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Changes of Soil Biogeochemistry under Native and Exotic Plants Species

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CHANGES OF SOIL BIOGEOCHEMISTRY UNDER NATIVE AND EXOTIC PLANT SPECIES

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Yujie Hua

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To: Dean Michael R. Heithaus
College of Arts and Sciences

This thesis, written by Yujie Hua, and entitled Changes of Soil Biogeochemistry under Native and Exotic Plant Species, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

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The thesis of Yujie Hua is approved.

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DEDICATION

I dedicate this thesis to my family. Without their patience, understanding, support, and most of all love, the completion of this work would not have been possible.

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I wish to thank the members of my committee for their support, patience, and guidance. Their gentle but firm directions were most appreciated. Dr. Krish Jayachandran accepted me as his student when I was in the most lost moment, and guided me towards a Master's thesis project. He had confidence in my abilities to not only complete a degree, but to complete it with excellence. Dr. Kateel Shetty helped me with my lab analysis, provided the facilities useful for my research, and gave me suggestions on both my proposal and thesis writing. Dr. Rosemary Hickey was particularly helpful in guiding me in my thesis writing and vocabulary. She also gave me great suggestions on my experimental design, which led the research to a publishable level. Also, I would like to thank my colleagues. Dr. Pushpa Soti helped me obtain the permit for soil sampling in the Tree Tops Park. Diana Johnson advised me on soil respiration experiments. Ramon Salazar conducted available phosphorus analysis with me. At last, I would like to my husband, Zhixuan Feng, for driving me to the sampling sites, companying me overnight in the lab, and teaching me the data analysis software.

I have found my coursework throughout Geosciences program to be inspiring and thoughtful, providing me with the tools with which to explore ideas and solve issues.

ABSTRACT OF THE THESIS

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Professor Krish Jayachandran, Major Professor

Invasive plant species are major threats to the biodiversity and ecosystem stability. The purpose of this study is to understand the impacts of invasive plants on soil nutrient cycling and ecological functions. Soil samples were collected from rhizosphere and non-rhizosphere of both native and exotic plants from three genera, *Lantana*, *Ficus* and *Schinus,* at Tree Tops Park in South Florida, USA. Experimental results showed that the cultivable bacterial population in the soil under Brazilian pepper (invasive *Schinus*) was approximately ten times greater than all other plants. Also, Brazilian pepper lived under conditions of significantly lower available phosphorus but higher phosphatase activities than other sampled sites. Moreover, the respiration rates and soil macronutrients in rhizosphere soils of exotic plants were significantly higher than those of the natives (Phosphorus, $p=0.034$; Total Nitrogen, $p=0.0067$; Total Carbon, $p=0.0243$). Overall, the soil biogeochemical status under invasive plants was different from those of the natives.

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Chapter 1 Introduction

The invasion of habitats by non-native species is a global phenomenon with serious consequences for ecological, economical, and social systems, and it constitutes a great threat to the global biodiversity (Davis et al., 2000; Lodge, 1993). In addition, invasive species are the second greatest cause of species extinction, just after habitat loss (Mooney and Cleland, 2001). Because of human activities such as plant material transportation worldwide, 10% of the introduced or transported species have successfully established themselves, and 10% of them have become very adapted to the new environment and were able to out compete the natives (Groves, 1991). A small proportion of introduced nonnative species become locally dominant and change relatively diverse communities into near monocultures (Levine et al., 2003). These species are generally referred to as 'invasive' species (Colautti and MacIsaac, 2004). In fact, the Florida Everglades is one of the locations most severely invaded by non-indigenous plants in the United States (Loope, 1992).

As exotic plant species invade ecosystems, ecologists have been attempting to assess the effects of these invasions on native communities and to determine what factors influence invasion processes. Much of the research on invasive plant has been focused on aboveground flora and fauna, while recent studies have shown that structurally and functionally diversified soil communities also can respond to, and mediate exotic plants

invasions. In numerous ecosystems, the invasion of exotic plant species has caused major changes in the soil community compositions and functions (Chapin, et al., 2000). Soil organisms, such as pathogenic or mutualistic fungi, have direct effects on the establishment, growth, and biotic interactions of exotic plants (Inderjit , 2010).

The actual invasion of an environment by new species is influenced by three factors: the number of species entering the new environment, the characteristics of the new species, and the susceptibility of the environment to invasion by new species (invasibility) (Lonsdale, 1999). Invasibility is an emergent property of an environment, such as the regional climate, the competitive abilities of native species in an ecosystem (Lonsdale, 1999). Rose and Fairweather (1997) suggested that invasive plants affect ecosystem structure and function adversely in habitats around the world by reducing the native species richness, and altering the soil nutrient contents and the biogeochemical processes. Although many studies have focused on explaining why some communities are more invasible than others, no general theory has yet merged, because results from field studies have been inconsistent (Williamson, 1999).

Most naturalized nonnative species appear to behave ecologically more or less like resident species, and occur at low to mid frequencies (Davis et al., 2000). There is a positive relationship between plant community diversity and susceptibility to invasion when the immigration is under low-intensity (Brown & Peet, 2003).

Vitousek (1990) first identified differences in resource acquisition by exotic plants and pointed out that exotic plants could alter soil processes. While Ehrenfeld (2001) pointed out that soil biota and/or soil physical conditions can indirectly affect the establishment of invasive plant species, rather than directly through the traits of the invading species. And it has been shown that once an invasive species established itself, it can actually change the soil biogeochemistry to be either less hospitable for the native species or more hospitable for its own species, thereby creating conditions that facilitate further invasion. Both mechanisms explain the rapid spread of the exotic species (Ehrenfeld, 2001 and Vitousek, 1990).

Habitat disturbance or destruction can open up opportunities for invasive species to enter an ecosystem and compete for sunlight, nutrients and space. In Florida, the isolated and subtropical climate of the region, as well as the natural disturbances such as hurricanes and fires provide ideal opportunities for the establishment of invasive species (Horvitz et al., 1995). A more challenging aspects of studying invasive plants is to use different parameters to predict the invasiveness of species and invasibility of habitats (Kareiva, 1996). Volin et al. (2004) built a model to show the distribution of one exotic plant species, *L. microphyllum*, with sets of parameters, and predicted that they will become established throughout the Everglades by 2014. Kourtev (2010) found that soils under two exotic understory plant species differed in microbial community structure and function, indexed by phospholipid fatty acid profile, enzyme activities and

substrate-induced respiration analysis. All the biogeochemical processes analyze results lead to the question of whether these soil changes facilitate further invasion, thereby exacerbating negative impacts on the ecosystem. An integrated understanding of how aboveground and belowground biotas interact with exotic plants is necessary to manage and restore communities invaded by exotic plant species. A firm scientific knowledge of the biological and biochemical process ongoing in the soil is also important in invasive plants studies (Aon and Colaneri, 2001). As land resource managers and policy makers may establish management scheme derived from experience results, this may has the potential to provide strategies and approaches to promote long-term ecosystem sustainability (Dick, 1994).

Continuous expansion of invasive species leads to degradation of ecosystem sustainability, loss of biodiversity and cause economic losses. Currently, the physical methods of cutting down and burning the invasive plants are widely used, but sometimes the species can recover within a short period of time. Chemical methods such as applying herbicides sometimes, though efficient, may be harmful to the entire ecosystem and cause even more serious contaminations, and extremely expensive to realize those approaches. Understanding the mechanism of soil biogeochemical processes will be applicable for eliminating invasive plants. Understanding the different impacts of various invasive plants on each specific type of soil will be an efficient and effective approach to deal with invasion problems.

Chapter 2 Literature review

2.1 Physiochemical properties

Although exotic plant species invasion as a major threat to the biodiversity and stability of ecosystem is well known, little attention had been paid to the nutrient cycling processes in the soil when invasive plants spread in those areas (Ehrenfeld, 2003). The literature on plant–soil interactions strongly suggested that the introduction of an invasive or exotic plant species had the potential to change many chemical components in soil, such as carbon (C), nitrogen (N), water, and others. Figure 1 showed the theory of fluctuating resource availability that controlled the relationship between invasibility and resource availability. Resource availability could increase because of resource supply; and it could also decrease with resource uptake. In the field, resource availability played an important role in exotic plant species invasibility. The invasibility increased as the trajectory moved right and/or below the supply/uptake isocline (Davis et al., 2000).

Lake and Leishman (2004) suggested that successful invasion by exotic species was greatly favored by nutrient addition. Bajpai (2013) found that an exotic plant tends to invade ecosystems where the soil had higher available nitrogen content. However, experimental studies in Sydney, Australia, suggested that native species grown in sandstone suffered high mortality with nutrient enrichment. Ehrenfeld et al. (2001) studied the soil pH, nitrification and net N mineralization rate in soil under native and

exotic plant species, and results suggested that higher values for all those three parameters in soils under exotic plants.

However, some opposite patterns also occurred. For example, in some cases, some exotic and native species did not show difference in the nutrient content in their understory soil; also, the same plant species could behave different under different type of soils (Ehrenfelf, 2003).

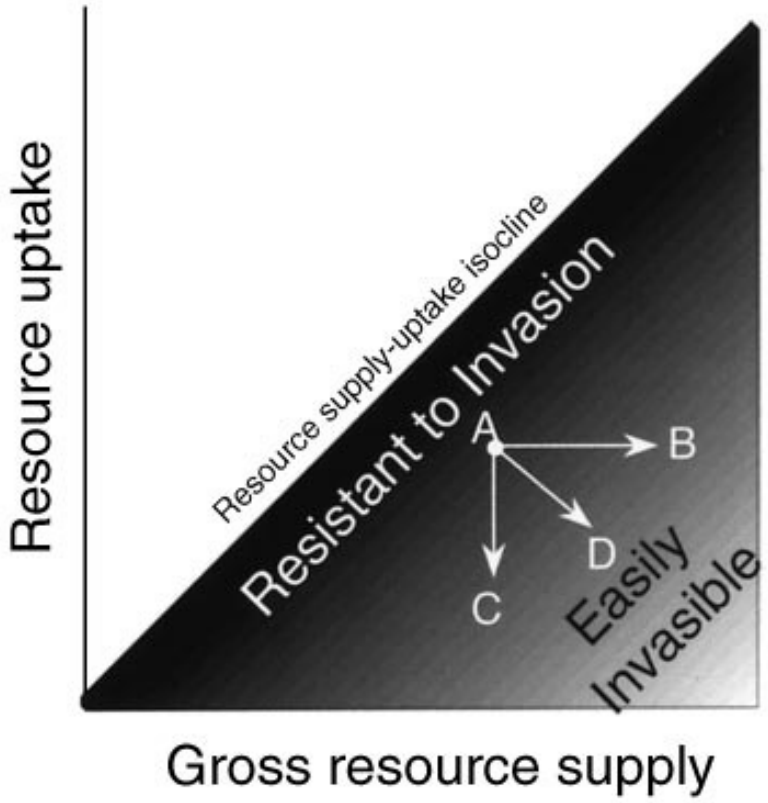

Figure 1 Relationship between invasibility and resource supply. In this plot, the resource availability play an important role in exotic plant species invasibility, it increases as the trajectory moves right and/or below the supply/uptake isocline (Davis et al., 2000).

