria in the termite gut. A termite's diet consist of carbon rich but nitrogen poor cellulose (Ohkuma et al., 1999; Lilburn et al., 2001). A symbiotic relationship between nitrogen fixing bacteria and termites exists and facilitates termite survival on dead wood (Ohkuma et al., 1999). The diet of wood feeding termites can contain as little as 0.05% nitrogen and nitrogen fixing bacteria can provide up to 60% of the nitrogen in termite biomass (Lilburn et al., 2001). Several nitrogen fixing bacterium have been cultured from the termite gut (*Citrobacter freundii*, *Pantoea agglomerans, Desulforibrio spp,* and *Enterobacter agglomerans*) (Ohkuma et al., 1999; Lilburn et al., 2001). Many more have been identified by molecular techniques including *Spirochaetes*, which accounts for up to 50% of all prokaryotes in the termite gut (Lilburn et al., 2001). The amount and diversity of nitrogen fixing bacteria varies between species, but a distinction can be made between lower and higher termites, with lower termites, which include *R. flavipes*, having more nitrogen fixing activity than higher termites (Ohkuma et al., 1999).

Termites could be consuming the organic matter in the soil and producing feces that has slightly more bioavailable nitrogen than organic matter not consumed by termites. The nitrogen fixing bacteria in the termite gut could be facilitating the conversion of non-bioavailable nitrogen in soil to bioavailable nitrogen that can be more easily absorbed and assimilated by plants. Termites could also be providing more nitrogen in the form of molted cuticles and other nitrogen compounds expelled from the gut. This increase in bioavailable nitrogen would improve plant growth in the vicinity of

subterranean termite tunnels. A more robust analysis of the bioavailable and non-bioavailable nitrogen in soil with and without termites needs to be performed.

Soil microbes, such as arbuscular mycorrhizae fungi and bacteria, can make nitrogen bioavailable for plants and increase phosphorus absorption (Artursson et al., 2006). These communities of soil microbes have been shown to work synergistically and improve plant growth through nutrient absorption (Artursson er al., 2006). Termites could be altering the soil microbiome and creating an environment that favors these communities and fosters synergistic interactions that improve plant growth. We cannot know the affects that termites have on the soil microbiome without using a more refined molecular tool, such as next generation sequencing technology. The results of this study warrant further investigation using next generation sequencing technology to support or rule out any affects termites might have on the soil microbiome that could impact plant growth. We plan on using next generation sequencing technology in the near future.

3.

Competitive interactions between temperate forest termites and two common wood-rot fungi

Abstract

Termites and fungi are the primary decomposers of dead wood. Interactions between termites and wood-rot fungi is inevitable given their shared food source. Termites have developed multiple defense strategies against infectious fungi, such as *Metarhizium*, that include antifungal proteins in their saliva and fungal inhibition properties in their gut. The antifungal properties of termite saliva is dependent on β-1,-3,-glucanase activity. Given the overlap in niches, there is opportunity for interference competition between termites and wood-rot fungi to occur. Here we demonstrate that the antifungal proteins in the saliva and the antifungal properties of the gut of the eastern subterranean termite *Reticulitermes flavipes* affects the growth of two common wood-rot fungi *Gloeophyllum trabeum* and *Phanerochaete chrysosporium*.

Introduction

The eastern subterranean termite *Reticuletermes flavipes* is a multi-site nesting species found throughout the east coast of the United States and inhabits temperate forest (Maynard et al., 2015). *R. flavipes* builds nests in the soil in order to expand existing nests and find new food sources (Bulmer et al., 2001). Colonies of *R. flavipes* can have hundreds, thousands, and potentially millions of individuals mostly consisting of the worker caste (Thorne et al., 1999). Workers actively feed on wood and build foraging galleries in soil that link together the multiple nests of the colony (Bulmer et al., 2002).

The multi-site nesting and foraging behavior of *R. flavipes* makes their colonies amoebae-like, probing from one piece of dead wood to another across the forest floor (Thorne et al., 1999).

Termites along with fungi are the primary decomposers of dead wood in temperate forest ecosystems (Bradford et al., 2014; Maynard et al., 2015; Lansdale et al., 2008; Fukami et al., 2010; Ulyshen et al., 2014). The ecology of termites and wood-rot fungi are still largely unknown and can have significant effects on nutrient cycling (Lansdale et al., 2008; Maynard et al., 2015). Local scale variations in carbon cycling need to be understood to properly investigate the effects and predict the consequences of global climate change (Bradford et al., 2014). Dead wood accounts for 5-10% of the world's carbon store, up to 73 Pg globally (Bradford et al., 2014; Maynard et al., 2015). Dead wood carbon is stored in the soil for later use by fungi and invertebrates, such as termites (Bradford et al., 2014). The interactions of fungi and invertebrates, such as termites, can determine the rate of decomposition and influence the productivity of a forest (Bradford et al., 2014). Identifying the interactions of wood decomposition organisms is essential to understanding regional and global carbon cycles (Maynard et al., 2015).

