THE INFLUENCE OF THE INVASIVE CHINESE TALLOW (TRIADICA SEBIFERA) LEAF LITTER ON AQUATIC CHEMISTRY AND MICROBIAL COMMUNITY COMPOSITION

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THE INFLUENCE OF THE INVASIVE CHINESE TALLOW (*TRIADICA SEBIFERA*) LEAF LITTER ON AQUATIC CHEMISTRY AND MICROBIAL COMMUNITY COMPOSITION

By

RAYMOND D. MONTEZ, BACHELOR OF SCIENCE

Presented to the Faculty of the Graduate School of

Stephen F. Austin State University

In Partial Fulfillment

Of the Requirements

For the Degree of

Masters of Science

STEPHEN F. AUSTIN STATE UNIVERSITY

December, 2016
THE INFLUENCE OF THE INVASIVE CHINESE TALLOW (TRIADICA SEBIFERA) LEAF LITTER ON AQUATIC CHEMISTRY AND MICROBIAL COMMUNITY COMPOSITION

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ACKNOWLEDGMENTS

I thank Dr. Alexandra Van Kley for allowing me to be apart this collaborative project. Her expertise in the field of biotechnology and her interest in explorative microbial ecology opened the door for me to undertake a challenging thesis in interdisciplinary science. I am grateful of her selection of me to integrate my personal interest of microbial ecology into my graduate research. I thank Dr. Dan Saenz and Cory Adams from the US Forest Service for all of their input, guidance and help throughout this thesis. I thank Gina Franke from the US Forest Service for providing assistance in finding additional funding, which was critical for undertaking a third sampling time period. I thank the Southern Research Station, US Forest Service, for funding the DNA sequencing portion of this research, and for providing the materials that were used for all of the experiments needed to complete this research. I thank the Division of Environmental Science for funding a portion of this research and for providing the technical resources needed to complete this project. I thank Dr. Farrish for his patience and editing, which helped me with this thesis. Much appreciation is given to Mr. Wayne Weatherford from the Soil, Plant, and Water Analysis Laboratory for his contributions, suggestions, and chemical analysis. I thank Dr. Mindy Shaw for serving as the non-departmental committee member. I
thank Dr. James Van Kley for all of his support during my undergraduate and graduate years at SFA. I thank Armen Nalian for assistance and interpretation of the advanced statistical programming that was required for the microbial community analysis. Finally, I thank Arthur Temple College of Forestry and Agriculture for a wonderful and fulfilling educational experience.
ABSTRACT

Global climate change and anthropogenic activity have facilitated the movement and invasive potential of nonnative plants in native environments. These invasions can have negative effects on ecosystem diversity and function. The nonnative and invasive plant, Chinese Tallow (Triadica sebifera), has already invaded much of the south eastern US where it is outcompeting native tree species and changing ecosystem diversity in a variety of habitats. Leaf litter from the Chinese tallow has been shown cause changes in dissolved oxygen and pH in the aquatic environment. Turbidity is also affected when Chinese tallow litter is present in water. A series of experiments were performed to determine the causes of these chemical changes in water when Chinese tallow litter is present. I determined that Chinese tallow litter has a different chemical composition from native litter, a different concentration of essential soluble nutrients from native litter, and a faster decomposition rate compared to native plant litter. Sterilization experiments suggest that Chinese tallow litter is promoting microbial activity through the rapid release of elemental nutrients, which subsequently influences a change in dissolved oxygen thorough stimulated microbial respiration. Changes in pH are not fully understood, but sterilization experiments suggest that unknown secondary chemicals, perhaps tannins and phenolic compounds, are the source of declining pH when Chinese tallow litter is
present in water. Using high throughput 16s and 18s rRNA mass parallel gene sequencing, followed by Non-metric Multidimensional Scaling ordination scatter plots, I determined that Chinese tallow litter can promote differences in microbial community composition from that of native plant litter. The NMDS ordination scatter plots demonstrate that both bacterial and fungal communities were different in Chinese tallow treatments when compared to native plant litter treatments. These results provide strong evidence that Chinese tallow litter can promote changes in the microbial community composition of an aquatic/wetland habitat.
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INTRODUCTION

Biological invasions of exotic species in native ecosystems are widely recognized as one of the major threats to biodiversity and ecosystem stability (Vitousek et al., 1997; Ehrenfeld, 2003). Many native plant and animal species are threatened by competition and/or major ecosystem changes caused by the introduction of non-native species (Pinmental, 2001; Webster et al., 2007). Plant invasions in particular have occurred around the world and have profoundly decreased biodiversity and altered the structure and function of many ecosystems (Radosevich et al., 2003). In the eastern forests of the United States, invasive plants have altered forest ecosystems through the displacement and competitive exclusion of native plants (Webster et al., 2007) and continue to degrade forest ecosystems and native plant diversity at an alarming rate (Wang et al., 2014).

The invasion of Chinese tallow (Triadica sebifera) in the southeastern forests of the United States has become a growing concern in the past few decades (Jubinsky and Anderson, 1996; Bruce et al., 1997; Pattison and Mack, 2008; Leonard, 2008; Gan et al., 2009; Camarillo et al., 2015). Since its introduction in the late 1700s (Bell, 1996; Randal and Marinelli, 1996), this invasive deciduous tree has become naturalized across the southeastern coastal
plains from North Carolina to Florida and into Texas, where it can be found throughout coastal forests, wetlands, and coastal prairies (Bruce et al., 1997; Wang et al., 2011).

The abundance of Chinese tallow has rapidly increased in Louisiana and Texas where it is now the fifth most common tree species (Oswald, 2010), and the most prevalent, stand-replacing, invasive tree in the forest lands of eastern Texas (Gan et al., 2009; Wang et al., 2014; USDA, 2014). Chinese tallow has altered native ecosystems by converting some coastal grassland into a new woodland habitat (Bruce et al., 1995) and reducing native tree species in bottomland hardwood forest habitat (Camarillo et al., 2015). It is has been a serious problem in all four of the East Texas National Forests, the Big Thicket National Reserve, and has contributed to the degradation of wetlands throughout the Gulf Coast (Herbert, 2005).

Chinese tallow is especially prevalent in the wetland environment (Zedler and Kercher, 2004). Several attributes contribute to the woody invader’s success in the wetland habitat. Although the Chinese tallow can be found in well-drained upland areas, the tree thrives in mesic to wet environments (Barrilleaux and Grace, 2000; Fan et al., 2012). It produces copious amounts of seeds that are primarily dispersed by birds (Renne et al, 2000), and flooding (Jubinsky and Anderson, 1996). Germination rates can be high and rapidly growing seedlings
may form mono-specific stands (Leonard, 2008), often outgrowing native species within the first year (Bruce, 1993).

Chinese tallow can grow well in a variety of soil and light conditions, tolerating salinity (Conner, 1994), flooding (Jones and Sharitz, 2000) and shade (Jones and McLeod, 1989). The invasive tallow tree also seems to have a high resistance to native herbivory (Rodgers and Siemann, 2003). This may be attributed to tannins present in its leaves (Cameron and LaPoint, 1978), as it seems to be relatively free of browsers, and has been reported to be toxic to domestic animals (Russell et al., 1969; USDA, 2014). Senesced leaf litter readily leaches tannins and phenolic compounds during rain events (Cameron and LaPoint, 1978), and has a rapid decomposition rate relative to native species, increasing nutrient cycling rates in soils and self-facilitating its own growth (Cameron and Spencer, 1989).

The rapid decomposition rate and tannin/phenolic leachate of Chinese tallow leaf litter can have an effect on diversity and the performance of aquatic populations through indirect changes in water quality (Leonard, 2008; Adams and Saenz, 2012; Cotton et al., 2012). Dissolved oxygen, pH and turbidity are major chemical and characteristic components that are important drivers of aquatic diversity; all of which are affected by Chinese tallow leaf litter. These indirect changes in water quality can occur within 48 hours after the addition of Chinese tallow leaf litter and the amount of chemical alteration is directly related to tallow
litter concentration. Adams and Saenz (2012) demonstrated that higher concentrations of tallow leaf litter contributed to lower dissolved oxygen concentrations and pH in simulated aquatic environments, with as little as 1 g tallow litter/L water caused dissolved oxygen concentrations to drop to hypoxic levels. Turbidity also increases as tannins and other unknown phenolic compounds are leached into the water column (Adams and Saenz, 2012). Although the exact mechanisms by which these chemical changes occur is unknown, it is suspected that microbial activity (bacteria and fungi) and/or secondary chemicals (tannins/phenolic compounds) are involved (Leonard, 2008). These chemical alterations, specifically in dissolved oxygen and pH, may have an influence on overall microbial community composition, leading to possible changes in the overall function of wetland habitat.

The impacts of Chinese tallow leaf litter on wetland/aquatic microbial community composition has not been explored. Besides competition, microbial populations and their communities are affected by environmental factors (temperature, pH, dissolved oxygen, salinity, turbidity, etc.) and direct changes in these chemical factors can contribute to changes in microbial community composition (Kirchman, 2012). Additionally, changes in microbial community composition may change specific ecosystem processes (van der Heijden et al., 2008). Therefore, Chinese tallow has the potential to alter wetland microbial
communities and ecosystem processes through the input of non-native leaf litter and subsequent changes in aquatic chemistry.