2.2 Viable Microbial population

Exotic plant species impact belowground process by influencing resource availability

through microbial activity. Most of the known bacterial species grow within the pH range of 4 to 9; and fungi are in moderately acidic soil, with pH range of 4 to 6. Thus, soil pH plays an important role in the growth and proliferation of soil microbes. So the pH of rhizosphere soil and non-rhizosphere soil has an indirect impact on the invasive plants through plant-microbe interaction.

There were different theories on how the exotic plant species survive in new ecosystems in terms of microbes in the soil. Some studies suggested that in the new living environment of the world, the exotics had low chances of encountering their soil-borne enemies while native plants had many enemies living in the soil restricting the growth of them (Reinhart and Callaway, 2006). Another explanation was that the invasive plants could have more mutualists in the soil than native plants, and the mutualists could enhance a successful invasion (Reinhart and Callaway, 2006). Van der Puttern et al. (1993) studied the pathogens accumulation in soil after invasion, and results suggested that some plants species accumulated pathogen quickly and maintained the plant population at a low level, as a result of the accumulation of species-specific pathogens. On the contrary, other plants accumulated their plants densities very quickly but kept the pathogens accumulation rate very low. These feedback relationships might facilitate invasion and inhibit reestablishment of native species by altering the soil biota-plants interactions.

There are 12 soil texture types classified on the basis of the percentages of sand, silt

and clay, and the texture could plays a significant role in the microbial community in the soil as well. Soil biotas were important regulators of plant community structure, and they had different abilities to influence both the soil and plant structures. So the soil texture may have the potential to affect plants invasion.

2.3 Biogeochemical functions

2.3.1 Soil respiration

Soil is a mixture of dead organic matter, air, water, and weathered rock that support plant growth (Buscot, 2005). Killham (1994) also include living organisms in the definition of soil, such as soil microbes and fauna.

Soil respiration is an ecosystem process that releases carbon dioxide from the soil to the atmosphere (Luo and Zhou, 2006). The carbon dioxide is released mainly via root respiration, faunal respiration and microbial decomposition of litter, and organic matter. Respiration is often studied in relation to energy supply at the biochemical and cellular levels of bioenergetics. However in this study, respiration is mainly used to describe CO_2 production by organisms and plants in soils. Soil respiration plays a critical role in regulating the atmospheric CO_2, the nutrient cycling, regional and global carbon cycling, also the climate change (Luo and Zhou, 2006).

Soil respiration rate is temperature dependent. Studies have shown that a distinct seasonal pattern of high soil respiration during summer and low respiration in winter. In a tall-grass prairie of Oklahoma, the summer peak of soil respiration reached nearly 6

$\mu molm^{-2}s^{-1}$ and less than 4 $\mu molm^{-2}s^{-1}$ during the winter in 2002 (Luo and Zhou, 2006). Similar seasonal patterns had also been observed in northern semiarid grasslands (Frank et al., 2002). Nonetheless, there were variations from year to year. Figure 2 showed a typical time course of CO_2 efflux in a tall-grass prairie of Oklahoma, USA from 1999 to 2005. Meanwhile, root respiration may become less sensitive to soil temperature over seasons of a year, resulting from its rapid thermal acclimation (Edwards et al., 2004). Luo and Zhou (2006) found the maximal rate of soil microbial respiration at a temperature of 23°C as showed in Figure 3. Since the current project aims to understand the variation of soil respiration caused by invasive plants species, the more significant differences would more desirable for showing the difference in soil respiration rate. The soil samples were collected in the summer of 2014 and incubated at 24°C.

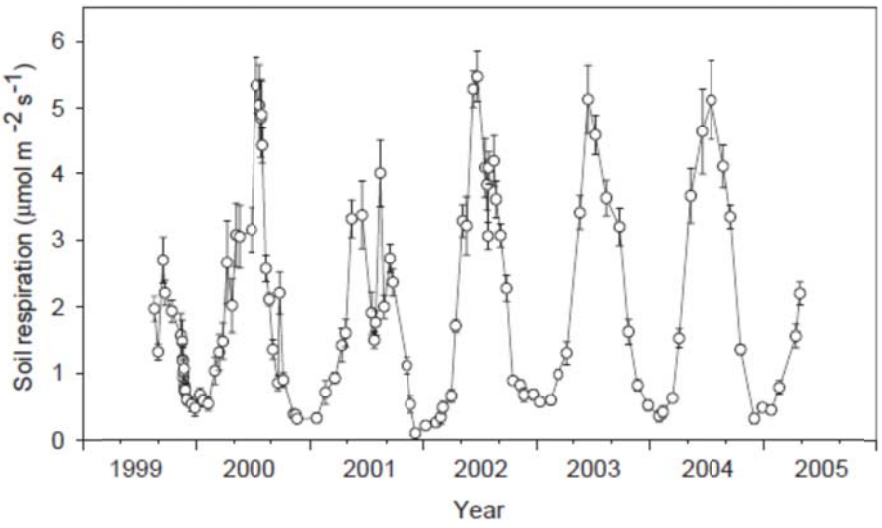

Figure 2 Soil carbon efflux in tall-grass prairie of Oklahoma, USA from 1999 to 2005. Open circles represented data points, and bars indicated the one standard error bellow and above the data points. Data were only form the measured soil CO_2 efflux in the control treatment in a warming and clipping experiment and adopted from Luo et al. (2001), Wan et al. (2005), and Zhou et al. (2006).

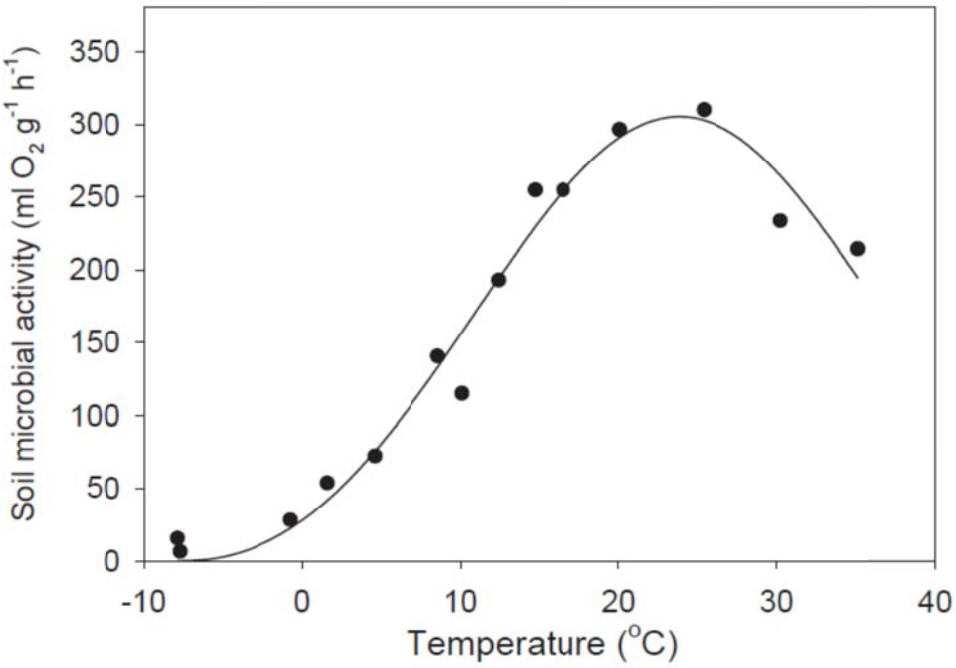

Figure 3 The relationship between soil microbial respiration and temperature (Flanagan and Veum, 1974)

The carbon cycle is initiated through photosynthesis by plants, where CO_2 from the air was fixed and converted into organic carbon compounds, and stored in the grown tissues. The CO_2 is released back in to the atmosphere through plant respiration and microbial respiration.

During the litter decomposition, the releasing of carbon combined with nitrogen immobilization gradually decreased with carbon-nitrogen ratio decreasing. Similarly, phosphorus and sulfur may also increase during initial phases of decomposition (Luo and Zhou, 2006).

Additionally, as a result of the carbon and nitrogen mineralization processes in the

litter and organic matter (SOM), the rate of nitrogen mineralization often tended to have a positive correlation with microbial respiration (Luo and Zhou, 2006). Zak et al. (1999) studied carbon and nitrogen released from labile SOM in five forests of Michigan, there was a correlation between the mineralized nitrogen (N_{min}) and microbial respiration (R_m): $R_m = 15.9\ N_{min}+27.4$ with r=0.853 and n=154. Similar relationships between net carbon and nitrogen mineralization were also found in organic soils (Eriksen and Jensen, 2001). So in the current study, a regression was also applied in between the nitrogen mineralization and microbial respiration to see the relationship between mineralization and microbial respiration.

The global soils contained as much as 3150 Pg C (1 Petagram = 1 billion tons), including 450 Pg C in wetlands, 400Pg C in permanently frozen soils, and 2300 Pg C in other ecosystems (Luo and Zhou, 2006). While the carbon pool size in the atmosphere was only 750 Pg C. So soil respiration plays a critical role in carbon cycling at both regional and global scales. Several studies had compiled data from field analysis and estimated the global respiration. A study estimated the global soil respiration rate at 77Pg C yr^{-1} with a global model (Raich and Potter, 1995). Soil respiration releases carbon from the soil pool at about four times that of the atmospheric pool (Luo and Zhou, 2006). Thus, a small change in the soil respiration could seriously change the CO_2 balance in the atmosphere. So in the present study, the respiration rate of soils under rhizosphere and non-rhizosphere of different plants were analyzed to see if there is a significant difference

between them.

Kourtev et al. (1999) found exotic plant species enhanced respiration of the soils and the potential rates of mineralization. However, Ehrenfeld et al. (2001) obtained completely opposite results for those two processes using a different set of exotic species. Moreover, adding different substrates could accelerate the respiration rate in soil. By adding different substrates to the soils can promote the respiration rates at different levels. Results had shown that adding carboxylic acids as the substrate, rhizosphere soils tended to have higher respiration activities than non-rhizosphere soils without substrates or with other substrates (Kourtev et al., 2002).