Wood-rot fungi and termites both inhabit and use dead wood as a food source. This makes interactions between termites and wood-rot fungi inevitable. The environmental conditions that are favored by termites are also favored by wood-rot fungi (Grant et al., 2012). Wood-rot fungi inhabit dead wood first followed by termites (Grant et al., 2012). Termites are attracted to wood inhabited by some wood-rot fungi while they avoid wood inhabited by other wood-rot fungi (Cornelius et al., 2002). It was originally

thought that brown rot fungi attracted termites while termites avoided white rot fungi, but it has become clear that this relationship is more complicated (Grant et al., 2012). When encountering two common wood-rot fungi, *R. flavipes* is preferentially attracted to wood decayed by *Phanerochaete chyrosporium* over wood decayed by *Gloeophyllum trabeum* despite *G. trabeum* producing the chemical (Z, Z, E)-3, 6, 8-dodecatrien-1-o1, which mimics the trail pheromone of *R. flavipes* (Cornelius et al., 2002). *P. chrysosporium* digest lignin, cellulose, and hemicellulose using extra cellular oxidative enzymes including oxidases and peroxidases (Kersten and Cullen, 2007). *G. trabeum* digest hemicellulose and cellulose while modifying lignin by demethylation and oxidation without metabolizing it (Cornelius et al., 2002). *R. flavipes* might be responding to differences in the chemical composition of wood and/or the chemical by products of lignin digestion by *P. chrysoporium* (Cornelius et al., 2005). The decay time of wood with *P. chrysosporium* does not affect the attraction or feeding preference of *R. flavipes* (Cornelius et al., 2002). Termites respond differently to wood decayed by different wood-rot fungi depending on the species of fungi, and there is no positive statistical correlation between the presence of termites and any group of fungi (Grant et al., 2012). However, termites do prefer wood decayed by certain wood-rot fungi over non-decayed wood (Cornelius et al., 2002). This may be due to the wood becoming more nutritious for termites after being decayed by certain wood-rot fungi (Cornelius et al., 2002). Some white rot fungi degrade all wood cell wall components while other white rot fungi are more selective, degrading only lignin and leaving large concentrations of cellulose (Cornelius et al., 2002). *R. flavipes* has also been observed feeding directly on basidiocarps (Zoberi and Grace, 1990).

Metarhizium is an endophytic insect pathogenic fungus found in soils around the world with concentrations up to 10^6 propagules per gram of soil (Behie and Bidochka, 2014). *Metarhizium* can infect over 200 species of insect (Sasan and Bidochka, 2012; Behie and Bidochka, 2013; Liao et al., 2013; Behie and Bidochka, 2014). *Metarhizium* spores attach to the insect cuticle infecting and then killing the insect (Behie and Bidochka, 2013; Behie and Bidochka, 2014). Soil dwelling insect pathogenic fungi like *Metarhizium* are potentially lethal to termites and pose a problem to nest survival. Chouvenc et al. suggest that Metarhizium is not a problem for subterranean termites because of their fungal defenses strategies. However genetic studies suggest that fungal pathogens (*Metarhizium*) impose strong selective pressure on subterranean termites. Termites have evolved several mechanisms to defend against infection of *Metarhizium*. The saliva of soil inhabiting termites contains two enzymes Gram-negative bacteria binding proteins (GNBPs) and termicins that have antifungal properties (Hamilton et al., 2011). The GNBPs break through the fungal cell wall via β-1,-3-glucanase activity allowing termicins to enter and interfere with fungal cell membranes (Hamilton et al., 2011). The termite gut also inhibits the germination of *Metarhizium*, facilitating the destruction of conidia ingested during the grooming of nest mates (Chouvenc et al., 2009).