This study focuses on the Chinese tallow (*Triadica sebifera*) leaf litter and its impacts on aquatic chemistry and microbial community composition (bacteria and fungi) in a simulated wetland environment, compared to the litter of native species of water oak (*Quercus nigra*) and loblolly pine (*Pinus taeda*). These two species are common in wetland habitat throughout East Texas and abundant across the forests of the southeastern United States.
OBJECTIVES

The objectives of this study were to determine the proximate cause(s) of chemical alteration by Chinese tallow leaf litter, and to determine if these chemical changes can have an influence on microbial community composition, compared to the native species of loblolly pine (*Pinus taeda*) and water oak (*Quercus nigra*) plant litter.

The objectives were met by the following:

1) Comparing aquatic chemistry changes associated with decomposing Chinese tallow, water oak, and loblolly pine litter.

2) Comparing plant litter quality and soluble extractable elements from Chinese tallow leaf litter to the two native species of water oak and pine plant litter.

3) Comparing the decomposition rate of Chinese tallow plant leaf litter to the plant litter of native species, water oak and loblolly pine.

4) Determine the proximate cause(s) of aquatic chemistry changes influenced by the presence of Chinese tallow plant leaf litter through sterilization tests.

5) Determine the impacts of Chinese tallow plant litter on aquatic microbial community composition in response to these aquatic chemistry changes, compared to the plant litter of two native tree species of water oak and loblolly pine.
LITERATURE REVIEW

The occurrence of nonnative species in new ecosystems is not a new or localized problem. Every nation now has thousands of nonnative, introduced species inhabiting their ecosystems (Pinmental, 2011). These biological invasions are not only widespread, but are considered as a component of anthropogenic environmental change (Vitousek et al., 1997). Biological invasions are a natural process (Renee, 2001), but the anthropogenic component is accelerating the rate at which these invasions occur (Vitousek et al., 1997). It has been documented that many nonnative species have been introduced through the anthropogenic activity of commerce and trade (Mack et al., 2000). Between the United States and Australia there is an estimated 2,000 species of nonnative plants that have been established through this route of introduction (Vitousek et al., 1997). It is also estimated that 100 million acres in the United States are affected by nonnative plant species (Herbert, 2005).

The negative impacts of nonnative tree species are not well understood, but are beginning to receive more attention (Leonard, 2008; Adams and Saenz, 2012). A growing body of scientific literature suggests that invasive plants are altering forest ecosystems through the displacement and competitive exclusion of native plants (Webster et al., 2007). The attention has been primarily focused on
the nonnative tree species have been known to be able to aggressively
outcompete other native tree species, leading to rapid community composition
change, the reduction of biodiversity and the alteration of native ecosystem
function (Vitousek et al., 1997; Webster et al., 2007).

There are many nonnative crop species that were intentionally introduced
into the United States that have since become a threat to agriculture and the
biodiversity of forests (Pinmental, 2011). The Chinese tallow (Tridica sebifera)
tree, a woody invader that has replaced native species in the southeastern
United States (Bruce et al, 1997) is an example of one of these crops. The
nonnative and invasive Chinese tallow, native to China, was introduced to South
Carolina, United States, in the 1700s by Benjamin Franklin, who though that the
seeds would be a profitable and viable source of wax and oil (Bell, 1966). Tallow
has been cultivated in China for 14 centuries for soap, fuel, candles, drying oils,
black dye, and protein (USDA, 2014).

The U.S. Department of Agriculture also introduced the Chinese tallow to
the Gulf of Mexico region in the 1900s to help establish soap and oil industries
(USDA, 2014). At the same time, the tree became, and remains a popular
ornamental tree to the public (USDA, 2014). Ornamental planting of Chinese
Tallow is thought to play a significant role on the local expansion of Chinese
Tallow within the Gulf coast region, since the tree displays vibrant color changes
during leaf senescence that are desired for aesthetic value. Since then it has
escaped cultivation and has thrived in the southeastern portion of the United States where it has altered species composition, community structure, and ecosystem processes in forests and disturbed habitats (Miller, 2003).

As of 2009, Chinese tallow has invaded a total of ten States, nine of which are located along the Gulf coastal plain regions in the southeastern region of the U.S, with an estimated 185,000 ha of forest occupancy (Gan et al., 2009). In this portion of the United States the tree has colonized riparian areas, coastal forests and grassland prairies (Bruce et al, 1997), and has also spread into the upland forests as far north as North Carolina, often found in the form of monocultures (Renne et al., 2002; Jubinsky and Anderson, 1996). There is also a small presence of Chinese tallow in California where it has invaded two northern counties (USDA, 2014). The tree has heavily invaded the State of Louisiana, and East Texas where is has gained considerable expansion. The current distribution of Chinese tallow in the southeastern region of the US is shown in Figure 1.

Oswalt (2010), estimates that the volume of Chinese tallow has increased a significant amount in both Louisiana and East Texas over the last two decades where it is now the fifth most abundant tree species in those areas. Only a few counties in Louisiana remain free of Chinese Tallow (USDA, 2014). Mississippi has also seen significant increases in the past decade but it has not yet made it on the top five most abundant list in that state (Oswalt, 2010). Jubinsky and
Anderson (1996) have also shown that Chinese tallow has invaded over half of Florida’s counties.

Figure 1. Current distribution of Chinese Tallow (*Triadica sebifera*) in southeastern United States counties as of 2014.

In Texas, forty-five counties have already been invaded by the nonnative Chinese Tallow. Chinese tallow has primarily become a problem in East Texas forests and along the Gulf Coast areas where it has displaced native grasses, and changed much of the upper coastal prairies into a new woodland type (Bruce
et al, 1995). The original coastal prairie community was once dominated by grasses, but is now covered with woody vegetation, 98% of which is Chinese Tallow (Bruce et al: 1995).

Chinese tallow has also become established in east Texas forestlands where it is now the most prevalent invasive tree (USDA, 2014). All but one East Texas County remains untouched by the invasive Chinese Tallow (Wang et al., 2014). It has been found in both private and public lands where it has become a serious problem in all four National Forests of east Texas (Herbert, 2005) and is often found along fence lines alongside transportation corridors (personal observation). Chinese tallow is also present in central and south Texas, where it seems to be expanding.

Invaded counties are spread across seven ecoregions in Texas, with the Piney Woods ecoregion almost entirely affected. Other ecoregions in Texas that the Chinese Tallow occupies are the Gulf Coastal Prairies and Marshes, the Blackland Prairies, the Oak Woods & Prairies, the Edwards Plateau, including the Llano Uplift portion, and the South Texas Brush ecoregion. The invasive nature of Chinese Tallow is a threat to native flora and vulnerable ecosystems. Chinese tallow is an aggressive woody invader that thrives in warm, moist climates (Pattison & Mack, 2008). It is a fast growing tree that may reach reproductive age in at little as three years (Jubinsky and Anderson, 1996). Additionally, it generates a great number of seeds that retain long term viability
(Bruce et al; 1997) and are primarily dispersed by birds (Renne et al., 2000) and flooding (Jubinsky and Anderson, 1996).

Once the tree becomes established it can aggressively out-compete native tree species, often generating monocultures (Oswalt, 2010). The tree has the ability to grow well in a variety of soils, but soil moisture appears to be the limiting factor in its invasive success (Barrilleaux and Grace, 2000), making it adaptable in different ecoregions with adequate soil moisture. It is also especially prevalent in wetlands and the margins of lakes (Zedler and Kercher, 2004). The range and invasive ability of the Chinese Tallow also seems to be limited by cold temperatures (Pattison and Mack, 2008; Wang et al., 2011).

With the formation of monocultures and the reduction of species diversity, Chinese tallow has potential to change ecosystem function and alter primary productivity (Cameron and Spencer, 1989). The leaves of Chinese tallow are known to have a rapid decomposition rate relative to native species (Cameron and Spencer, 1989; Leonard, 2008) and contain plant secondary compounds (tannins and phenols) that may negatively affect populations of both terrestrial and aquatic species (Cameron and Spencer, 1989).

Past studies have detected tannins and other secondary chemicals in tallow leaves and bark (Conway and Smith, 2002). Tannins are the most abundant secondary plant metabolite made by plants and can defend leaves against insect herbivores by deterrence or by toxicity (Barbehenn and Constabel,
Secondary chemicals in Chinese tallow litter may give it an ecological advantage over native species. The competitive success of Chinese tallow in the United States may be attributed to these secondary compounds, (but more likely due to lack of its native insect herbivores & diseases) as the tree loses very small amounts of leaf area to herbivory (Rogers et al; 1995) and seems to be free of grazers in its nonnative environment (Rodgers et al, 1995).

Chinese tallow leaf litter also alters the chemistry of ecosystems it invades (Cameron and Spencer, 1989). Chinese Tallow leaf litter deposited in terrestrial systems has been shown to alter soil nutrient dynamics (Cameron and Spencer, 1989). Soils in a Tallow forest contained significantly higher amounts of P, K, NO$_3$-N, Zn, Mn, and Fe, and significantly lower amounts of Mg and Na than soils in coastal prairie vegetation (Cameron and Spencer, 1989). The rapid decomposition rate of Chinese tallow litter, relative to native deciduous species, has also been found to contribute to higher mineralization and release rates of Ca, N, K, Mg, and P, in ecosystems than native deciduous trees in temperate regions, perhaps due to lower lignin content in Chinese tallow litter (Cameron and Spencer, 1989). This is especially noteworthy because litter decay rates are associated with rates of nutrient mineralization and primary productivity (Cameron and Spencer, 1989).
In aquatic environments the leaf litter has also been found to alter aquatic chemistry (Leonard, 2008, Adams and Saenz, 2012; Saenz et al., 2013). Dissolved oxygen and pH of surface water are affected when tallow leaf litter is present; rapidly changing the pH to more acidic conditions (pH < 5.5), and depleting dissolved oxygen concentrations (DO < 2 mg/L), (Adams and Saenz, 2012: Saenz et al., 2013). This rapid chemical change in aquatic chemistry from Chinese tallow leaves was determined to have a detrimental effect on the hatching success of the southern leopard frog, where as little as 1mg/L of Chinese tallow leaf litter caused aged tap water to become hypoxic and unsuitable for the hatching of eggs of frog (Adams and Saenz, 2012). The Southern Leopard frog is a common species in eastern Texas and breeds in a variety of aquatic habitats ranging from ephemeral to permanent (Adams and Saenz, 2012).