2.3.2 Enzyme activity

Early indicators of ecosystem stress may function as “sensors”; sensitively warn us about soil degradation or changes (Aon and Colaneri, 2001). The classical and slowly changing soil properties under the native plant species have significant difference compare to exotic soils, such as organic matter contents and soil enzyme activities (Dick, 1994; Aon and Colaneri, 2001). Soil enzymes are mainly produced by plants and microorganisms (e.g., bacteria and fungi). They are proteins made from amino acids. When they are formed, they make stringing together between 100 and 1,000 amino acids in a very specific and unique order. Then the amino acids chain folds into a unique shape. The specific shape allows the enzyme to carry out specific reactions, and it can be very rapidly and efficiently. Figure 4 showed how maltose (it is made of two glucose

molecules bonded together) broke down into two glucoses with reaction on a maltase enzyme.

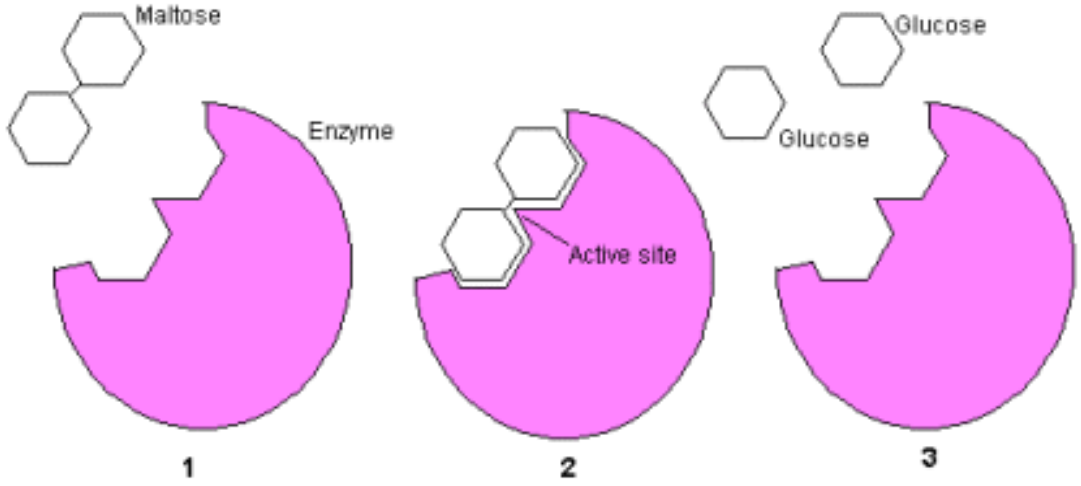

Figure 4 Mechanism of enzyme breaking down cell molecules. Maltose is made of two glucose molecules bonded together. The maltase enzyme is a protein that is perfectly shaped to accept a maltose molecule and break the bond. A single maltase enzyme can break in excess of 1,000 maltose bonds per second; this is a very efficient reaction.

It was mostly known that bacteria grew in environment of pH range from 4 to 9, and fungi grew better in more acidic environments, pH range from 4 to 9. Each type of those enzymatic reactions takes place at a certain pH. Thus, the soil pH plays a important role of the enzyme activity of the soil microbe (Aon and Colaneri, 2001).

Studies had shown specific relations between enzyme activity and certain microbes. Some enzymes functions were associated with the microbes themselves, such as dehydrogenase activity of which mainly located in the membranes of fungi. Other enzymes, such as phosphatase are secreted extracellularly by bacteria or fungi. These extracellular enzymes may involve in biogeochemical process in the soil matrix. β-glucosidases are enzymes widely distributed in nature and are involved in the

saccharification of cellulose (Tabatabai, 1994); phosphatases are involved in the transformation of organic and inorganic phosphorus compounds in soil (Amador et al., 1997).

Most studies on soil enzymes are representatives of major nutrients cycles (Carbon, Nitrogen, Phosphorus) (Aon and Colaneri, 2001). Three physicochemical properties of soil, organic carbon content, total nitrogen, and water-filled space, exhibited strong relationships with the enzymatic activity (acid and alkaline phosphatase, and β-glucosidase) measured irrespective of season and presence of crops. Also some enzyme activities are most sensitive in the soil depth of 5 to 10 cm (Aon, 2001). Higher enzyme activities were shown under native plant in Hawaii, specifically, 50 umol pNP g^{-1} soil h^{-1} compared to 150 umol pNP g^{-1} soil h^{-1} of acid phosphatase activity; 3 umol pNP g^{-1} soil h^{-1} compared to 8 umol pNP g^{-1} soil h^{-1} of N-releasing enzyme activity. Different enzymatic activity showed different relationships between the nutrients content. Organic carbon and total nitrogen had strong links between enzymatic activities since both are main constituents of soil organic matter and thus of substrates for enzyme degradation. β-glucosidase and urease are enzymes related to C and N cycles in soil. Urease activity increases with a decreasing of C/N ratio. Respectively, it correlates negatively with organic carbon (r= -0.51) and positively with total nitrogen (r= 0.72). Aon (2000) found that the fluorescein diacetate hydrolysis activities correlated positively to organic carbon

(r=-0.58) and negatively to total nitrogen (r=-0.787). Dehydrogenase activities whereas correlated negatively to organic carbon (r=0.68) and positively to total nitrogen (r=-0.63).

2.4 Native and invasive plants

2.4.1 *Ficus*

Ficus aurea is the strangler fig. It tends to establish on a host tree in which it gradually encircles and "strangles", eventually taking the place of that tree in the forest canopy. Its native range includes Florida, northern Caribbean, Mexico, and south central America (Swagel et al., 1997).

Ficus microcarpa was widely distributed as an ornamental plant and is one of the most common street trees in warm climates. The tree is considered a major invasive species in Hawaii, Florida, Bermuda, Central America, and South America (Carauta et al., 2002).

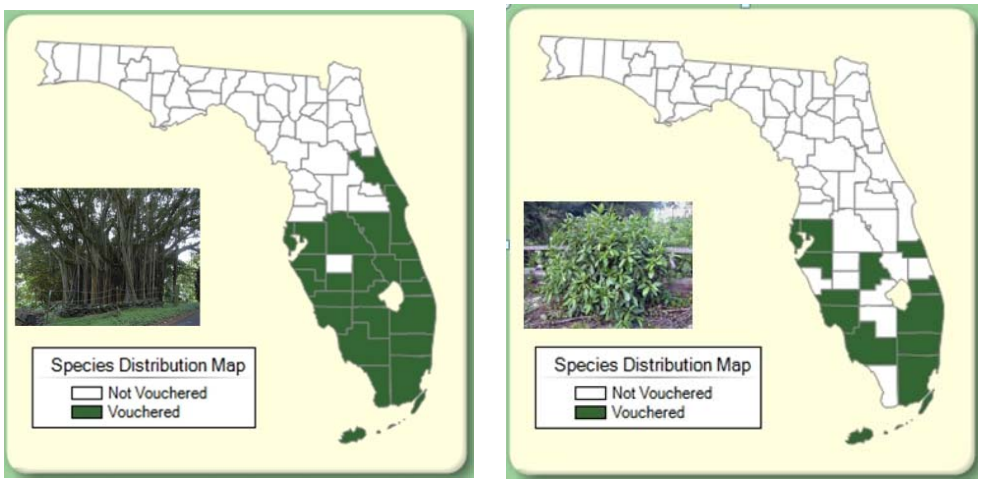

Figure 5 Distribution of *Ficus microcarpa* (exotic) and *Ficus aurea* (native) in Florida

2.4.2 *Lantana*

Lantana camara has been introduced into other parts of the world as an ornamental plant and is considered an invasive species in many tropical and sub-tropical areas (Sanders, 2012). It has been naturalized in the United States, particularly in the Atlantic coastal plains, from Florida to Georgia, where the climate is close to its native climate, with high heat and humidity (Florida Exotic Pest Plant Council, 2005).

Lantana involucrata is supported by many stiff lateral roots and abundant fine roots. The native range of *Lantana involucrata* includes southern Florida, the West Indian islands, Mexico through northern South America bordering the Caribbean, and the Galápagos Islands (US Forest Service).

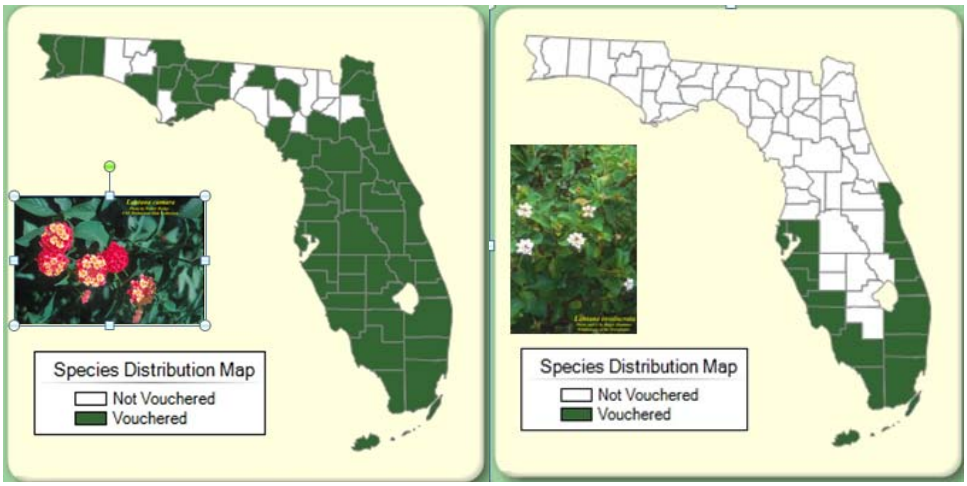

Figure 6 Distribution of *Lantana camara* (exotic) and *Lantana* involucrata (native) in Florida

2.4.3 *Schinus*

Schinus terebinthifolius (common name is Brazilian pepper) is a species of flowering plant in the cashew family, Anacardiaceae, is native to subtropical and tropical South

America. It was introduced to Florida and has spread rapidly since about 1940 (Ewel 1986), replacing native plants, like mangroves, with thousands of acres occupied.