Termites along with ants are the most abundant macroinvertebrates in dead wood (King et al., 2013). Termites affect wood decomposition directly by feeding and indirectly by interacting with fungi (Maynard et al., 2015). The niche opportunities for wood-rot fungi are influenced by interactions with other organisms, such as termites (Lansdale et al., 2008). The overlap of niches in dead wood by fungi and termites

provides a situation where competition for resources could exist. The same antifungal defenses used against *Metarhizium* may be used by termites to exclude wood-rot fungus from foraging and nest galleries in dead wood. This exclusion could alter wood decomposition rates that affect carbon cycling, and developing a better understanding of subterranean termite interactions with wood-rot fungi is required for determining their impacts on carbon cycling and sequestration.

Materials and Methods

Termite colony collection and survival assay

Colonies of *R. flavipes* with a minimum of 200 termites each were collected from Massachusetts (6 colonies), North Carolina (6 colonies), and Maryland (4 colonies). Wood containing an appropriate size colony was cut in to manageable lengths and put in a plastic container. These colonies were kept in the laboratory under dark moist conditions at 25°C. Collection sites were separated by approximately 60 meters to help ensure that the collections were from different colonies (Vargo and Husseneder, 2009).

The effectiveness of each colony's pathogenic fungal defenses was assessed by a survival assay. Termite workers from each colony were placed in petri dishes with filter paper moistened with 300µl of filtered and sterile H_2O for 48 hours. This allowed termites to groom each other and remove cuticular microbes (Hamilton et al., 2011). Termites were infected by submerging 12 workers from each colony in 120 µl of 10^7 *Metarhizium brunneum* conidia ml^{-1} of 0.1% Tween 80. The isolation and preparation of *M. brunneum* conidia from soil is described in Denier and Bulmer, 2015 (isolate 002). Termites were submerged and gently shaken for 10 seconds and placed on filter paper to

remove excess solution. Termites used for the control treatments were submerged in a 0.1% Tween 80 solution with no *Metarhizium* conidia. Once the excess solution was removed the termites were placed in a petri dish with filter paper moistened with 300µl of sterile H_2O. The termites were checked daily and any dead termites removed. These cadavers were surface sterilized with 70% ethanol and put in a petri dish with filter paper moistened with 300µl of sterile H_2O. The dead termites were monitored daily for evidence of *Metarhizium* infection. *Metarhizium* infection is evident by the growth of white mycelium from the dead termite body followed by a green muscardine appearance associated with sporulation. Only termites that died of *Metarhizium* infection were counted. After 15 days of observation, four colonies with high survivorship were selected and used to test the effects of termites on wood rot fungi.

Wood rot fungi

Wood-rot fungi were obtained from the Center for Forestry Mycology Research United States Forest Service Northern Research Station. Two species of fungi were used, one brown rot (*Gloeophyllum trabeum*) and one white rot (*Phanerochaete chrysosporuim*).

Spore incubation assay

The effects of termite antifungal enzymes found on the cuticle were investigated by incubating fungal spores with termite cuticular wash. The conidia were suspended in sterile H_2O, and the concentration was determined with a hemocytometer. The suspension was then diluted to a 10^4 conidia ml^{-1} sterile H_2O. The cuticular wash was prepared by placing worker termites, which had been briefly cold immobilized on ice, in

sterile H_2O and agitating for 20 seconds (1 termite per 4µl of sterile H_2O). The termites were removed and the remaining liquid centrifuged through a silica column (EconoSpin™) at 12,000g for 2 minutes. The filtered cuticular wash (10µl) was incubated with the conidia solution (10µl) and 100ug ml^{-1} ampicillin (20µl) to make a 40µl incubation solution. Sterile H_2O (10µl) was used in place of the filtered cuticular wash for the control. The solutions were plated on agar (Fisher Scientific) after 48 hours of incubation and the area covered by mycelial growth was measured after 72 hours. The area coverage was measured by marking the boundary of the mycelium growth with a black marker and counting the number of squares on a 2.5mm grid that were free of mycelium. This method was employed for the two wood-rot fungi and modified for *Metarhizium*. *Metarhizium* was used to confirm the antipathogenic actions of the termite cuticle wash (Hamilton et al., 2011). CFU's were counted instead of area coverage of mycelium given the different growth patterns of wood-rot fungi and *Metarhizium*. Eight replicates were used for each treatment and control for each fungi. This procedure was performed for each of the four colonies selected from the survival assay used to assess the effectiveness of fungal pathogen defenses.