Dissolved oxygen depletion in surface water is thought to be a result of increased microbial activity as the rapid decomposition rate of the tallow leaves quickly supports microbial respiration and eventually depletes the available dissolved oxygen. Nitrogen and phosphorous are often limiting nutrients in terrestrial environments and the rapid decomposition rate of Chinese tallow may be providing the nitrogen and phosphorous needed to support accelerated respiration for microbial respiration. This change in chemical ratios between
nutrients and oxygen becomes a factor in the development of hypoxia, where oxygen may become the limiting nutrient as respiration increases.

Increased respiration is also thought to play a role in pH change through the formation of higher concentrations of carbon dioxide. As carbon dioxide is increasingly released through active aerobic metabolism, it reacts with water to produce carbonic acid. It is also speculated that tannins or other secondary compounds may also play a role in the change in pH and dissolved oxygen. It is also possible that secondary compounds like tannins are playing a role in removing dissolved oxygen from the system and somehow changing the pH through unknown chemical reactions. The proximate mechanisms of chemical alteration remain unknown to due to the variety of tannins that can be produced and the limited research on this subject.

The negative effects of Chinese tallow in a variety of habitats create a great concern over the presence of the non-native Chinese tallow in the southeastern US and East Texas. Shifts in microbial community structure due to the chemical changes, brought about by the presence of Chinese tallow, are possible in aquatic systems. The effects of this possible change are unknown, but can pose a threat to aquatic habitat diversity, food chain dynamics, and overall ecosystem function. The leaf litter from the invasive Chinese tallow tree
is known to have an effect on amphibian populations residing in aquatic zones (Cotton et al., 2012; Adams and Saenz, 2012; Saenz, 2013).

Amphibians are important components of aquatic ecosystems; they represent a high quality food source for predators and contribute to ecosystem stability through bottom up control of consumer abundance and diversity (Leonard, 2008). The Southern Leopard frog is a common species in eastern Texas and breeds in a variety of aquatic habitats ranging from ephemeral to permanent (Adams and Saenz, 2012). The presence of Chinese tallow in wetland habitats generates the potential for food web and trophic structure disruption in aquatic environments (Zedler and Kercher, 2004).

Shifts in microbial community structure can be another mechanism for bottom up control in aquatic systems, or may contribute to changes in biogeochemical cycles, thus, the Chinese tallow may have effects that can ripple through the ecosystem, decreasing diversity, and changing community structure in both aquatic and terrestrial environments. Wetlands, a transitional zone between terrestrial and aquatic systems may also be affected by the presence of Chinese tallow. Invasions by this plant may not only affect biodiversity and ecosystem function, but also the human use and enjoyment of wetlands (Zedler and Kercher, 2004).
Pattison and Mack, 2008, suggest that the invasion range of Chinese Tallow may be restricted to wet and warm climates. Figure 2 shows the current extent of Chinese Tallow in the southeastern US, along with annual precipitation averages. Figure 2 shows that precipitation is a dominating factor in the distribution range of Chinese tallow, with the higher concentrations of counties invaded having annual precipitation ranges from 40 to 70 inches, yearly.

Aquatic ecosystems and wetlands in this region are at risk, with the annual loading of Tallow leaves that are associated with rapid decay rate, secondary chemicals and chemical alterations. The formation of monoculture stands can produce tallow leaf litter concentrations of 1.27 g of leaf litter/L of water (Leonard, 2008), and is suggested to be much higher in more dense monocultures stands that reside in and around wetlands (Adams and Saenz, 2012). Many of the wetlands of the United States exist within this region and are already infested with Chinese tallow (Zedler and Kercher, 2004).
Figure 2. Annual average precipitation ranges of the US (1981-2013) and the current extent of Chinese Tallow invasion on the counties in the southeastern portion of the US.
MATERIALS AND METHODS

Leaf Collection

Leaf litter from the non-native Chinese tallow (*Triadica sebifera*), and the native species of water oak (*Quercus nigra*), and the needles of loblolly pine (*Pinus taeda*) were collected and used in six separate studies to compare litter species influence on aquatic chemistry and microbial community composition. All litter was hand collected off the ground from under multiple trees located throughout Nacogdoches, Texas, during the period of November-December, 2014. The collection of leaves was from ten different trees from each species. Recently senesced leaves and needles that looked fresh were selected over those that looked too damaged or weathered. Much of the litter collected was not green, but rather brown, red, and yellow, especially in the case of tallow litter.

All litter samples were air-dried and stored (separated by species) in plastic containers (30cm diameter x 28cm deep) and kept in a climate – controlled room for 60 days before being used for the following experiments; An outdoor mesocosm study, including aquatic chemistry monitoring, and water sampling for DNA analysis, a litter quality and soluble extractable elements analysis, a decomposition comparison, and a sterilization test (tallow only).

Laboratory studies by Saenz et al, (2013) showed that storing tallow leaves for
12 months was found to have no effect on measured chemical changes when compared to freshly fallen Chinese tallow leaves that had been submerged in water, therefore, the short-term storage should not have influenced results.

**Mesocosm Design**

To examine the effects of litter input on aquatic chemistry (objective 1) and subsequent effects on microbial diversity and community composition, an outdoor mesocosm approach was employed. Based on prior experiments, the mesocosm approach was helpful in determining the effects of Chinese tallow leaf litter on aquatic amphibian survival (Saenz et al., 2013). Based on this approach a similar set-up was used for this study.

Experimental units were set up using forty, 100-L plastic wading pools (1.2m diameter x 0.3m deep) that served as pond/wetland mesocosms. These experimental units were set up at a site approximately 13.0m x 18.0m in size, in four rows of 10, at the Stephen F. Austin Experimental Forest. All units were located in a semi-open area, exposed to the same light and weather conditions, and were covered with 30% shade cloth to reduce debris input from surrounding trees. Four, 9mm holes were drilled near the top in each pool to prevent overflow of leaves during rain events.

Each of the pools was filled with 80 L of well water on 28 February 2015 and the pools were allowed to settle for three days before the addition of leaf litter to verify structural stability of the experimental units, and to allow
temperature equalization. The source of the well water was from a local well within the Stephen F. Austin Experimental Forest.

Mesocosm Treatment

A completely randomized design with four treatments, (each replicated 10 times), was used for these 40 experimental mesocosms. The four treatments included three litter species monocultures (*Triadica sebifera*, *Quercus nigra*, *Pinus taeda*) and a control treatment containing no leaves.

All litter treatments contained 160g of dry, single species, leaf or needle litter, for a litter concentration of 2g/L, and was added to the mesocosms on 2 March 2015. Leaf litter concentrations were chosen based on Adams and Saenz (2012), who found that as little as 2g/L of tallow leaf litter submerged in aged- tap water can depress dissolved oxygen concentrations within a 48 hour period, causing it to fall to as low as 1.1mg/L.

Mesocosm Aquatic Chemistry Monitoring and Analysis

To complete objective 1, mesocosm aquatic chemistry parameters and changes were recorded in all 40 mesocosms. Using a Quanta® Hydrolab water quality monitoring system, aquatic chemistry measurements (dissolved oxygen, pH, turbidity, salinity, specific conductance ORP, and temp) were recorded for 16 weeks.

Initial recordings began immediately after the addition of leaf litter (2 March 2015) and continued daily for 72 hours, then weekly for 16 weeks, with
final readings on 22 June 2015. Water quality measurements were taken in the same mesocosm sample order for every sample period, in the mornings, between 7am and 12pm.

An analysis of variance of the treatments was determined at the initial, 48 hour and 16 week periods with the PROC GLM program of SAS 9.2 (SAS Institute, Inc., 2002). Means were compared at the α = 0.05 level using Tukey’s Studentized Range Test.

**Litter Quality Comparison and Analysis**

To accomplish objective 2, the collected leaf litter from each species was used to determine the quality of litter. The quality of litter is, for this study, defined as the chemical composition of litter. Leaf litter from each species was analyzed for a litter quality comparison.

Five grams of each litter species was oven-dried, ground, and digested in a nitric acid solution (SPC Scientific; EPA Method 200.7) and analyzed for fourteen elements (P, K, Ca, S, Mg, Na, Mn, Al, B, Mo, Zn, Fe, Cu, As) using an inductively coupled plasma optical emission spectrometer (ICP-OES 7400 Thermo Scientific, Dual View). Carbon and nitrogen concentration was determined using a C/N analyzer (Leco C/N model 628). Ten replications from each species were used. An analysis of variance (ANOVA) of the litter quality results was determined with the PROC GLM program of SAS 9.2 (SAS
Means were compared at the $\alpha = 0.05$ level using Tukey’s Studentized Range Test.

**Soluble Extractable Element Comparison and Analysis**

A bench-top lab study was employed to determine and compare the concentration of soluble extractable elements leached from decomposing leaf litter submerged in water (objective 2). Experimental units were constructed at the U.S. Forest Service Southern Research Station Laboratory, Nacogdoches, Texas using 30, white plastic pails (18 cm diameter x 18 cm deep) capable of holding 2.25L.