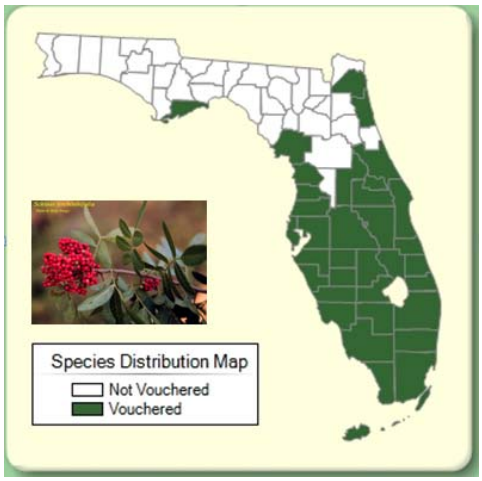

Figure 7 Distribution of *Schinus terebinthifolius* (exotic) in Florida

Chapter 3 Materials and methods

3.1 Sampling design

In this study, all soil samples were collected from Tree Tops Park in Broward County, FL, on May 6, 2014. Five plant species were chosen from 3 genera, as shown in Table 1. Within each genus, 1 to 2 species were chosen, at least one exotic plant for each genus. Three replicates of each soil sample were taken from both rhizosphere and nonrhizosphere of each plant. Roots and rhizosphere soils were carefully separated. The roots and rhizosphere soils were transferred to separate plastic bags, transported to the laboratory. A portion of the soils for nutrient analysis were stored at -20°C until being processed. Another portion of soils for enzyme activity and microbial respiration analysis were processed within 24 hours after sampling. The sampling location for each plant and sampling time was listed in Table 2 and Figure 8.

Table 1 Description of native and exotic plant species used in the study.

Genus	Species	Common Name	Native status
Lantana	*camara*	Lantana	Exotic
Lantana	*involucrata*	Buttonsage	Native
Ficus	*microcarpa*	Indian laurel	Exotic
Ficus	*aurea*	Strangler fig	Native
Schinus	*terebinthifolius*	Brazilian pepper	Exotic

Table 2 Sampling sites in Tree Tops Park of Broward County, FL.

Plants names	Latitude	Longitude	Sampling time
Lantana *camara*-1	-80.27981166	26.07053472	2014/05/06 13:59
Lantana *camara*-2	-80.27968383	26.07055086	2014/05/06 15:30
Lantana *involucrata*-1	-80.27906165	26.06534326	2014/05/06 12:07
Lantana *involucrata*-2	-80.27945132	26.06967738	2014/05/06 15:03
Ficus *microcarpa*-1	-80.27665663	26.06899258	2014/05/06 14:38
Ficus *microcarpa*-2	-80.27608734	26.06632194	2014/05/06 13:15
Ficus *aurea*-1	-80.27717766	26.06574975	2014/05/06 14:31
Ficus *aurea*-2	-80.27645764	26.06877352	2014/05/06 14:02
Schinus *terebinthifolius*-1	-80.27948577	26.06930853	2014/05/06 15:16
Schinus *terebinthifolius*-2	-80.27915544	26.06888202	2014/05/06 15:08

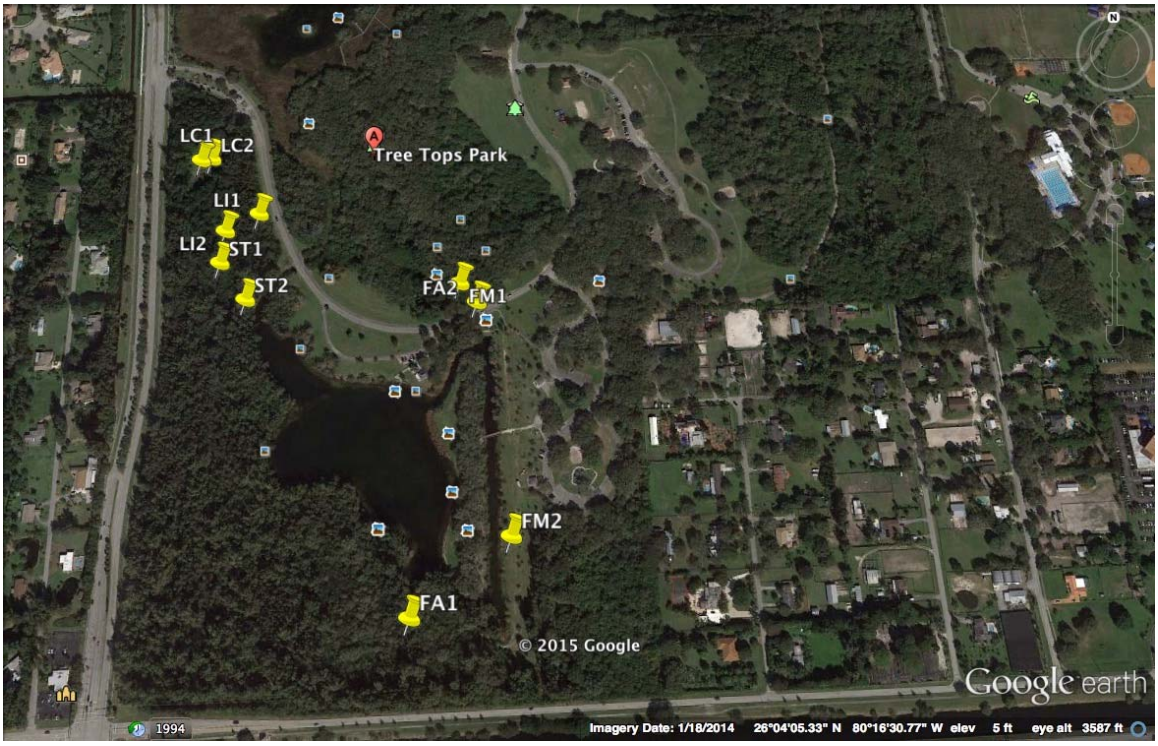

Figure 8 Distribution of sampling sites in Treetops Park, FL.

3.2 Lab analysis

3.2.1 Total Carbon and Nitrogen

I dried 5 grams of each wet soil sample in a drying oven at 65 degree Celsius for 24 hours. All samples were cooled to room temperature before taking out from the oven to avoid moisture absorption. Then dry soil samples were sieved and ground with sieve at 20um. I weighed 1 to 1.5 grams of dry soil into aluminum foil and wrapped the samples into a small ball. I entered the weight of the corresponding sample and sample IDs into computer connected with gas chromatography. Before each run of the gas chromatography, I checked for leaks and tested the accuracy of the machine with factory-made soil samples. The standard curve I created had a nearly perfect correlation

(r^2 >0.99). Then total carbon and nitrogen were measured automatically. During the measuring process, proper care was taken every 30 minutes to check if the samples passed through the cylinder and went into the furnace successfully.

3.2.2 Available Phosphorus

The available phosphorus was extracted by Olsen's sodium bicarbonate method (Olsen et al., 1954). The reagents were extraction reagent, mixed reagent, coloring-developing reagent and phosphorus standards. The extraction reagent was a 0.5 N sodium bicarbonate ($NaHCO_3$): I dissolved 42.0 g sodium bicarbonate in water in a 1,000mL volumetric flask and adjust the pH to 8.5 by adding sodium hydroxide (NaOH). The mixing reagent was a mixture of ammonium molybdate solution (12.0 g dissolved into 250 mL DI water) and antimony potassium tartrate solution (0.2908 g dissolved into 500 mL 5 N sulfuric acid), then mixed in a 2,000 mL volumetric flask and brought to the volume with DI water. The coloring-develop reagent was made by dissolving 0.739 g ascorbic acid into 140 mL mixing reagent. I dissolved 0.4394 g commercially prepared monobasic potassium phosphate standard into 1,000 mL DI water and added 5 drops of toluene to make the standard solutions. The solution contained 100 mg P mL^{-1}. Then I pipetted 1, 2, 5, 10, 20, and 30 mL of the 100 mg P mL^{-1} solution into 100 mL volumetric flasks and brought to the volume to produce 0.1, 0.2, 0.5, 1.0, 2.0, and 3.0 mg P mL^{-1} working P standard, respectively.

The soil samples were extracted by mixing 2.5 g of air-dry, 2 mm mesh sieved soils with 50 mL extraction reagent into a 250 mL extraction bottle. Then the bottles were shaken for 30 minutes on a reciprocating shaker. I filtered and collected the filtrate with 8 μm Fisherman filter paper. Then I pipetted 5 mL extracted soil sample solutions and working phosphorus standards into test tubes. All test tubes were washed with liquid-Nox, 0.1 N HCl, and DI water. I added 5 mL of coloring-develop reagent slowly and carefully to prevent loss of the samples due to excessive foaming. I added 15 mL DI water and mixed thoroughly. Let them stand for 15 minutes and measured the color intensity at 880 nm. If the color were too intense (upper limit is 2.5 % T), I diluted the solution 10 times by adding 1 mL of the color-developed samples into 9 mL DI water.

3.2.3 Viable Heterotrophic Microbial Population

The most common procedure for the enumeration of viable heterotrophic bacteria and fungi was the viable plate count (Van der Puttern et al., 1993). In this method, serial dilutions of a sample containing viable microorganisms were plated onto a suitable growth medium. The plates were then incubated under conditions that allow microbial reproduction so that colonies developed to a size that can be seen without the aid of a microscope. It is assumed that each bacterial colony arose from an individual cell that had undergone cell division. Therefore, by counting the number of colonies and accounting for the dilution factor, the number of bacteria in the original sample could be determined.

To prepare Tryptic Soy Agar media (TSA), I mixed 2 g TAS and 7.5 g Bacto Agar with 500mL of DI water in a 1000mL glass bottle thoroughly, autoclaved the bottle with liquid setting. After I took out the bottle with gloves, and left it cool in water bath set at 60 degree Celsius. I added a sterilized magnet into the bottle and mixed the media well. Then I dissolved 50mg of cyclohexamide with 10mL DI water in a 10mL beaker and filtered the cyclohexamide solution with 0.2μm nylon membrane and 25mm syringe in the hoods, then covered with parafilm. I added the cyclohexamide into agar and mixed again and poured plates in the sterilized hood.

To prepare Rose Bengal Agar media (RBA), I mixed 15.8 g RBA with 500 mL of DI water in a 1000 mL glass bottle thoroughly, autoclaved the bottle with liquid setting. After I took out the bottle with gloves, and left it cool in water bath. Then I poured plate in the sterilized hood.

Note that before autoclaving, I checked the water level of the autoclave. Also, I loosed all the bottle lids to balance the pressure. After pouring plates, I left all the petri dishes in the hood for at least 30 minutes to avoid condensation.