Spore incubation assay with GDL

The potential for fungal inhibition by cuticular β-1,3-glucanases was tested by incubating *P. chrysosprium* conidia with cuticular washes. The assay followed the procedure described above using cuticular wash and control treatments and a treatment that included D-δ-gluconolactone (GDL), which is an inhibitor of termite β-1,3-glucanase activity (Hamilton et al. 2011). The GDL treatment was prepared by adding 400mM GDL (10µl) to filtered cuticular wash (10µl), conidia suspension (10µl), and 200µg/ml

ampicillin (10µl) to make a 40µl incubation mix. The control GDL treatment was prepared by adding 400mM GDL (10µl) to conidia suspension (10µl), and 100µg/ml ampicillin (20µl) to make a 40µl incubation mix. The cuticular washes from each of the four colonies were not tested individually, but were pooled and mixed in an equal ratio for this assay.

Wood disk assay

Small disks of wood cut from a pine dowel rod 3.2cm in diameter and 0.5cm in height were sterilized by boiling in H_2O for 30 minutes. These wood disks were flame sterilized for 20 seconds on each side and placed in a sterile petri dish. Plugs of agar containing mycelium were placed in the center of each wood disk and allowed to grow for 72 hours. A group of 160 termite workers was placed on filter paper moistened with 300µl of sterile H_2O. The termites were allowed to feed on the moist filter paper for 48 hours. Ten workers were then placed in each treatment dish and control dishes did not receive termites. Eight replicates of treatments and controls were used for each wood-rot fungi (*G. trabeum* and *P. chrysosporium*). The dishes were kept in the dark and close to 100% humidity at 25°C for 6 days. After 6 days the dishes were visually inspected under a stereo dissecting scope and each dish was scored as inhibition or no inhibition of fungal growth. Inhibition was determined by comparing the amount of mycelium growth from the agar plug in the center of the wood disk to the edge of the wood disk and/or the presence of fungal spores between treatment and control dishes. Termites were removed, cold immobilized, surface sterilized by brief submergence in 70% ethanol and their alimentary tracts including the hindgut were removed with fine forceps. The alimentary tracts were combined with 20µl of sterile H_2O and 20µl 100µg/ml ampicillin. This

solution was vortexed for 30 seconds and then plated on agar and spread with a glass stir rod. This was repeated for each wood-rot fungus independently. Agar plates were kept in the dark and close to 100% humidity at 25°C. After 6 days the plates were inspected for the presence of fungal growth. Spore samples from distinct sporing bodies were streaked with a sterile inoculation loop on new plates to obtain fungal isolates. Spores were taken from these isolates and their DNA was extracted with a QIAGEN DNAeasy blood and tissue kit using the animal tissue protocol with a 2 hour proteinase K-digest. Partial 18S ribosomal DNA was PCR amplified (PN3 and PN34 primers, Denier and Bulmer, 2015) and the products were Sanger sequenced (Macrogen USA). The identification of fungi found in the gut of termites that were in the presence of wood-rot fungi was used to determine if the wood-rot fungi was ingested by the termites and if the termite gut inhibited the wood-rot fungi.

Fungal competition assay

Termite workers were placed on a sterile wood disk with an agar plug that contained no fungal mycelium and kept in the dark and close to 100% humidity at 25°C. The same procedure was followed as above.

Three pure cultures were obtained and tested against two wood-rot fungi (*G. trabeum* and *P. chrysosporium*). An inoculation loop immersed in conidia from the gut isolates was stabbed into agar at one end of a plate and a loop immersed in wood-rot spores was placed at the opposite end of the plate. Five replicates were prepared for each pure culture and wood-rot combination. The plates were kept in the dark and close to 100% humidity at 25°C. After 72hrs the furthest extent of fungi mycelium growth from the inoculation point in the agar was measured for *G. trabeum* and *P. chrysosporium*.

Statistical analysis

Mycelium coverage and colony forming unit (CFU) counts for spore incubation data were analyzed with Shapiro-Wilk tests for normality followed by Mann-Whitney U test. Mycelium coverage and CFU counts for the spore incubation with GDL data were analyzed with Shapiro-Wilk tests for normality followed by ANOVA and Tukey's HSD for multiple comparisons. Mycelium growth for fungal competition data was analyzed with Shapiro-Wilk tests for normality followed by ANOVA and Tukey's HSD for multiple comparisons.

Results

Spore incubation assay

Significant differences in mycelium growth were detected between spore incubation treatments and controls of *G. trabeum* and *P. chrysosporium*. Cuticular wash treatments appear to accelerate the growth of *G. trabeum* mycelium compared to controls ($Z = 4.75993$, $p < 0.001$) and inhibit the growth of *P. chrysosporium* mycelium compared to controls ($Z = -4.75993$, $p < 0.001$). A statistically significant reduction in CFU's was detected between spore incubation treatments and controls of *Metarhizium*. Cuticular wash treatments appear to inhibit the growth of CFU's compared to controls ($Z = -3.50449$, $p < 0.001$).