Three treatments were assigned to these experimental units using single species, air-dried, litter (oak, pine, tallow) and aged-tap water. (Aged-tap water is tap water that was allowed to sit in large containers for chlorine removal through surface-atmosphere exchange). Each treatment received 4g of single species litter and 2L of aged tap water for a final concentration of 2g/L of single species litter. Each treatment was replicated 10 times.

After a 48 hour decomposition period at room temperature, water samples were taken and processed at the Soil, Plant and Water Analysis Laboratory (Nacogdoches, Texas), using nitric digestion techniques (SPC Scientific; EPA Method 200.7) and analyzed for total soluble extractable elements (P, K, Ca, S, Mg, Na, Mn, Al, B, Mo, Zn, Fe, Cu, As) using an ICP-OES 7400 (Thermo Scientific, Dual View analyzer). An analysis of variance (ANOVA) of the soluble
extractable elements results was determined with the PROC GLM program of SAS 9.2 (SAS Institute, Inc., 2002). Means were compared at the $\alpha = 0.05$ level using Tukey’s Studentized Range Test.

Litter Decomposition Analysis

An *in-situ* litterbag approach was used to test and compare the decomposition rates (objective 3) of all three litter species (*Triadica sebifera*, *Quercus nigra*, *Pinus taeda*). Nylon litterbags (20 x 25cm) with 2mm$^2$ mesh received 5g of air dried, single species litter. This was replicated 50 times for each species for a total of 150 litter bags. All litter bags were deployed simultaneously in a local wetland located within the Stephen F. Austin Experimental Forest, Nacogdoches County, Texas.

After 1 week of decomposition, 30 litterbags (10 replicate litterbags from each species) were retrieved, air-dried, and weighed. This was repeated after 2, 4, 8, and 16 weeks of *in-situ* decomposition until all of the 150 litter bags were retrieved from the wetland. An analysis of variance (ANOVA) of the litter decomposition results was determined with the PROC GLM program of SAS 9.2 (SAS Institute, Inc., 2002). Means were compared at the $\alpha = 0.05$ level using Tukey’s Studentized Range Test.

Sterilization Test

To test the relative influence of microbial activity, and to determine the proximate causes of depressed dissolved oxygen and pH changes in water
chemistry when tallow leaves are present (objective 4), a bench-top lab SET-UP was constructed to compare sterile versus non sterile treatments using 10% formalin as a sterilization agent. Forty plastic pails (18cm diameter x 18cm deep), each filled with 2L of aged tap water were set up at the U. S. Forest Service Southern Research Station Laboratory, Nacogdoches Texas. Using these 40 experimental units, formalin (10%), and 2g/L of air-dried tallow leaves, the following four treatments were assigned, with each treatment replicated 10 times.

1.) Aged tap water only (control)
2.) Aged tap water + air-dried tallow litter (4g)
3.) Aged tap water + air-dried tallow litter (4g) + formalin (15ml,10%)
4.) Aged tap water + formalin (15ml,10%) (secondary control)

All experimental units were set up at the same time and allowed to decompose for 48 hours. Dissolved oxygen (mg/L) and pH concentrations were measured and recorded after the 48 hour period using the portable Quanta© Hydrolab water monitoring system.

An analysis of variance (ANOVA) of the treatments was determined with the PROC GLM program of SAS 9.2 (SAS Institute, Inc., 2002). Means were compared at the α = 0.05 level using Tukey’s Studentized Range Test.
Mesocosm Microbial Community Sampling and Analysis

In addition to taking water quality readings, water samples were also taken from the mesocosms to assess microbial diversity and community composition over time to determine and compare possible changes associated with Chinese tallow litter with that of native species (objective 5). Water samples were taken by drawing 30mL of individual mesocosm water into sterile syringes and pushed through sterile cellulose acetate syringe filters (0.45µm, VWR). The filters were placed in individual WHIRL-PAC® bags and then stored in a -80°C freezer.

Water samples were taken at the same time water quality readings were taken, in the same sample order, every period, for a total of 40 water samples per period. Due to funding constraints, only three periods (initial, 48hour, and 16 week) were used for this study to assess microbial diversity and community composition.

To determine differences in microbial diversity and community composition associated with litter type and aquatic chemistry, the Shannon-Weiner Index and R- 3.2.3 (Longo et al, 2015) statistical software was used. Mesocosm water samples (120) were sent to the Research and Testing Laboratory, Lubbock, Texas, for DNA extraction, qPCR amplification, and mass parallel sequencing (Illumina MiSeq) for microbial identification and diversity analysis (bacteria and fungi). The generated data were then compared to validated microbial sequencing databases (GenBank). From this data, two
operational taxonomic unit (OTU) matrices were delivered as the product, listing bacterial and fungal operational taxonomic units that were found per sample, for all three time periods. Richness (number of OTU) was then calculated from these matrices. The Shannon-Weiner Index was used to score microbial diversity. In the R-3.2.3 software, the VEGAN package and allies were used to test differences in overall microbial community composition among samples by using a Bray-Curtis Distance matrix and permutation multivariate analysis (PERMANOVA). A stress plot function in R was used to determine non-metric or linear fit. Package VEGAN supports all basic ordination methods, including Non-Metric Multidimensional Scaling, and supports functions for fitting environmental variables and for ordination graphics (Oksanen, 2015). A redundancy analysis (RDA), and Non-metric Multidimensional Scaling ordinations using Bray-Curtis dissimilarities function in R was used to determine dissimilarities of microbial composition. Permutation testing was set to 1,000. An indirect gradient analysis was also performed to determine collinearity of environmental factors. Graphical representations of the Non-metric Multidimensional Scaling ordination scatter plots were produced using R software.
RESULTS AND DISCUSSION

Mesocosm Aquatic Chemistry Results

One of the mesocosms was damaged during the experiment; therefore, the total number of mesocosms was reduced to 39. Initial aquatic chemical parameters are listed in Table 1. There were significantly different effects on water quality based on leaf type over the sixteen week experiment after the initial introduction of leaf litter. Chinese tallow litter treatments had the greatest effect on dissolved oxygen, pH, and turbidity at the 48 hour and 16 week time periods, compared to native species (Tables 2 and 3, respectively).

Dissolved Oxygen. Average dissolved oxygen concentrations were initially recorded between 12.23 and 12.34 mg/L for all treatments. After the first 48 hours of decomposition in the mesocosm pools, the average dissolved oxygen concentration in all treatments declined. Mean dissolved oxygen in the control treatment remained close to the initial value after 48 hours, eventually dropping to 10.22 mg/L, while oak and pine treatments caused dissolved oxygen concentration to decline to 8.71 and 6.02 mg/L, respectively. Dissolved oxygen in tallow treatments were found to be significantly lower than the control and both native litter treatments, with an average dissolved oxygen content of 3.45 mg/L.
Table 1. Results and summary of One-way ANOVA and post-hoc Tukey-Kramer test comparing the means of the aquatic chemistry parameters found in Chinese tallow, water oak, and loblolly pine litter treatments at the initial period.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>One-Way ANOVA Results</th>
<th>Single Species Treatment Means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>DF</td>
</tr>
<tr>
<td>Temp °C</td>
<td></td>
<td>39</td>
</tr>
<tr>
<td>SpC</td>
<td></td>
<td>39</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td></td>
<td>39</td>
</tr>
<tr>
<td>pH</td>
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<td>39</td>
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<tr>
<td>Salin (PSS)</td>
<td></td>
<td>39</td>
</tr>
<tr>
<td>DO (%)</td>
<td></td>
<td>39</td>
</tr>
<tr>
<td>ORP (mv)</td>
<td></td>
<td>39</td>
</tr>
<tr>
<td>Turb (NTU)</td>
<td></td>
<td>39</td>
</tr>
</tbody>
</table>

Note: Means with the same letter in a row are not significantly different.

***, ***, ****, **** = Chinese tallow is significantly different from 1, 2, and 3 other treatments.
Table 2. Results and summary of One-way ANOVA and post-hoc Tukey-Kramer test comparing the means of the aquatic chemistry parameters found in Chinese tallow, water oak, and loblolly pine litter treatments at the 48 hour period.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>One- Way ANOVA Results</th>
<th>Single Species Treatment Means (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>DF</td>
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<tr>
<td>Temp (°C)</td>
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<tr>
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<td>*** DO (mg/L)</td>
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<td>3, 35</td>
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<td>*** pH</td>
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<td>3, 35</td>
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<tr>
<td>* Salin (PSS)</td>
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<td>3, 35</td>
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<tr>
<td>*** DO (%)</td>
<td>39</td>
<td>3, 35</td>
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<tr>
<td>ORP (mv)</td>
<td>39</td>
<td>3, 35</td>
</tr>
<tr>
<td>*** Turb (NTU)</td>
<td>39</td>
<td>3, 35</td>
</tr>
</tbody>
</table>

Note: Means with the same letter in a row are not significantly different

***, **** = Chinese tallow is significantly different from 1, 2, and 3 other treatments.
Table 3. Results and summary of One-way ANOVA and post-hoc Tukey-Kramer test comparing the means of the aquatic chemistry parameters found in Chinese tallow, water oak, and loblolly pine litter treatments at the 16 week period.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>One-Way ANOVA Results</th>
<th>Single Species Treatment Means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>DF</td>
</tr>
<tr>
<td>Temp °C</td>
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<td>3, 35</td>
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<tr>
<td>SpC</td>
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<tr>
<td>DO (mg/L)</td>
<td>39</td>
<td>3, 35</td>
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<tr>
<td>pH</td>
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<td>3, 35</td>
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<tr>
<td>Salin (PSS)</td>
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<tr>
<td>DO (%)</td>
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<td>3, 35</td>
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<tr>
<td>ORP (mv)</td>
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<td>3, 35</td>
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<tr>
<td>Turb (NTU)</td>
<td>39</td>
<td>3, 35</td>
</tr>
</tbody>
</table>

Note: Means with the same letter in a row are not significantly different.