To dilute the soil samples, I pooled together three replicates of each soil sample, and mixed the pooled samples thoroughly. Then I weighed 1 g of each mix fresh soil sample and poured them into the test tubes. I added 9 mL of autoclaved tap water into the tube to make 10 times dilution of the soil sample. I used vortex to mix the solution well and kept diluting till 10^{-5} under aseptic environment. Then I used sterilized pipettes transfer 0.1 ml

of diluted solution to plates filled with agar. The selection of dilution, types of media, incubation period, and counting frequency were different as shown in table 3. I spread the inoculum on the surface of the agar with sterilized spreader and concealed the petri dishes with clean parafilm. I incubated bacteria in incubator set at 65°C, and RBA at room temperature in dark for 7 days. I counted the number of colonies each day until the number became stable.

The final unit of measure was the number of colony-forming unit (CFU) g^{-1} ds.

$$\text{CFU/g ds} = \text{CFU} \times \text{Dilution}/0.1\text{g} \times \text{Fraction Dry}$$

Table 3 Experimental design of viable microbial population analysis

Microbial type	Dilution	Frequency	Media	Days
Bacterial	10^{-5}	24h	TSA	7
Fungus	10^{-4}	24h	RBA	7

For bacteria counts, if the number of colonies was greater than 300, I used 10^{-6} dilution of soil samples. For fungi, if the number of colonies was greater than 300, I used 10^{-4} dilution of soil; if the number of colonies was less than 50, I used 10^{-2} dilution of soil samples.

3.2.4 Respiration rate

Since the soil samples were generally dry, they were diluted with DI water and into 1:1(weight based) slurry before adding them into 20 mL vials. I weighed and kept record of the vials weights, labeled them with their sample names. I weighed 3 g of soil samples to 10 mL plastic cups, then added 3 mL of DI water into the soil samples and mixed well. I pipetted 2mL of the slurry into its vial, then weighted and kept record of the wet soil

and vial weights. I capped the vials with rubber lids, and then seal them with aluminum caps. After 4 days of incubation, I ran the samples on gas chromatography machine. This gave me the CO_2 and CH_4 concentration in each vial.

3.2.5 Enzyme activities

The objective was to determine the enzyme activity in dilute soil solutions using a rapid fluorometric assay with the fluorescent model substrate 4-methylumbelliferone (MUF) (Sinsabaugh et al., 1997). I used assays to determine 3 enzyme activities: alkaline phosphatase (P), β-1-4-glucosidase (C), and β-N-acetylglucosaminidase (N). The substrates for each of these enzyme assays were: 4-methylumbelliferyl-phosphate (MUF-P), MUF-β-D-glucoside (MUF-C) and MUF-N-acetyl-β-D-glucosaminide (MUF-N). Enzyme activity was determined by subtract the amounts of fluorescent substrate liberated after incubation time from the amounts of fluorescent substrate liberated at the initial time.

I made two types of buffers (Morpholinoethanesulfonic acid and Trisaminomethane) , 3 types of substrates (MUF-C, MUF-N and MUF-P), and 1 series of standards.

To prepare the Morpholinoethanesulfonic acid (MES) stock solution (100 mM), I dissolved 1.953 g MES in 100mL distilled, deionized water ($DDIH_2O$) and adjusted pH to 6.0 (±0.1) by dropwise addition of sodium hydroxide (NaOH) and hydrochloric acid (HCl). Approximately 1 drop equals 0.05 mL. I pipetted 25 mL of MES stock solution into a 500 mL volumetric flask and brought to the mark with $DDIH_2O$ to get MES

working solution (5mM). To make the Trisaminomethane (Trizma) stock solution (100 mM) I dissolved 1.280 g of Trizma pre-set crystals (pH=8.7) in 100 mL $DDIH_2O$. Then I adjusted the solution pH to 8.7 (±0.1). I pipetted 25 mL of Trizma stock solution into a 500 mL volumetric flask and brought to the mark with $DDIH_2O$ to get Trizma working solution (5mM).

For making substrates solutions, all substrates powders were dissolved in buffer solutions of 5 mM. I dissolved 1.692 mg MUF-C (fw=338.3 g $mole^{-1}$) in 100 mL MES working solution; 1.897 mg MUF-N (fw=379.4 g $mole^{-1}$) in 100 mL MES working solution; 1.281 mg MUF-P (fw=256.2 g $mole^{-1}$) in Trizma working solution.

For MUF standard stock solution (1 mM), I dissolved 1.982 mg MUF (fw=198.2 g $mole^{-1}$) in 10 mL methanol within a plastic scintillation vial. I made sequential dilutions using $DDIH_2O$ and started with the stock standard following the Table 4. All standards were kept in freezer in plastic scintillation vials.

Enzyme activity analyses were conducted within 24 hours of sample collection. I removed substrates from the freezer and melt completely. Soil samples were made into 1:1 slurry and then diluted into 1×10^{-3} solutions. I added 200 µL of diluted soil solution and 50µL of each substrate solution into the multiwell plate. Each multiwell plate had 8 rows and 12 columns. Each sample was filled into the same column, which means 8 replicates for each sample. Incubate in the dark at 25 °C. The incubation periods for

MUF-N and MUF-C were 24 hours; the incubation time for MUF-P was 2 hours. At the end of the incubation time, I added 10 μL of 0.1 M NaOH to all cells to stop the reaction.

Then the samples were run on the Biotek plate reader. The settings for the plate reader and well assignment for the standard curve are shown in table 5. The amount of Absolute Fluorecence Units (AFU) were calculated referring to the standard curve.

Table 4 Standard solutions procedures.

Initial concentration of MUF (μM)	**Volume of initial MUF (mL)**	**Volume of DDI water (mL)**	**Final concentration of MUF ((μM)**
1000	2	18	100
100	2	18	10
100	1.25	18.75	6.25
10	5	15	2.5
10	2.5	17.5	1.25
10	1.875	18.125	0.9375
10	1.25	18.75	0.625
10	0.625	19.375	0.3125
10	0.25	19.75	0.125
0.625	2	18	0.0625
0.125	2	18	0.0125
0.0625	2	18	0.00625

Table 5 Well assignment for the standard curve. This table shows the first row.

blank	0	0.00625	0.0125	0.0625	0.125	0.3125	0.625	0.9375	1.25	2.5	2.5

Each plate had 12 columns and 8 rows. Table 5 shows the first row of the plate I designed for calculating the standard curve. All the 7 other rows were replicates of the first row. I used the average value of the first column as blank, which is V_b. Then I subtracted all AFU values of the last 11 columns from V_b to get the real AFU values of the standards. I used the value I got from the second step to get the average fluoresces of

each column. When making the standard curve, the Y-axis was the concentration of the substrate (uM or umole/L), and the X-axis was the means of AFUs (without any unit or the unit is 1). I used the linear regression to get the R^2, the slop and Y- intersection.

On the basis of the value I got from the soil, they were relatively low, so I changed the range of X-axis smaller to make sure in the regression step the values were in the middle of the standard curve.

3.3 Data statistical analysis

The purpose of the study is to find out the soil characteristics that change under native and exotic, so the statistical analysis can show the significance level of changes.

The correlation coefficients between two different parameters are calculated in Matlab 2012a (Mathworks, Natick, MA) with a p-value less than 0.05 regarded as significant.

One-way analysis of variance (ANOVA) is conducted using Matlab (2012a) statistical toolbox to determine whether there are significant differences between pairs of native and invasive soil samples. The pairs include: all native versus invasive samples, LI versus LR, FA versus FM, LI versus ST, and FA versus ST.

Chapter 4 Results

Statistical analyses were conducted on the rhizosphere soil geochemical properties between native and exotic plant species, including available phosphorus, organic matter, total nitrogen, total carbon, and pH. Their means and standard deviations are shown in Figure 9. The P-values calculated from ANOVA are shown in Table 6. Because there were no rhizosphere soil samples of native *Schinus* genus, exotic *Schinus terebinthifolius* (ST) were compared with native plants of two other genera, *Lantana involucrata* (LI) and *Ficus aurea* (FA).

The overall available phosphorus contents in rhizosphere soils under native plants were significantly lower than those in rhizosphere soils under exotic plants (P-value=0.0340). The soil available phosphorus content under *L. involucrata* (LI) (0.226%) was significantly higher than that under *Lantana camara* (LC) (0.178%)(P-value<0.0001). While rhizosphere soils under FA and *Ficus microcarpa* (FM) had no significant difference in available phosphorus content. However, rhizosphere soil under ST contains significantly lower available phosphorus than that of FA (P-value=0.0035).

Generally, the rhizosphere soil organic matter contents under native plants were significantly higher than soil under exotic plants (P-value=0.039). Brazilian pepper was able to survive in soils of much lower organic matter content (10.2%) and available phosphorus content (0.196%) than other native plants. While the native and exotic

Lantana both lived in soils of higher organic matter soils, and there were no significant difference between the organic matter content in their rhizosphere soils (P=0.2672).

Both TN and TC content in soil also were higher in rhizosphere soils under exotic plants than native plants (TN, P-value=0.0067; TC, P-value=0.0243). Though soils under LI (native) was presenting similar TN values compare to LC(exotic), all the other native plants tended to have more TC and TN in their rhizosphere soils.

The rhizosphere soil under LC (exotic) was found to have higher pH than the native soils (P=0.0216). While, rhizosphere soil under FM (exotic) had lower pH values than those under FA (natives) (P=0.0487). And soil under Brazilian pepper lived in soil more alkaline than other plants, but the pH value was not statistically significant.

ANOVA was conducted on the soil's biogeochemical properties between native and exotic plant species, such as enzyme activities, soil microbial respiration and viable microbial population. Their means and standard deviations are shown in Figure 10. P-values calculated from ANOVA are shown in Table 7.

The phosphatase activity of soil under Brazilian pepper was much higher than all soils under other plants (FA vs ST, P=0.0263; LI vs ST, P= 0.0258). Compared this result to the available phosphorus content in soil, I found out that the lower the phosphorus availability, the higher the enzyme activity will be.

The glucosidase activity did not have much difference between native and exotics soils. While the acetylglucosaminidase activities between native and exotic soils were all

significant difference. For *Lantana*, the exotic species had higher glucosidase activities. While, the exotic *Ficus* species had lower enzyme activities. Different plants had different levels of influences on the soil microbial behaviors.

Soil microbial respiration rate also varied in between different plant-soil interactions. The native and exotic plants of *Lantana* and *Ficus* changed the soil microbial respiration rate. The natives had significant higher rates than the exotic species (*Lantana,* $P<0.0001$; *Ficus*, $P<0.0001$).