Spore incubation assay with GDL

A significant difference in *P. chrysosporium* mycelium growth was detected between the cuticular wash (CW) treatment and either the control (C), GDL, and control GDL (CGDL) treatments ($F = 16.0090$, $p < 0.001$, Tukey's HSD $p < 0.01$ for each

comparison) (Figure 3.1). The results were similar for *Metarhizium* CFU's between the cuticular wash treatment and control, GDL, and control GDL treatments ($F = 22.9720$, $p < 0.001$, Tukey's HSD $p < 0.01$) (Figure 3.2). There appeared to be a synergistic effect in inhibition when the four colonies cuticular washes were pooled and mixed. Any visible growth of *P. chrysosporium* mycelium was delayed by at least 24 hrs in the cuticular wash treatment when compared to control, GDL, and control GDL treatments. This delay was not detected when the colony cuticular washes were tested individually.

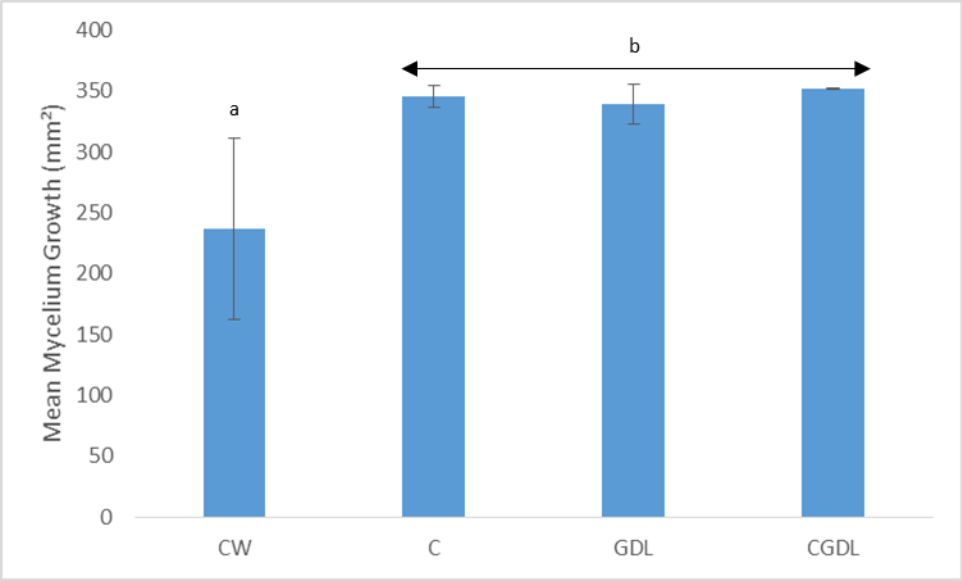

Figure 3.1. Inhibition of cuticular wash anti- *P. chrysosporium* activity by GDL. Control (C) used sterile H_2O in place of cuticular wash (CW). GDL and control GDL (CGDL) treatments contained 100mM GDL. Error bars represent ±SD of mean mycelium growth (mm^2). Different letters indicate statistically significant differences in fungal growth.