***, ***, **** = Chinese tallow is significantly different from 1, 2, and 3 other treatments.
After the 48 hour period tallow treatments continued to cause the most reduction in dissolved oxygen for nine consecutive weeks, causing dissolved oxygen to decline to as little as 2mg/L, followed by oak litter, pine, then control treatments. This strongly suggests that microbial respiration and heterotrophic activity is highest in tallow treatments during these time periods. 

Dissolved oxygen in the control treatments never reached a concentration below 6.18mg/L or above 12.43mg/L during the 16 weeks. Oak litter treatments ranged from 4.22 to 9.49mg/L, while pine litter treatments had a mean dissolved oxygen range from 5.13 to 10.44mg/L. Tallow treatments exhibited the lowest dissolved oxygen with means ranging from 2.00 to 12.21mg/L. The lowest dissolved oxygen concentration at 2.00mg/L was recorded on week four, while the highest dissolved oxygen concentration of 12.21mg/L was recorded on week 10. After week 10, dissolved oxygen concentrations remained above 6.00mg/L in all treatments.

Perhaps the differences in initial litter quality, more water soluble nutrients and faster decay rate of Chinese tallow litter (described later in the results) promoted higher microbial respiration and growth than native litter treatments, contributing to a higher biological oxygen demand that depleted dissolved oxygen. This demonstrates that tallow litter may have an effect on the activity of microbes in terms of biological respiration, which may be the cause of depleted dissolved oxygen concentrations in these mesocosm pools. At these low oxygen...
concentrations, it would be difficult for many aerobic heterotrophic organisms to function, but some bacteria in particular can use other electron acceptors when oxygen is not available. Fungi on the other hand may be more significantly affected, as they require oxygen.

There were several rain events throughout the 16 week experiment. These rain events may have caused dissolved oxygen to be replenished to some degree in the mesocosm pools. Dissolved oxygen increased the most in tallow treatments at week ten, reaching an average dissolved oxygen concentration of 12.21 mg/L. All litter and control treatments declined after this period, but tallow treatments remained highest among treatments until the end of the experiment. It is not clear why dissolved oxygen at this time period increased to these levels. Perhaps the many rain events replenished dissolved oxygen with increased atmospheric/surface interaction. An alternative hypothesis is that microbial activity may have been hindered by high pH during this time period. Variation in pH based on leaf type is described later in the section.

At the 16 week period dissolved oxygen concentrations in tallow treatments were found to be significantly higher than the control and native litter treatments. Mean dissolved oxygen concentration in tallow treatments was found to be 7.85 mg/L. The control was found to have a mean dissolved oxygen concentration of 7.51 mg/L, while oak and pine litter treatments had a mean
dissolved oxygen content of 7.17 and 7.30 mg/L. Graphical results of dissolved oxygen trends based on leaf type are shown in Figure 1.

![Graphical results of dissolved oxygen trends based on leaf type](image)

**Figure 3.** Mean dissolved oxygen (mg/L) trend over the 16 week mesocosm experiment from Chinese tallow, water oak, loblolly pine, and control treatments.

Although tallow treatments caused dissolved oxygen to decline just after 48 hours of decomposition, (most likely due to microbial respiration), the chemical composition of Chinese tallow litter also seems to hinder microbial respiration at later stages of decomposition. An alternative possibility is that microbes capable of photosynthesis like photoautotrophic bacteria and algae may have increased oxygen in these mesocosms as tallow litter released necessary nutrients through decomposition. Anoxic levels of dissolved oxygen
may be associated with increased levels of dissolved carbon dioxide, providing the means to increase photosynthesis.

Tannins and other phenolic compounds are also known to exist in tallow leachate. Secondary chemicals may be affecting microbes in a manner we do not understand, or perhaps carbon sources are diminished at this these later stages of decomposition, leaving microbes without an organic carbon source. Either way, microbial heterotrophic activity seems to be affected at these later stages of tallow decomposition, allowing dissolved oxygen to accumulate in these mesocosms. More research, including respiration monitoring and soluble carbon availability during this period should be addressed in the future to answer these questions.

**pH.** Initial pH values for all treatments were recorded at 8.9. Mean pH was found to be significantly lower in tallow treatments than native litter and control treatments at the 48 hour period (Table 6). The pH in the control treatments at the 48 hour period was found to be 8.92. Oak and pine treatments showed values of 8.43, and 8.64, respectively, while tallow treatments caused pH to drop to 7.89. Mean pH in every week after the 48 hour period remained relatively stable until week 10. All treatments had mean pH ranges from 8-9. Tallow treatments remained the lowest among all treatments until week ten. At week ten, pH values increased in all treatments with tallow producing the highest pH values, reaching a pH of 9.50 and above for rest of the experiment. Tallow
treatments exhibited the highest pH at week 10 and every week thereafter. At week 16 there were significant differences in pH, with tallow treatments having a pH of 10.36 (Table 7). The control treatments had a pH of 9.54, while oak and pine treatments were found to be 7.17 and 7.30, respectively. Graphical results of pH trends based on leaf type are shown in Figure 2.

![Figure 4](image)

Figure 4. Mean pH trend over the 16 week mesocosm experiment from Chinese tallow, water oak, loblolly pine, and control treatments.

The high pH found during week ten through sixteen in tallow treatments may be attributed to the higher calcium content in tallow litter. Leached, or water soluble calcium content was not determined at the sixteen week period, but it is possible that the disassociation of calcium from plant structural materials may
have caused pH in these mesocosms to become more alkaline. As described later in the results, Chinese tallow litter does have significantly more calcium than native tree litter. Magnesium was also found to be significantly higher in tallow litter (presented later). Perhaps the combination of both calcium and magnesium contributed the much higher pH that was found in the latter weeks of decomposition in tallow treatments. These sudden changes in pH are correlated with the sudden changes in dissolved oxygen. This suggests that there may be a relationship between dissolved oxygen and pH that are not entirely understood. Additionally, the highly alkaline pH could have contributed to decreases in microbial diversity and activity at these extreme alkaline conditions. Results presented later in this study demonstrate that the sixteen week time period did have different microbial communities present. Whether the cause was alkaline conditions is a matter of debate.

**Turbidity.** Turbidity was significantly higher in tallow treatments than in the control and native litter treatments after 48 hours in water (Table 6). Tallow litter produced a mean turbidity value of 7.60 NTU, while the oak, pine and control treatments produced mean turbidity values of 5.54, 4.90, and 4.82 NTU, respectively. Mean turbidity after the 48 hour period increased in all treatments through the end of the 16 week experiment. Tallow treatments had the greatest effect on turbidity, causing NTU units to rise in week 9 and 10, then again in week 15. Additionally, mean turbidity results in tallow treatments were
significantly different at the 16 week period (Table 7). By the end of the 16 weeks, tallow treatments had produced a mean turbidity of 38.5 NTU, while the control treatment means had an NTU value of 14.1. Both pine and oak litter treatments had an NTU value of 21 at the end of the 16 week experiment. Graphical results of turbidity trends based on leaf type are shown in Figure 3.

![Graphical results of turbidity trends based on leaf type](image)

**Figure 5.** Mean turbidity (NTU) trend over the 16 week experiment from Chinese tallow, water oak, loblolly pine, and control treatments.

Higher turbidity produced by decomposing Chinese tallow litter may have an effect on phototrophic microbes. Wetlands and other water bodies have a photic zone in which can vary in depth based on turbidity (Horne et al., 1994). Higher turbidity can reduce the photic zone and alter primary productivity in the
Vertical patterns of bacterial distribution may be disrupted by waters having alterations in this photic zone, causing the natural balance of respiration and photosynthesis to be altered, thus changing microbial driven processes in areas in which higher turbidity affects light penetration (Horne et al., 1994). With increases in turbidity found in tallow treatments, it is suggested that tallow trees in and around wetlands can create potential for increasing turbidity in these aquatic habitats, therefore, potentially altering the photic zone and primary productivity of the limnetic zones of the wetland habitat.

**Oxidation Reduction Potential.** All treatments caused oxidation reduction potential (ORP) to decline after the 48 hour period. Initial readings were for the control, pine, and oak treatments were recorded at 83 to 89 mv. Tallow treatments had a different initial value of 55 mv. After the 48 hour period, ORP dropped to a range of 20 to 31 mv. These results were not significantly different among treatments at the 48 hour period (Table 6). Oxidation reduction potential (ORP) varied throughout the rest of the experiment after the 48 hour period, but the variance trend was similar in all treatments. By the end of the 16 week experiment tallow treatments did produce lower mean ORP values than the control and native litter treatments, with negative values. Although lower than the other treatments, these differences are were not significant (Table 7). Graphical results of oxidation reduction potential trends based on leaf type are shown in Figure 4.
These lower OPR values may indicate that electron acceptors other than oxygen were used in tallow treatments. Although we did not find anoxic conditions at these later time periods, the lower ORP suggests that the conditions created by tallow litter may favor anaerobic respiration over aerobic respiration, at least in the short term.