Variation in viable heterotrophic fungal and bacterial population (CFU) due to host induced rhizosphere factors was expected under different plant species. The heterotrophic bacterial population was found to be highest under Brazilian pepper than in soils under other plants. In case of *Lantana,* both native and the exotic showed no significant difference in heterotrophic microbial population (Fungi, $P=0.1503$; Bacteria, $P=0.1753$).

ANOVA was also used to compare the difference between the rhizosphere and nonrhizosphere soil geochemical status of each plant species, each single plant soil sample had three replicates. The results also suggested that the rhizosphere and nonrhizosphere soil under both native and exotic plants were significantly different. As shown in Figure 11 and Table 8, available phosphorus contents were different between different plant species in both rhizosphere and nonrhizosphere. At the mean time, there was no significant difference between rhizosphere and nonrhizosphere of each plant species (*Lantana*, $P=0.6664$; *Ficus*, $P=0.3879$; *Schinus*, $P=0.1439$). In general, the

available phosphorus did not vary much from rhizosphere to nonrhizosphere. The organic matter, total carbon, total nitrogen content and pH for rhizosphere and nonrhizosphere of *Lantana* were not different (OM, P=0.7499; TC, P=0.4843; TN, P=0.9619; pH, P=0.4052). This result may need to correct, because some of the nonrhizosphere soil were collected relatively close to the plants roots. The plant stands were much smaller than other plants, so I assumed the rhizosphere were smaller. This assumption might be wrong. The other plants all showed significantly higher TN, TC and OM content in rhizosphere than those in nonrhizosphere. The nonrhizosphere soils had more acidic pH compared to that of the bulk soil or nonrhizosphere soils. This was the result of plant soil interactions. The plants were capable of secret nutrients from their roots system, during which increased the nutrients contents in soil. Also it was generally known that Florida soils were mostly alkaline as the result of having the bedrock of limestone. So plant-soil interaction was able to adjust the soil to a more neutralized pH. The average pH in rhizosphere is 6.93, while 7.35 in nonrhizosphere.

ANOVA was used to compare the difference between the rhizosphere and nonrhizosphere soil biogeochemical status of each plant species, and all results are shown in Figure 12 and Table 9. The average phosphatase activity and acetylglucosaminidase activity were significantly different. Overall, the EAP was higher in nonrhizosphere soil (P=0.0018), while the EAN was higher in the rhizosphere (P=0.0002). And EAC did not show significant difference between rhizosphere soil and nonrhizosphere soil (P=0.1001).

This suggested that plants tended to live in soils content higher available phosphorus. This also explains the limiting nutrients for plants to grow in Florida is phosphorus. There was no significant difference between EAC in nonrhizosphere and rhizosphere. This suggested the available carbon content was very much enough, so that the rhizosphere biota didn't need to take extra effort to supply the plants with more carbon compounds. The respiration rate in rhizosphere and nonrhizosphere was very different ($P<0.0001$). In the study, I only analyzed aerobic respiration rate, because the plants were living in high lands and soils were collected from the soil surface layer. The rhizosphere soil produced more carbon dioxide than nonrhizosphere soil. This was the result of microbial respiration. The soil-biota interaction supplied plants with nutrients, broke down the organic form of nutrients, decomposed the dead leaves and roots, and in these processes, more carbon was released. There was no significant difference between the viable fungi amount in soil ($P=0.317$). But the difference between bacteria amount was very significant for all the plants species (*Lantana*, $P=0.0044$; *Ficus*, $P=0.0410$; *Schinus*, $P<0.0001$). This result was only an indicator showing the viable microbes. There were also much more microbes not viable for my experiment. And Brazilian pepper as a very invasive plant species, the bacteria amount in rhizosphere soil was about 10 times higher than those of the others plant species. This might be a major reason for this plant to spread so rapidly.

Correlation coefficients were calculated between each two parameters for all the plant species. The scatterplots and histograms were shown in Figure 13, and the correlation coefficients were shown in Table 10. The total carbon had a strong positive relationship with total nitrogen (r^2=0.6618) and organic matter content (r^2=0.5692). This maintained the soil with a constant carbon and nitrogen ratio, and plants benefited from the certain C: N in their growth. Also organic matter correlated positively with both total nitrogen and total carbon. Soil microbial respiration rate correlated with the organic matter content, total nitrogen content and total carbon content positively, while negatively correlated with EAC. This shows that soil respiration could be a good indicator for nutrient enrichment. They other parameters were not strongly correlated.

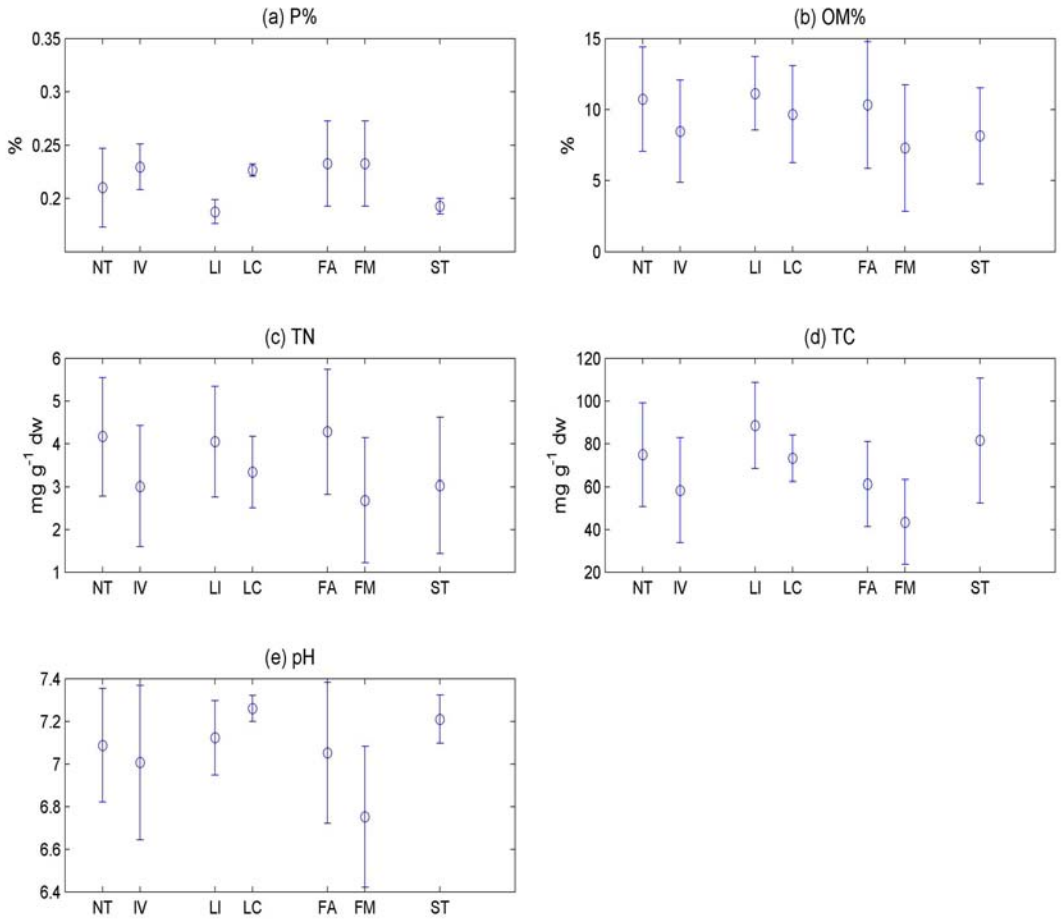

Figure 9 Comparison between soil geochemical statuses in rhizosphere underneath native and exotic plants species. NT is native species; IV is invasive species; LI is *Lantana involucrate*; LC is *Lantana camara*; FA is *Ficus aurea*; FM is *Ficus microcarpa*; ST is *Schinus terebinthifolius*. Circles indicate the arithmetic means and vertical bars are standard deviations. P% is available P percentage in soil; OM% is organic matter percentage in soil; TN is total nitrogen content in soil; TC is total carbon content in soil.

Table 6 P-values for ANOVA between native and invasive plant species. P<0.05 are considered significant different.

P-values	%P	OM%	TN	TC	pH
Native vs Invasive	**0.0340**	**0.0391**	**0.0067**	**0.0243**	0.3837
LI vs LC	**<0.0001**	0.2672	0.1366	**0.0365**	**0.0216**
FA vs FM	0.9957	**0.0806**	**0.0273**	**0.0742**	**0.0487**
LI vs ST	0.2229	**0.0296**	0.1119	0.5155	0.1757
FA vs ST	**0.0035**	0.2107	**0.0672**	**0.0694**	0.1490

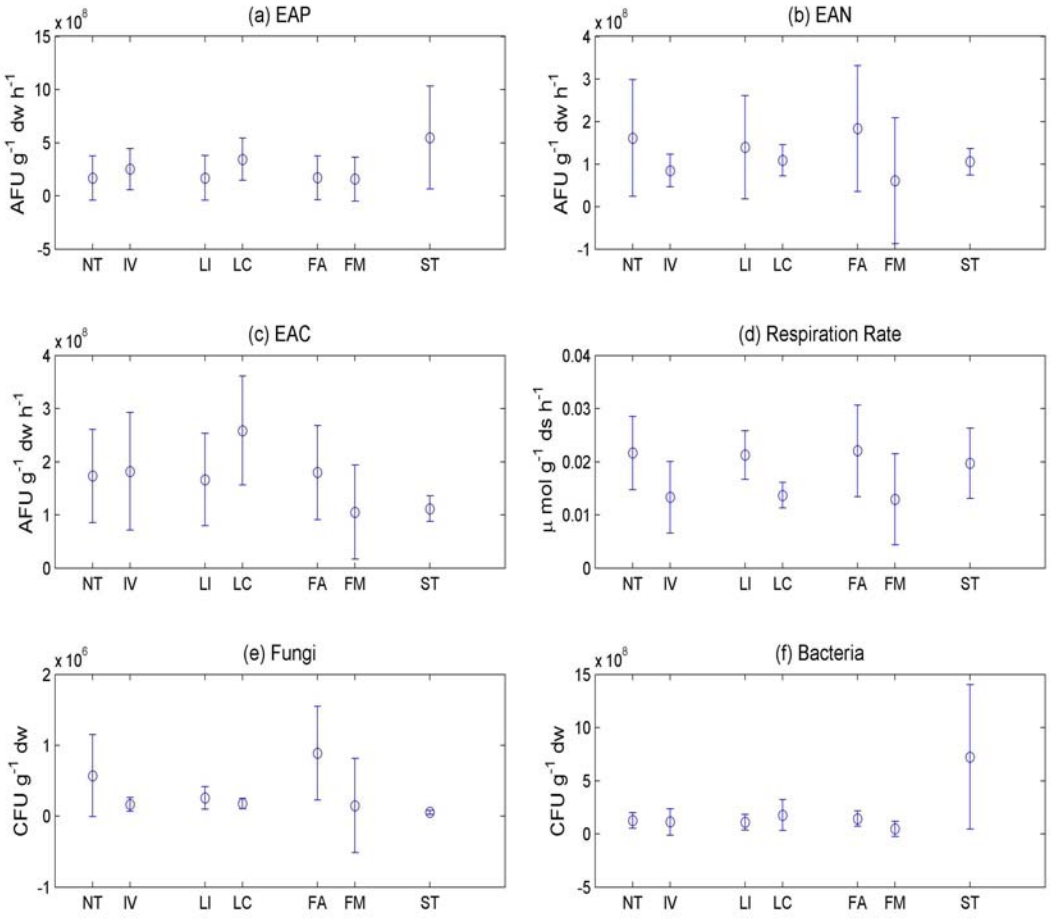

Figure 10 Comparison between soil biogeochemical statuses in rhizosphere underneath native and exotic plants species. NT is native species, IV is invasive species, LI is *Lantana involucrate*, LC is *Lantana camara*, FA is *Ficus aurea*, FM is *Ficus microcarpa*, ST is *Schinus terebinthifolius*. Circles indicate the arithmetic means and vertical bars are standard deviations. EAs are all enzymatic activities, P is for alkaline phosphatase, N is for β-N-acetylglucosaminidase, C is for β-1-4-glucosidase.