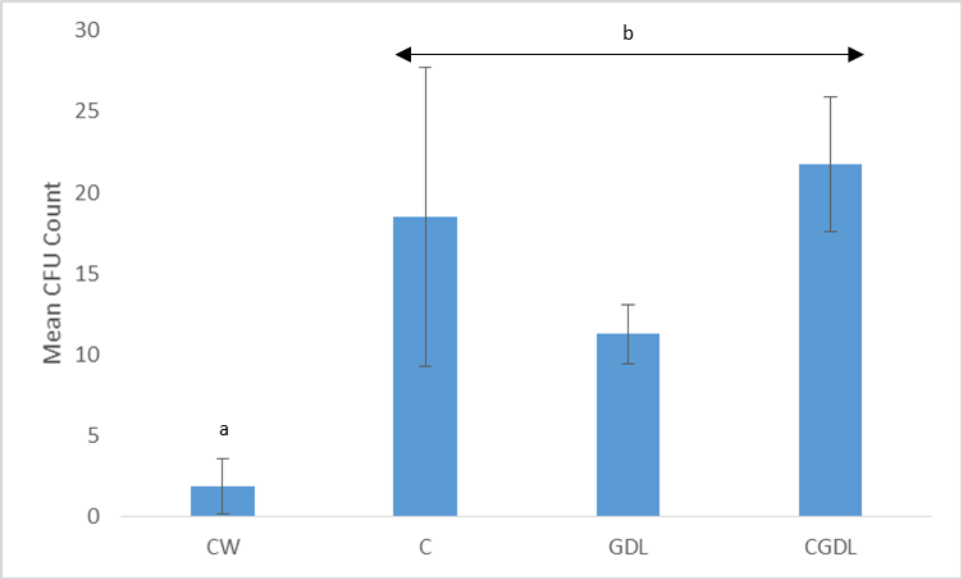

Figure 3.2. Inhibition of cuticular wash anti- *M. brunneum* activity by GDL. Control (C) used sterile H_2O in place of cuticular wash (CW). GDL and control GDL (CGDL) treatments contained 100mM GDL. Error bars represent ±SD of mean mycelium growth (mm^2). Different letters indicate statistically significant differences in fungal growth.

Wood disk assay

The presence of termites inhibited the growth of mycelium and the formation of spores for all eight replicates of both *G. trabeum* and *P. chrysosporium*. Seven of the eight agar plugs used in the *G. trabeum* trials appeared to be consumed by the termites, while only one of the eight agar plugs used in the *P. chrysosporium* trials appeared to be consumed by the termites. The fungi isolated from the termite gut was identified as *Trichoderma* by comparison of 18S ribosomal DNA sequence with the nucleotide collection in the National Center for Biotechnology Information's (NCBI) databases. Neither of the wood-rot fungi was identified in the termite gut.

Fungal competition assay

Fungi isolated from the termite gut exhibited three distinct conidiophore morphotypes; small <.5mm (sm), medium .5-1.5mm (md), and large >1.5mm (lg). The fungi isolated from the termite gut was identified as previously described. A significant reduction in mycelium growth was detected for *G. trabeum* ($F = 243.0578$, $p < 0.001$) and *P. chrysosporium* ($F = 15.7530$, $p < 0.001$) when in the presence of termite gut fungi. There was significant inhibition of *G. trabeum* mycelium growth when in the presence of termite gut fungi compared to controls (sm: Tukey's HSD $p < 0.01$; md: Tukey's HSD $p < 0.01$; lg: Tukey's HSD $p < 0.01$) (Figure 3.3). There was also significant inhibition of *P. chrysosporium* mycelium growth when in the presence of termite gut fungi compared to controls (sm: Tukey's HSD $p < 0.05$; md: Tukey's HSD $p < 0.01$; lg: Tukey's HSD $p < 0.01$) (Figure 3.4).

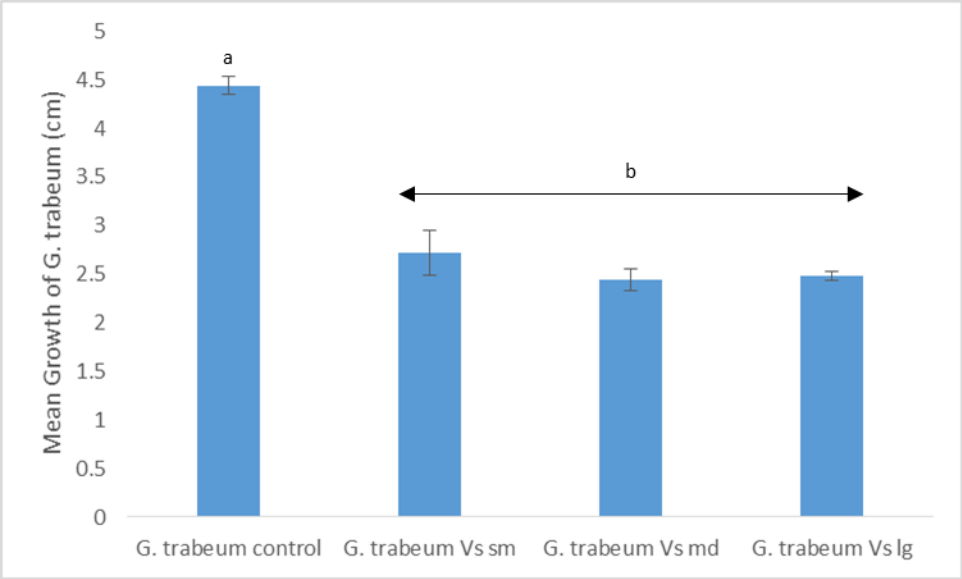

Figure 3.3. Inhibition of *G. trabeum* by different conidiophore morphotypes of *Trichoderma spp.* Bars represent mean growth of *G. trabeum* mycelium (cm) (±SD). Different letters indicate statistically significant differences in fungal growth.