Changes in oxidation reduction potential can have effects on the solubility of metals, as the oxidation state of many metals and some nutrients are affected by redox potential (Lampert and Sommer, 1997). Chinese tallow in dense and developed monocultures may influence the solubility of metals found in native soils and wetlands. It may also influence the microbial communities found. Bacteria are the main organisms effecting oxidation reduction reactions; perhaps those populations will see the greatest change when tallow litter is present. Results on microbial community composition may provide insight on these possibilities.

Salinity and Specific Conductance. Salinity and specific conductance trends in all treatments were the same, displaying an overall declining trend during the course of the 16 week experiment. After the initial period salinity and specific conductance did increase slightly in all treatments, but after the 48 hour period both salinity and specific conductance decreased until the end of the 16 week time period. There were no significant differences among treatments at the
48 hour or 16 week time period for salinity or specific conductance. Salinity values were initially 0.17 PSS and diminished to 0.02 PSS.

Figure 6. Mean oxidation reduction potential (mv) over the 16 week mesocosm experiment from Chinese tallow, water oak, loblolly pine, and control treatments.

Specific conductance values were initially 0.37 in all treatments, and diminished to a range of 0.010 to 0.015, with oak having the highest specific conductance, followed by tallow, pine, then the control. Graphical results of salinity and specific conductance trends based on leaf type are shown in Figures 5, and 6, respectively.

The initial salinity and specific conductance of the mesocosms most likely reflected the natural chemical composition of the well water used in this experiment. The declining trends of salinity and specific conductance found over
the 16 week experiment may indicate resource decline through microbial assimilation. The dilution of salinity in these mesocosms may have also been caused by the various rain events that occurred over the 16 week experiment.

This experiment used the same amount of each litter by weight to determine differences in the chemical alteration of water. The chemical composition of natural waters is regulated by processes of rainfall, erosion, and sedimentation. Daily, seasonal and long-term cycles of the major elements are also influenced by the biological components of the watershed, stream, and wetland waters.

In natural wetland systems the most important structural and chemical resource is the vegetation. Plant litter in particular is a major source of chemical nutrients for a wetland system. In Chinese tallow infested wetlands, the amount of litter produced by each species would not be equal. Areas of higher Chinese tallow density would produce higher amounts of tallow specific litter. However, Chinese tallow tree invasions may not have a considerable impact on salinity and specific conductance of the wetland when compared to native plant species.
Figure 7. Mean salinity (PSS) trend over the 16 week mesocosm experiment from Chinese tallow, water oak, loblolly pine, and control treatments.

Figure 8. Mean specific conductance (SpC) trend over the 16 week mesocosm experiment from Chinese tallow, water oak, loblolly pine, and control treatments.
Litter Quality Analysis Results

The litter quality analysis showed significant differences in a variety of elements among leaf litter types. Significant differences were found in mean carbon, calcium, boron, and copper (Table 4). Mean carbon content was lowest in tallow litter, followed by oak litter, and highest in pine litter. Mean calcium content was highest in tallow litter, with two and three times more calcium than oak and pine litter, respectively. Boron content in tallow litter was also much higher than in oak or pine litter. All other elements in tallow litter were found to be statistically different than either pine or oak litter, but not both.

Overall, tallow litter was more similar to oak litter than pine litter. Tallow and oak species contained a higher nitrogen concentration and was statistically different from pine litter, but not from each other. Potassium content in tallow litter was also more like that of oak litter than pine. Other differences include zinc and manganese, which was highest in oak litter. Aluminum and sodium was highest in pine litter. Molybdenum was below the detection limit in all of the three litter species. All other elements in tallow litter were statistically different than either pine or oak litter, but not both.

The Chinese tallow litter quality results were also consistent with Cameron and Spencer, (1989), where they found that the rank of total nutrients from tallow litter was Ca > N > K > Mg. Oak litter also contained the same ranking for those specific elements, but not the pine litter. Pine litter contained more nitrogen than
calcium, while the rest of the ranking of elements followed tallow and oak litter trends.

Table 4. Results and summary of One-way ANOVA and post-hoc Tukey-Kramer test comparing the means from the litter quality (chemical composition) comparison of Chinese tallow, water oak, and loblolly pine litter, in mg/kg.

<table>
<thead>
<tr>
<th>Element</th>
<th>Tallow</th>
<th>Oak</th>
<th>Pine</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>3.07E+03</td>
<td>4.50</td>
<td>0.0299</td>
</tr>
<tr>
<td>Ca</td>
<td>1.01E+04</td>
<td>4.50</td>
<td>0.0299</td>
</tr>
<tr>
<td>N</td>
<td>3.81E+03</td>
<td>0.16</td>
<td>0.8506</td>
</tr>
<tr>
<td>K</td>
<td>3.25E+03</td>
<td>0.16</td>
<td>0.8506</td>
</tr>
<tr>
<td>Mg</td>
<td>1.01E+04</td>
<td>4.50</td>
<td>0.0299</td>
</tr>
<tr>
<td>P</td>
<td>1.01E+04</td>
<td>4.50</td>
<td>0.0299</td>
</tr>
<tr>
<td>S</td>
<td>1.01E+04</td>
<td>4.50</td>
<td>0.0299</td>
</tr>
<tr>
<td>Mn</td>
<td>1.01E+04</td>
<td>4.50</td>
<td>0.0299</td>
</tr>
<tr>
<td>Fe</td>
<td>1.01E+04</td>
<td>4.50</td>
<td>0.0299</td>
</tr>
<tr>
<td>Na</td>
<td>1.01E+04</td>
<td>4.50</td>
<td>0.0299</td>
</tr>
<tr>
<td>Al</td>
<td>1.01E+04</td>
<td>4.50</td>
<td>0.0299</td>
</tr>
<tr>
<td>B</td>
<td>1.01E+04</td>
<td>4.50</td>
<td>0.0299</td>
</tr>
<tr>
<td>Zn</td>
<td>1.01E+04</td>
<td>4.50</td>
<td>0.0299</td>
</tr>
<tr>
<td>As</td>
<td>1.01E+04</td>
<td>4.50</td>
<td>0.0299</td>
</tr>
<tr>
<td>Mo</td>
<td>1.01E+04</td>
<td>4.50</td>
<td>0.0299</td>
</tr>
</tbody>
</table>

These results show that the inorganic chemical composition (litter quality) of Chinese tallow litter are different from the inorganic chemical composition of water oak and loblolly pine species. The ratio of carbon to nitrogen was found to be 46:1 in tallow, 42:1 in oak, and 72:1 in pine litter. Lower carbon to nitrogen
ratios have been found to have to promote faster microbial growth with 20-30:1 being optimal (Barton and Northrup, 2011). Tallow litter was thought to have a much lower C:N ratio than what was found in this study. Many of the leaves collected had been weathered by heavy precipitation events before and during the collection phase. Some of the leaves used had to be carefully separated and dried individually. Noticeable leachate was visible during this process. With this noted, it is possible that some of this leachate could have been soluble nitrogen, which have an effect on the overall carbon to nitrogen ratio found in tallow litter.

These differences in litter quality (chemical composition) are important to understand as tallow continues to encroach upon wetland habitat, eventually changing canopy composition and releasing a source of litter that is different from native species. Past studies have shown that initial leaf litter quality can have an effect on the community composition and relative abundance of microbial communities (Meler and Bowman, 2008; Stoler and Reyea, 2015). Furthermore, shifts in relative abundance and composition of microbial communities can have an effect on ecosystem function (McGuire and Treseder, 2010).

**Soluble Extractable Element Analysis Results**

Significant differences in soluble extractable elements were found among litter species (Table 5). Chinese tallow litter leachate contained detectable concentrations of twelve of the 14 elements for a total of 11.80mg/L of soluble extractable elements after a 48 hour period. Oak litter leachate contained
detectable concentrations of nine of the 14 elements for a total of 5.77mg/L, while pine litter leachate was found to contain only five detectable concentrations of the 14 elements after 48 hours of being submerged in water, for a total of 0.86mg/L.

Significant differences were found in mean potassium, sodium, phosphorous, boron, zinc, and copper. Tallow litter had the highest amount of all significantly different elements, followed by oak, then pine. Soluble organic carbon and total nitrogen were the only two elements that were not determined.

Potassium was found to be the most abundant element in all litter leachate, also making up the majority of what was leached out of pine needles (0.81mg/L). Of the 11.80mg/L of soluble extractable elements in tallow leachate, 8.52mg/L of this was potassium. Sodium and phosphorous was also found to be relatively abundant in Chinese tallow litter leachate, with average concentrations of 1.58mg/L and 1.09mg/L detected, respectfully. All other elements found in tallow leachate were found to be below 1mg/L. Calcium and magnesium were not detectable in any of the litter leachate samples. Perhaps it takes much longer for calcium and magnesium to be degraded into its elemental form, as these elements are essential structural components of the cell wall and the chlorophyll molecule, respectively (Barbour, 1987).
Table 5. Results and summary of One-way ANOVA and post-hoc Tukey-Kramer test comparing the means from the water soluble element found in Chinese tallow, water oak, and loblolly pine litter, in mg/L.