Table 7 P-values for ANOVA between native and invasive plant species. P<0.05 are considered significant different.

P-value	EAP	EAN	EAC	Respiration	Fungi	Bacteria
Native vs Invasive	0.1670	**0.0131**	0.7639	**<0.0001**	**0.0016**	0.6219
LI vs LC	0.0533	0.4347	**0.0329**	**<0.0001**	0.1503	0.1753
FA vs FM	0.8635	**0.0123**	**0.0173**	**0.0238**	**0.0014**	**0.0003**
LI vs ST	**0.0258**	0.3827	0.0559	0.5336	**0.0004**	**0.0068**
FA vs ST	**0.0263**	0.1004	**0.0225**	0.4844	**0.0004**	**0.0100**

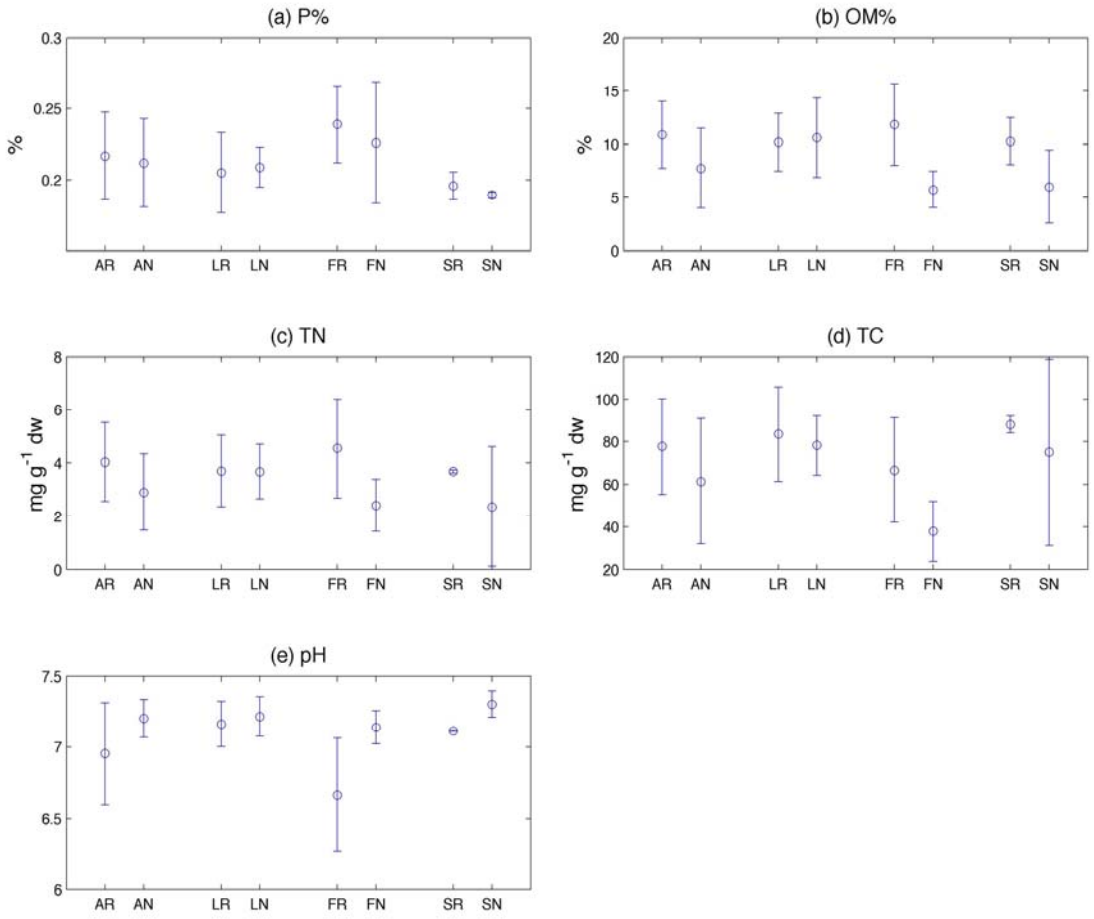

Figure 11 Comparison between soil geochemical statuses in both rhizosphere and nonrhizosphere of native and exotic plants species. AR is average of rhizosphere; AN is average of nonrhizosphere; LR is rhizosphere of *Lantana*; LN is nonrhizosphere of *Lantana*; FR is rhizosphere of *Ficus;* FN is nonrhizosphere is *Ficus;* SR is rhizosphere of *Schinus*; SN is nonrhizosphere of *Schinus*. Circles indicate the arithmetic means and vertical bars are standard deviations. P% is available P percentage in soil; OM% is organic matter percentage in soil; TN is total nitrogen content in soil; TC is total carbon content in soil.

Table 8 P-values for ANOVA between rhizosphere and nonrhizosphere of native and invasive plant species. P<0.05 are considered significant different.

P-value	%P	OM%	TN	TC	pH
AR vs AN	0.5446	**0.0009**	**0.0038**	**0.0191**	**0.0008**
LR vs LN	0.6664	0.7499	0.9619	0.4843	0.4052
FR vs FN	0.3879	**<0.0001**	**0.0019**	**0.0019**	**0.0007**
SR vs SN	0.1439	**0.0283**	**0.1793**	0.4848	**0.0005**

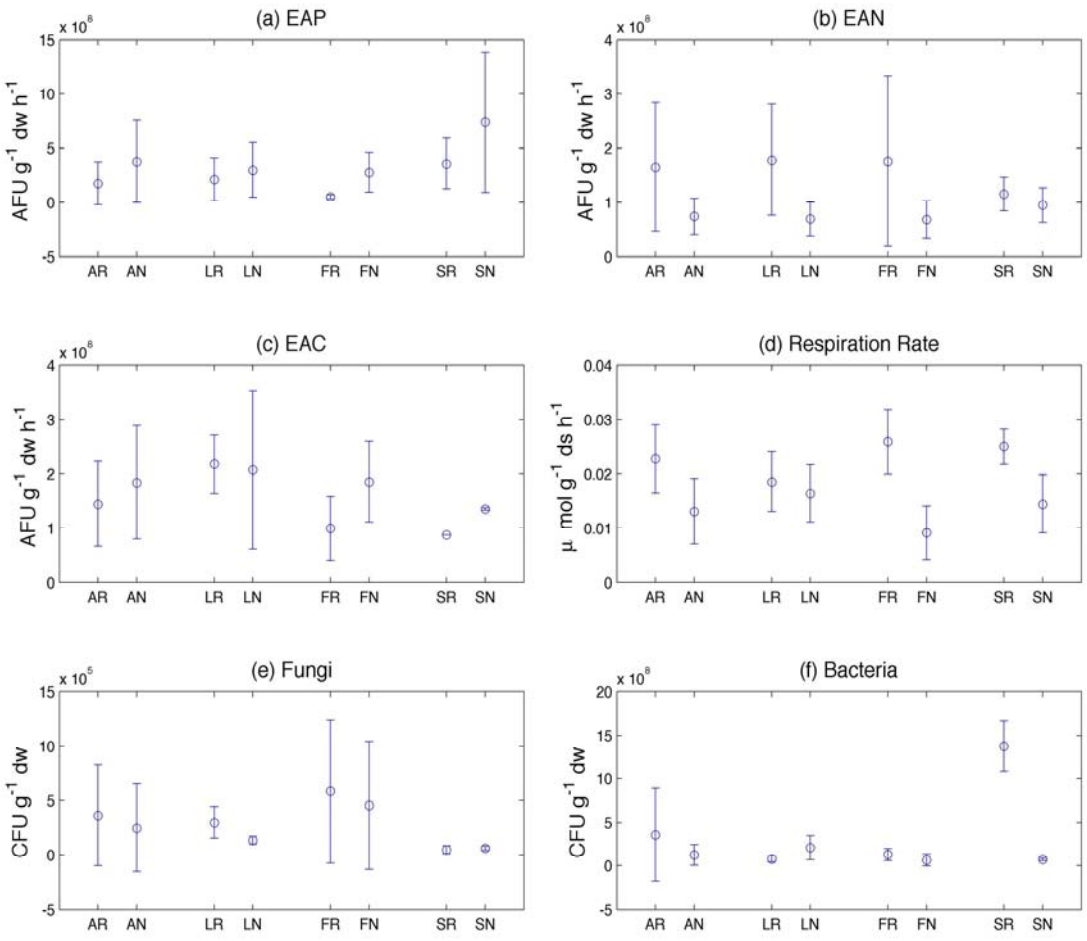

Figure 12 Comparison between soil biogeochemical statuses in both rhizosphere and nonrhizosphere of native and exotic plants species. AF is average of rhizosphere; AN is average of nonrhizosphere; LR is rhizosphere of *Lantana*; LN is nonrhizosphere of *Lantana*; FR is rhizosphere of *Ficus;* FN is nonrhizosphere is *Ficus;* SR is rhizosphere of *Schinus*; SN is nonrhizosphere of *Schinus*. Circles indicate the arithmetic means and vertical bars are standard deviations. EAs are all enzymatic activities, P is for alkaline phosphatase, N is for β-N-acetylglucosaminidase, C is for β-1-4-glucosidase.