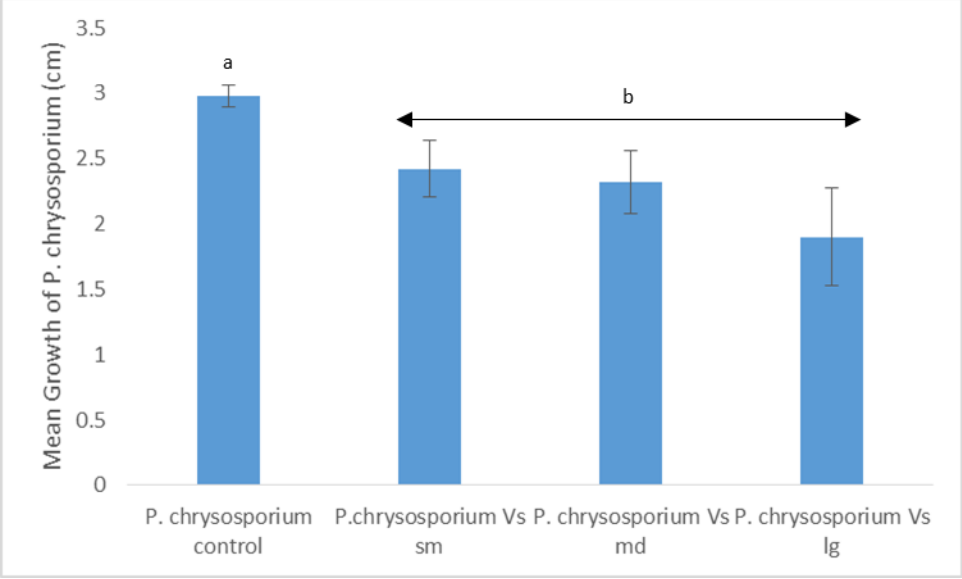

Figure 3.4. Inhibition of *P. chrysosporium* by different conidiophore morphotypes of *Trichoderma spp.* Bars represent mean growth of *P. chrysosporium* mycelium (cm) (±SD). Different letters indicate statistically significant differences in fungal growth.

Discussion

Antifungal proteins of termite saliva have been shown to be effective inhibitors of infectious fungi including *Metarhizium* (Hamilton et al., 2011). The active antifungal components of termite saliva are Gram-negative bacteria binding proteins (GNBPs) and termicins, which are highly expressed in termite salivary glands (Hamilton et al., 2011). These GNBP's have β-1,3-glucanase activity that break down the β-1,3-glucan of the fungal cell wall allowing termicins to enter and interfere with fungal cell membranes (Hamilton et al., 2011). Termites appear to spread saliva on their cuticle during self and allogrooming, and incorporate saliva into their gallery walls. The termite gut also has specific antifungal properties that allow for the ingestion and inhibition of infectious fungal spores, including *Metarhizium*, during the grooming of nest mates (Chouvenc et al., 2009).

The saliva and the antifungal proteins it contains were removed from the cuticle and tested against other fungi, including two wood-rot fungi. The antifungal peptides found in the saliva of *R. flavipes* are effective against the white rot fungi *P. chrysosporium* but not the brown rot fungi *G. trabeum*. The mycelium growth of *P. chrysosporium* was significantly inhibited while the mycelium growth of *G. trabeum* was enhanced. This discrepancy might be due to the antifungal peptides working against *P. chrysosporium* but not affecting *G. trabeum*, which could have used nutrients in the cuticular wash solution as a food source, giving the cuticular wash treatments of *G. trabeum* an advantage over the control treatments. The addition of GDL to cuticular wash treatments of *P. chrysosporium* significantly decreased the inhibition of mycelium growth, suggesting that the β-1,-3-glucanase activity of the GNBP's in the saliva is a

contributing factor to the inhibition effect of the cuticular wash treatments. The synergistic effect observed with the pooling of multiple colonies cuticular washes might be explained by variation in the effectiveness of antifungal enzymes from colony to colony.