<table>
<thead>
<tr>
<th>Element</th>
<th>One-Way ANOVA Results</th>
<th>-----------mg/L----------</th>
</tr>
</thead>
<tbody>
<tr>
<td>** K</td>
<td>30 2, 27 0.867 171.70 &lt; 0.0001</td>
<td>8.522 a 4.953 b 0.813 c</td>
</tr>
<tr>
<td>** Na2</td>
<td>30 2, 27 0.745 10.00 0.0006</td>
<td>1.580 a 0.189 b 0.000 b</td>
</tr>
<tr>
<td>** P</td>
<td>30 2, 27 0.008 387.32 &lt; 0.0001</td>
<td>1.099 a 0.334 b 0.041 c</td>
</tr>
<tr>
<td>** Mn</td>
<td>30 2, 27 0.001 196.16 &lt; 0.0001</td>
<td>0.295 a 0.147 b 0.007 c</td>
</tr>
<tr>
<td>S</td>
<td>30 2, 27 0.052 0.88 0.4250</td>
<td>0.135 a 0.061 a 0.000 a</td>
</tr>
<tr>
<td>Al</td>
<td>30 2, 27 0.019 0.74 0.4879</td>
<td>0.069 a 0.059 a 0.000 a</td>
</tr>
<tr>
<td>** B</td>
<td>30 2, 27 0.0004 37.54 &lt; 0.0001</td>
<td>0.072 a 0.017 b 0.003 b</td>
</tr>
<tr>
<td>Mo</td>
<td>30 2, 27 0.0004 1.69 0.2030</td>
<td>0.016 a 0.009 a 0.000 a</td>
</tr>
<tr>
<td>** Zn</td>
<td>30 2, 27 9.73E-06 15.61 &lt; 0.0001</td>
<td>0.007 a 0.001 b 0.000 b</td>
</tr>
<tr>
<td>Fe</td>
<td>30 2, 27 4.80E-05 1.00 0.3811</td>
<td>0.004 a 0.000 a 0.000 a</td>
</tr>
<tr>
<td>** Cu</td>
<td>30 2, 27 4.47E-06 15.16 &lt; 0.0001</td>
<td>0.005 a 0.000 b 0.000 b</td>
</tr>
<tr>
<td>As</td>
<td>30 2, 27 1.73E-07 1.08 0.3550</td>
<td>0.001 a 0.0006 a 0.0005 a</td>
</tr>
<tr>
<td>Ca</td>
<td>30 - - - -</td>
<td>ND ND ND</td>
</tr>
<tr>
<td>Mg</td>
<td>30 - - - -</td>
<td>ND ND ND</td>
</tr>
<tr>
<td>** Total</td>
<td>30 - - - -</td>
<td>11.8 5.8 0.9</td>
</tr>
</tbody>
</table>

Note: Means in the same row with the same letter are not significantly different. ND = Non detect; " * " = Tallow found to be significantly different from at least one other treatment; " ** " = Tallow found to be significantly different from both other treatments.

These results suggest that after 48 hours in water, Chinese tallow litter can leach more soluble extractable elements from senesced leaf litter than native species. The increased amount of nutrient resources made available by Chinese tallow litter may be a contributing factor that is promoting the growth of microorganisms, leading to decreases in dissolved oxygen through higher biological oxygen demands via respiration, and possibly diminishing dissolved oxygen into a limiting resource when macro and micronutrients become available.
from Chinese tallow litter. Potassium, phosphorous and manganese are mineral nutrients that can promote microbial growth.

The highly soluble components of Chinese tallow leaves seems to stimulate microbial activity, possibly promoting microbial community composition changes in native wetland systems. Although water samples were not analyzed for soluble organic carbon or nitrogen, the observed yellowish-brown color (Muncell value range of 10YR 5/8 to 10YR 6/8) of Chinese tallow litter leachate in Figure 7 suggests that there was soluble carbon in solution. The metabolic use of this possible carbon source may also contribute to the growth and activity of microorganisms when tallow litter is present in water. Further research is recommended to determine possible soluble carbon and nitrogen compounds that may exist in tallow leachate water.

Figure 9. Image of Chinese tallow\(^1\), water oak\(^2\), and loblolly pine\(^3\) litter leachate samples after 48 hours of decomposition in aged-tap water. Muncell value range of Chinese tallow litter leachate; 10YR 5/8 to 10YR 6/8.
Chinese tallow grows well in and around wetlands and riparian areas (Zedler and Kercher, 2004). Monoculture stand formation of Chinese tallow trees in native habitat may change nutrient cycling rates and alter primary productivity. Monoculture stands can produce tallow leaf litter concentrations of 1.27g of leaf litter/L of water, and is suggested to be much higher in dense monoculture stands that reside in the wetland habitat (Adams and Saenz, 2012). Higher concentrations of mineral nutrients in an ecosystem may have effects on microbial composition and species diversity, possibly causing dissolved oxygen to become a limiting resource in the wetland environment, much like the recognized problem of anthropogenic eutrophication in water bodies.

**Litter Decomposition Comparison**

Throughout the 16 week experiment, rising water levels in the wetland made it difficult to find every sample that was deployed. In the latter weeks of the decomposition experiment, not all of the litter bags were retrieved, leaving 26 samples to work with in week 8 and 16. There were significant differences in decomposition rates (measured as mass loss) among litter species for every week (Table 6) Chinese tallow litter decomposed at a significantly faster rate than both oak and pine litter, losing 2-3 times more mass than both native litter species every week. After one week of decomposition tallow litter had lost an average of 29.8% of the total 5g that was placed in the wetland, while oak and pine species had only lost 13.1% and 5.6%, respectively.
Throughout weeks 2, 4, and 8 of decomposition, Chinese tallow litter continued to lose significantly more mass than both oak and pine litter. By the end of the 16 week experiment Chinese tallow had lost 72% of the original 5 grams that was placed in the wetland, while oak and pine litter had only lost 19.8%, and 14.2%, respectively.

Table 6. Results and summary of One-way ANOVA and post-hoc Tukey-Kramer test comparing the means from the decomposition experiment of Chinese tallow, water oak, and loblolly pine litter, at each week, recorded as mean mass loss in grams.

<table>
<thead>
<tr>
<th>Week</th>
<th>One-Way ANOVA</th>
<th>Mean Mass Loss (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>DF</td>
</tr>
<tr>
<td>**1</td>
<td>30</td>
<td>2, 27</td>
</tr>
<tr>
<td>**2</td>
<td>30</td>
<td>2, 27</td>
</tr>
<tr>
<td>**4</td>
<td>30</td>
<td>2, 27</td>
</tr>
<tr>
<td>**8</td>
<td>26</td>
<td>2, 23</td>
</tr>
<tr>
<td>**16</td>
<td>26</td>
<td>2, 23</td>
</tr>
</tbody>
</table>

Note: Means with the same letter in a row are not significantly different. Chinese tallow mean is significantly different from 1"***" and 2"****" other treatments.

The decomposition comparison results demonstrate that Chinese tallow litter decomposes at a faster rate than the native species of water oak and pine litter. Other research suggests that initial litter quality, (lignin content, C:N ratio, and P, K, Ca, and Mg concentrations) may interactively influence litter decomposition processes, with litter quality being the most important and direct regulator of litter decomposition at the global scale (Gholz et al., 2000; Zhang et al., 2008). The litter quality (chemical composition) of Chinese tallow litter was
found to be significantly different. Differences in chemical composition from that of native litter may have an effect on rate at which Chinese tallow decomposes.

Higher lignin to nitrogen ratios, or higher carbon to nitrogen ratios in plant litter have been linked to slower degradation rates (Zang et al., 2008). Although this study did not determine lignin content, past research has shown that the ratio of lignin to nitrogen in Chinese tallow litter is relatively lower than native temperate deciduous tree leaves (Cameron and Spencer, 1989). Furthermore, it is documented that lignin content is highest in coniferous needles, followed by broadleaf species litter (Rahman et al., 2013). The faster decomposition rates of Chinese tallow litter suggest that this litter type may have a lower lignin content contributing to faster turnover rates than the native species of oak and pine litter in the wetland habitat.

Fungi and bacteria play a major role in the decomposition of organic matter. Specific enzymes that are produced by different microorganisms are required to decompose plant structural material. In the case of lignin degradation fungi contain the necessary enzymes, rather than bacteria, to degrade the lignin component of plant litter. The lack of, or relatively little lignin content in Chinese tallow litter suggests that microbial community composition that are fostered during the decomposition phase may be different from oak and pine litter since lignin content is higher in these native species.
Studies have shown that substances released by leaf litter during the decomposition process can have an impact on microbial community composition and subsequently influence other functions within the ecosystem (Waldrop and Firestone, 2004). These substances are largely dependent on litter species type (Stoler and Relyea, 2011). Additionally, the total litter produced and the rate at which it is decomposed can have an effect on nutrient cycling rates in the ecosystem in which those litter types exist, exhibiting differences in biogeochemical cycling rates such as carbon, nitrogen and sulfur cycles. Overall ecosystem response from these possible changes is unknown, but may affect vegetation and animal diversity in the wetland and riparian habitat where Chinese tallow is encroaching.

While decomposition is a naturally occurring process, to heterotrophic microorganism, it is key to their existence. Bacteria and fungi are important in the decomposition of biological material and without these organisms there would be an accumulation of dead plants in every habitat that has terrestrial vegetation present. Differences in litter quality that lead to differences in decomposition rate, such as in the Chinese tallow leaf litter, may have an effect on microbial community composition. Possible changes in microbial community composition may have effects on ecosystem function and processes that are important to native ecosystems. Many chemical processes are mediated by specific microbial species.
The decomposition process itself is an ecosystem process, regulated by specific heterotrophic microorganisms. The knowledge of turnover rates of plant material and organic carbon are a prerequisite for understanding availability and cycling of nutrients such as carbon, nitrogen, sulfur, and phosphorous (Paul, 2015), which can influence which microbe species are present, and the functions they carry out.