Table 9 P-values for ANOVA between rhizosphere and nonrhizosphere of native and invasive plant species. P<0.05 are considered significant different.

	EAP	EAN	EAC	Respiration	Fungi	Bacteria
AR vs AN	**0.0118**	**0.0002**	0.1001	**<0.0001**	0.3127	**0.0244**
LR vs LN	0.3530	**0.0019**	0.8197	0.3348	**0.0010**	**0.0044**
FR vs FN	**0.0003**	0.0298	**0.0050**	**<0.0001**	0.6207	**0.0410**
SR vs SN	0.2061	0.2904	**<0.0001**	**0.0021**	0.4594	**<0.0001**

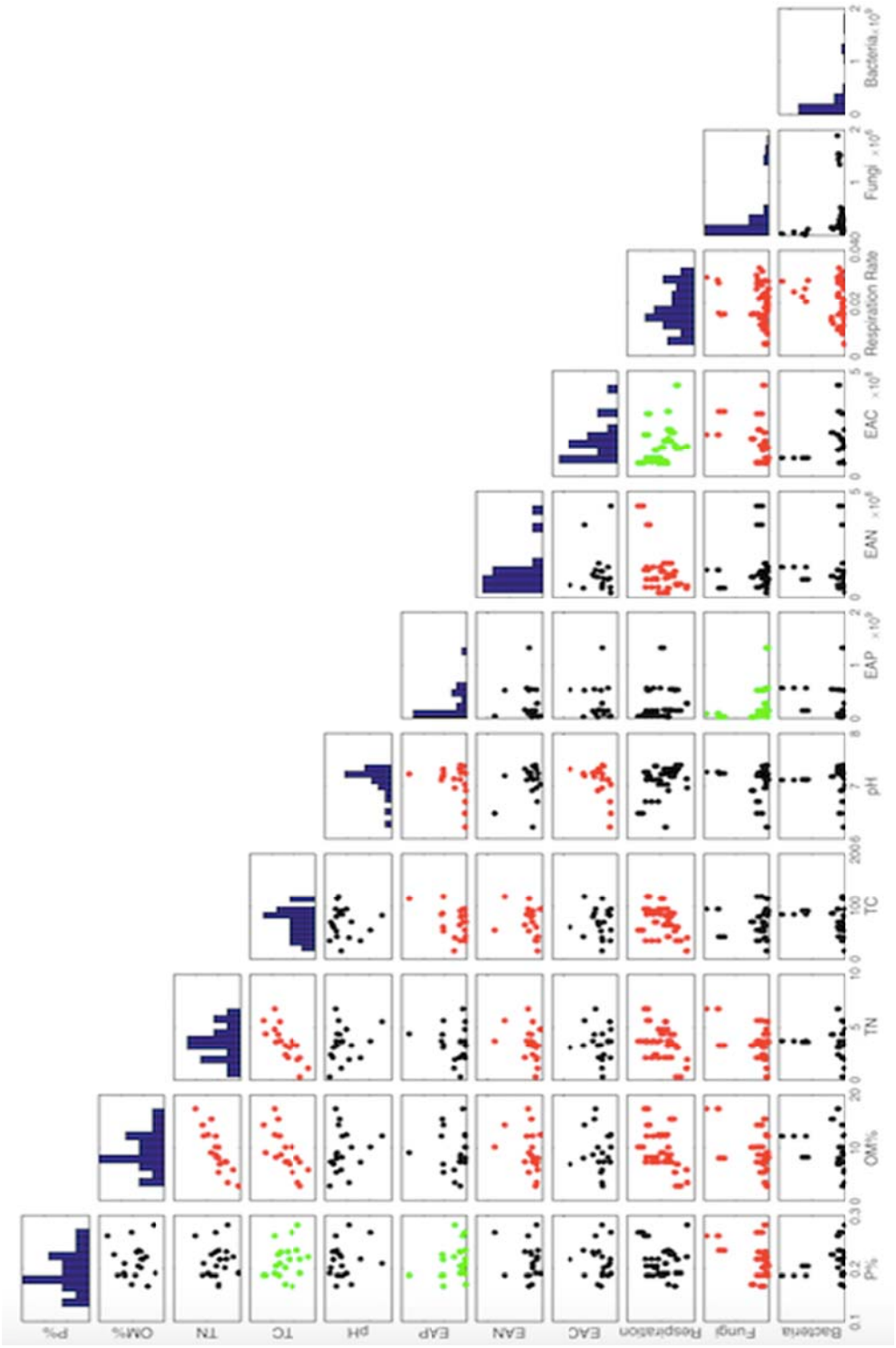

Figure 13 Correlation analysis between each other parameter. The red dots plots mean the correlations are positive and significant. The blue dots plots mean the correlations are negative and significant. The black dots plots mean the correlations are not significant.

Table 10 The correlation coefficients and significance values between parameters.

	P	OM	TN	TC	pH	EAP	EAN	EAC	RR	Fungi
Bacteria	r=-0.1617 p=0.2170	r=0.2094 p=0.1084	r=0.1027 p=0.4350	r=0.2532 p=0.0509	r=0.0797 p=0.5449	r=0.1232 p=0.3482	r=0.0182 p=0.8904	r=-0.1791 p=0.1709	**r=0.3459** **p=0.0068**	r=-0.1189 p=0.3654
Fungi	**r=0.3548** **p=0.0054**	**r=0.3509** **p=0.0060**	**r=0.3638** **p=0.0043**	r=-0.0077 p=0.9534	r=0.1293 p=0.3274	**r=-0.3058** **p=0.0175**	r=-0.0105 p=0.9366	**r=0.3398** **p=0.0079**	**r=0.2605** **p=0.0444**	—
RR	r=0.0079 p=0.9520	**r=0.5751** **p<0.0001**	**r=0.5446** **p<0.0001**	**r=0.5110** **p<0.0001**	r=-0.2466 p=0.0575	r=0.1129 p=0.3903	**r=0.5835** **p<0.0001**	**r=-0.2542** **p=0.0500**	—	—
EAC	r=0.0592 p=0.6530	r=0.0397 p=0.7636	r=0.0950 p=0.4703	r=0.0580 p=0.6600	**r=0.4939** **p=0.0001**	r=0.2092 p=0.1086	r=-0.0224 p=0.8651	—	—	—
EAN	r=0.1508 p=0.2501	**r=0.3271** **p=0.0108**	**r=0.3216** **p=0.0122**	**r=0.2994** **p=0.0201**	r=-0.2427 p=0.0617	r=0.0952 p=0.4691	—	—	—	—
EAP	r=-0.3076 p=0.0168	r=0.0422 p=0.7490	r=0.1413 p=0.2814	**r=0.4016** **p=0.0015**	**r=0.3153** **p=0.0141**	—	—	—	—	—
pH	r=-0.1905 p=0.1449	r=-0.1836 p=0.1603	r=-0.2279 p=0.0799	r=0.0661 p=0.6160	—	—	—	—	—	—
TC	**r=-0.2842** **p=0.0277**	**r=0.7139** **p<0.0001**	**r=0.8033** **p<0.0001**	—	—	—	—	—	—	—
TN	r=-0.0026 p=0.9843	**r=0.8788** **p<0.0001**	—	—	—	—	—	—	—	—
OM	r=-0.0148 p=0.9106	—	—	—	—	—	—	—	—	—

Chapter 5 Conclusion and discussion

This study showed that different invasive plants changed the soil biogeochemical processes in different ways and at different levels. Even the plants from the same invasive plant species were differ in those biogeochemical processes, if the underneath soils were different. Brazilian pepper is a very wide spread invasive plant in south Florida. The viable heterotrophic bacteria population in the soil under Brazilian pepper was 10 times greater than the others. Also they could live under conditions of significant lower available phosphorus than other plants. In addition, the phosphatase activity in soil under Brazilian pepper was significantly higher than other plants. This suggested Brazilian pepper might be able to support population increase and activity of phosphorus solubilizing bacteria to grow. The bacterial have huge community population, and they secret enzymes to break down organic phosphorus in soil into available phosphorus, thus supply the plant with the nutrient. This process assists Brazilian pepper to spread in barren soils where other plants can barely survive.

For later study, the nutrient amount in plants root, branches and sprout should also be analyzed. Because just by looking at the nutrient content in soil is only part of the nutrient cycle, which is not complete. In that way, we can tell if the plant is really in need of phosphorus to survive. Also I will do experiment to test if there is such specific type of mutualism bacteria in the rhizosphere soil to assist survival of invasive plant species.

The respiration rates in soils under native and invasive plants were significantly different. And all the soils under invasive plants had higher respiration rate. This showed that invasive plant help the soil released more carbon into the air. In this way, the carbon pool in soil become smaller with the increase amount of invasive plants. This indicated that invasive plants were possibly able to stimulate increased biological activity in the soil and took advantage of it for their growth and spread. For further study, the anaerobic respiration rate should also be included. Some bacteria decompose soil organic matter under anaerobic conditions; this may be of relevance to invasive plant species growing under submerged or semi-submerged conditions.

The enzyme activity for study did not give us much results. Only a small portion of the results gives me low significant P values.

In this study, the plants were originally chosen in pairs, each genus had two species (one native and one invasive). This could help make better comparison than the *Shcinus*. The native *Schinus molle* was present in California, in South Florida related species such as *Toxicodendron* sp. and *Metopium* sp. within the family Anacardiaceae sp. were not safe to handle and collect samples. The sampling location should be chosen away from the street to avoid disturbance. I sampled one soil sample of the strangler fig tree by the roadside which may introduce lots of disturbance to my results. Additionally, it is important to have a good understanding of the range of rhizosphere of each plant. The results of *Lantana* soil samples are not much significantly different. This was contrary to

the result obtained from others. So the nonrhizosphere might be too close to the root system. Small plants could have a huge root system and rhizosphere. Also the sampling size needed to be bigger for each plant species, so that it would be better for statistical analysis and the results can be more trustable.

Understanding the mechanism of soil biogeochemical processes will be applicable for eliminating invasive plants. Understanding the different impacts of various invasive plants on each specific type of soil biogeochemical process will be an efficient and effective approach to understand the invasion process. Any direct or indirect nutrient enrichment of natural area soil, particularly if the area is prone invasion by exotic plant species should be paid additional attention, because this may help the invasive plants overcome the limitation for their growth. Application of herbicides and pesticides should also be reviewed, since that may create imbalance in the soil microbial population and may favor invasive plants.

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