The growth of *G. trabeum* and *P. chrysosporium* on wood was inhibited by the presence of *R. flavipes*. Yet our assays suggest that antifungals associated with the cuticle and derived from the salivary gland, promote rather than inhibit *G. trabeum* growth. Termite ingestion of wood-rot fungi is likely to be critical for limiting fungal growth and cuticle antifungal activity is more likely to be critical for limiting the growth of pathogenic spores that can attach to cuticular surfaces. Neither *G. trabeum* nor *P. chrysosporium* was found in the gut of *R. flavipes* after their apparent ingestion. These results are consistent with Jayasimha and Henderson (2007) who showed that the growth of *G. trabeum* was inhibited in the presence of *Coptotermes formosanus*, and there was no evidence of *G. trabeum* in the gut of *C. formosanus*. Fungi isolated from the gut of *R. flavipes* appears to be *Trichoderma spp. Trichoderma spp.* was also isolated from the gut of *C. formosanus* (Jayasimha and Henderson, 2007). This genus is a free living fungus found in temperate and tropical soils with concentrations of 10^1-10^3 propagules per gram of soil (Harmen et al., 2004). *Trichoderma* is parasitic on other fungi and can express β-1,-3-glucanase activity (Benitez et al., 2004). This suggests that the antifungal activity of *R. flavipes* and other subterranean termites, such as *C. formosanus*, may be attributable to intrinsic antifungals of termites and their symbionts as well as extrinsic antifungals derived from ingested microbes such as *Trichoderma spp*.

These results suggest that the antifungal peptides in termite saliva and the fungi found in the termite gut can be used to exclude some wood-rot fungi from areas inhabited by termites. This can give termites the upper hand when competition for resources arise. It has been proposed that wood decayed by wood-rot fungi is more nutritious for termites (Cornelius et al., 2002). Yet, termites respond differently to decayed wood depending on the species of fungi, wood species, and environment (Grant et al., 2012).

The relationship between wood-rot fungi and termites is more complicated than interference competition. Termites' ability to exclude some wood-rot fungi changes the decomposer community structure in dead wood. Community structure is considered a key factor of ecosystem functioning, and the species diversity and composition of decomposers is a critical component of ecosystem carbon cycles. The interactions of fungi and invertebrates, such as termites, can determine the rate of decomposition and influence the productivity of a forest (Bradford et al., 2014). Identifying the interactions of wood decomposition and soil organisms is essential to understanding regional and global carbon cycles (Maynard et al., 2015). The interactions investigated here are but a small part of a much larger, more complicated picture.

4.

Conclusion

Reticulitermes flavipes is well understood as a pest but has a poorly understood ecological role in forests. In a laboratory experiment, the presence of *R. flavipes* in soil significantly increased phosphorus and nitrogen concentration in sunflower plants resulting in increased plant productivity. *In vivo* and *in vitro* experiments also showed that *R. flavipes* affects the growth of a common brown and white wood rot fungi (*Gloeophyllum trabeum* and *Phanerochaete chrysosporium*). Further investigation is needed to determine if the impact of *R. flavipes* on plant productivity is due to the increase of nitrogen and phosphorus facilitated by gut symbionts or interactions with the soil microbiome. Based on this laboratory research, *R. flavipes* has the potential to affect its surrounding soil and dead wood environment, and influence primary production and decomposer communities. These influences could have significant out comes for global carbon cycling models due to the global distribution of *Reticulitermes*. This research suggests that temperate subterranean termites are over looked organisms that can have significant ecological influence in soils and decomposing wood.

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EDUCATION

M.S.: Biology, Towson University, Towson, MD. Expected December 2017

Awards: *Graduate Student Association Research Award*

B.S.: Biology, Towson University, Towson, MD. May 2014

A.A.: Liberal Arts, Northern Virginia Community College, Alexandria, VA. August 2011

Relevant Coursework: Molecular Ecology, Entomology, Community Ecology Biostatistics, Multivariate statistics, Ecosystems Ecology, Population and Community Ecology, Conservation Biology

PROFESSIONAL EXPERIENCE

Graduate Teaching Assistant, Towson University 2015-2017

Taught three laboratory sections of undergraduates each semester for an introductory course focused on non-science majors. Materials covered basic biological concepts including evolution, ecology, and basic molecular and cellular function.

Master's Student, Towson University 2015-2017

Collected *Reticulitermes flavipes* termites from multiple states. Grew wood-rot and pathogenic fungi in a controlled laboratory setting. Analyzed effects of termites on plant and wood-rot fungal growth. Extracted soil and fungal DNA for PCR and sequencing.

Undergraduate Intern, Anacostia Watershed Society 2012

Assisted in wetland restoration and invasive plant removal. Participated in storm water outflow remediation projects. Surveyed native bee population to analyze the effectiveness of a riparian meadow restoration area.