**Sterilization Test Results**

Dissolved oxygen concentrations were significantly different among sterile and non-sterile tallow treatments (Table 7). Mean dissolved oxygen in the control treatment was found to be 8.69mg/L, while the tallow litter treatment results were found to be significantly lower, at 1.28mg/L. Treatment 3, containing both tallow litter and formalin also had a significantly different result from the control treatment with a mean dissolved oxygen concentration of 7.87mg/L. Mean dissolved oxygen results in Treatment 4 (formalin only) showed similar results to the control, at 8.61mg/L, and were not significantly different.

These results suggest that dissolved oxygen is affected by microbial activity. The control treatments (Treatment 1) containing no tallow litter had no effect on dissolved oxygen. Additionally, the similar results from the treatments containing formalin only (Treatment 4) show that formalin also had no effect on dissolved oxygen. In the tallow treatments without formalin (Treatment 2), dissolved oxygen was clearly reduced to near hypoxic levels. The addition of
formalin as a sterilization agent in Treatment 3, containing both tallow litter and formalin, showed that when formalin was added, the activity of microbes (most likely respiration) was hindered, also significantly reducing the amount of dissolved oxygen that was consumed in these treatments.

Although dissolved oxygen results in Treatment 3 did showed significant differences from the control treatment, the amount of dissolved oxygen remained close to values of the control treatment. It is possible that the formalin was not 100 percent effective at killing all of the microbes present. It may also suggest that the chemical composition (secondary chemicals) in tallow litter may also have an influence on dissolved oxygen. Treatment 2 and treatment 3 both produced a solution that was brown in color (identical to the color in Figure 7). The leachate from Chinese tallow litter may be the source of needed nutrients, carbon, tannins and other phenolic compounds that influence the activity of microbes when tallow litter is present in water. Results from the soluble extractable elements experiment described earlier in this section showed that tallow litter does have significant differences in leached substances compared to native species.

Means for pH were also found to be significantly different among treatments (Table 7). The control treatment produced a mean pH of 7.80, while the litter only treatment produced a statistically different mean pH of 5.38. The litter + formalin treatment (Treatment 3) produced similar results as the litter only
treatment (Treatment 2), with mean pH of 5.37. Treatment 4, the formalin in aged tap water treatment, produced results similar to the control treatment with a measured mean pH of 7.77.

Table 7. Results and summary of One-way ANOVA and post-hoc Tukey-Kramer test comparing the means from the sterilization experiment, testing treatment influence on dissolved oxygen and pH in a bench top lab study, using 30 X30cm units, and formalin (15ml of 10%) as the sterilization agent.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>One- Way ANOVA</th>
<th>Treatment Means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>DF</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>40</td>
<td>3, 36</td>
</tr>
<tr>
<td>pH</td>
<td>40</td>
<td>3, 36</td>
</tr>
</tbody>
</table>

Note: Means in a row with the same letter are not significantly different at the 0.05 level.

The pH results suggest that secondary chemicals and leachate substances, rather than microbial respiration are significantly affecting pH when Chinese tallow litter is present. The possibility of carbonic acid formation from respiratory carbon dioxide may also have an influence on the pH results in Treatment 3, but not likely since the pH results in Treatment 2 and 3 are the same. This further suggests that other chemicals in Chinese tallow litter have an effect on pH. Both the control treatments (Treatment 1) and the formalin only treatments (Treatment 4) produced pH results that are normally found in tap water. It also shows that formalin had no effect on pH. These results were also consistent with the pH results found by Adams and Saenz (2012).
Microbial Community Analysis Results

Over 500 operational taxonomic units (OTU) of bacteria and 185 OTU of fungi were found among all treatments. Eleven of the bacterial samples were not readable due to low DNA presence, reducing bacterial DNA samples to 109. Most of these non-detects were from the control and pine litter treatments at the initial and 48 hour time periods. A total of four (one control; three pine) bacterial DNA samples were not readable in the initial time period while a total of seven (3 control; 3 pine; 1 oak) bacterial DNA samples were not readable in the 48 hour time period. All fungi samples contained readable and amplifiable DNA.

Bacteria. Four, Non-metric Multidimensional Scaling (NMDS) ordination scatter plots were generated to visualize PERMANOVA and Bray-Curtis distance matrix results from bacterial community samples. The first ordination was of all 109 bacterial samples (all three time periods), while the other three were of each time period alone. The NMDS ordination scatter plot of all time periods (Figure 8) show three distinct clusters formed from the 109 bacterial samples. These clusters are grouped by time period, which strongly suggests that bacterial community composition from treatment samples differed more among sampling time periods than among treatment within each time period. However, looking at each time period alone (Figures 9-12) it was discovered that Chinese tallow treatments differed in bacterial community composition from those of native litter treatments at the 16 week time period.
Figure 10. Non-metric Multidimensional Scaling (NMDS) ordination scatter plot of 109 bacterial samples from Chinese tallow, water oak, loblolly pine, and control treatments and the relative aquatic chemistry parameters found at the initial, 48 hour and 16 week time periods.
The NMDS of all bacterial samples (Figure 8) also shows the relative aquatic chemistry parameters at each time period. Each measure aquatic chemical parameter is defined by a blue line and is correlated with the strength of the relationship by the length and direction of the line. For example, the initial period can be characterize by having a relatively high mean dissolved oxygen concentration, high mean oxidation reduction potential, and high salinity. Perhaps the differences in aquatic chemistry (dissolved oxygen, pH, and turbidity) found at each time period were the driving forces that caused major differences in bacterial community composition seen among each of these time periods. What is interesting is the fact that the bacterial community composition in almost all of the control treatment samples at the 48 hour period did not change from the initial time period despite changes in aquatic chemistry.

This demonstrates that leaf litter, regardless of litter species type, caused a change in bacterial community composition at the 48 hour period from those that were found at the initial time period. These changes in bacterial community composition were similar among litter treatments when looking at all samples together. Bacterial community composition from samples that came from the control treatments at the 16 week period eventually changed into communities that were very much like the rest of the litter treatments at the 16 week time period, when looking at all of the samples at the same time.
However, looking within each time period alone tells a more detailed story of how leaf litter affects bacterial community composition. Although Shannon-Weiner Indices reveal that there was no difference in bacterial richness, evenness, or diversity at the initial time period, among treatments (Table 8), the Non-metric Multidimensional Scaling (NMDS) ordination scatter plot of the initial time period (Figure 9) shows the 36 treatment samples clustered into two groups.

Table 8. Results and summary of One-Way ANOVA and post-hoc Tukey-Kramer test comparing Chinese tallow, water oak, loblolly pine and control treatment means of the Shannon-Wiener Diversity Index values of bacteria at the initial time period.

<table>
<thead>
<tr>
<th>Shannon-Wiener Index</th>
<th>One-Way ANOVA Results</th>
<th>Treatment Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>DF</td>
</tr>
<tr>
<td>Richness (S)</td>
<td>36</td>
<td>3, 32</td>
</tr>
<tr>
<td>Evenness (E)</td>
<td>36</td>
<td>3, 32</td>
</tr>
<tr>
<td>Diversity (H)</td>
<td>36</td>
<td>3, 32</td>
</tr>
</tbody>
</table>

Note: Means with the same letter in a row are not significantly different

These two clusters in Figure 9 reveal that there are differences in bacterial community composition among the 36 samples. The two clusters shown in the NMDS scatter plot (Figure 9) suggests that differences in microbial community composition in each sample are not from litter type, but are from unexplained variables that are difficult to determine. The samples of microbial community composition are not grouped in any kind of treatment arrangement. They are arranged in two loose groupings in an almost random nature.
Figure 11. Non-metric Multidimensional Scaling (NMDS) ordination scatter plot of Chinese tallow, water oak, loblolly pine and control treatment samples at the initial time period, \((n=36; C = 9, O = 10, P = 7, T = 10)\) \((p = 0.8125; r^2 = 0.0381)\). Each sample represents is own bacterial community. Groups of samples are based on bacterial community similarities \((\alpha = 0.05)\) at the phylogenetic level.
No differences in aquatic chemistry among treatments at the initial time period were observed (see Table 1). This suggests that the 36 bacterial samples that form two distinct clusters found at the initial period could possibly be explained by potential differences in geological substrate from the groundwater source. The extraction of the groundwater that was used for the mesocosms took a few hours to extract. Geological substrate differences within the aquifer are possible and could have contributed to differences in bacterial community composition found at this time period. Outliers of microbial community composition were also found in a few samples (1 control, 1 tallow, 2 pine) at this time period.

The clustering of these samples indicates that the bacterial community composition of these samples fall into two distinct communities. The cluster of samples on the left side of the NMDS scatter plot, in Figure 9, are dominated by *Deltaproteobacteria*, while the second cluster of samples that are grouped together on the right of the NMDS scatter plot are dominated by unclassified taxa. Many other taxa also contributed to differences in bacterial community composition within these two clusters of samples. Bacterial communities from samples that are dominated by the *Deltaproteobacteria* also contained taxa that were not found in the second cluster of samples. These bacterial taxa include *Lactobacillus, Gamaproteobacteria, Pedobacter, Escherichia coli, Roseococcus, Acidobacterium, Aquabacterium, Chloroflexi*, and many more (see Figure9).
Bacterial communities from samples dominated by the unclassified taxa also contained many taxa that were not found in the prior described cluster of samples. These include; *Clostridia, Desulfovibria, Betaproteobacteria, Burkholderia, Hymenobacter, Oxalobacteraceae, Citrobacter, Novosphingobacter,* and many more. There was one control sample in the initial time period that contained *Ralstonia sp.* A few pine and tallow treatment sample outliers produced bacterial communities not like the rest of those found within the loose cluster. These pine and Chinese tallow sample outliers included *Spirochaetia, Firmicutes, Corynebacterium, Geobacter, Chryseobacterium, Streptococcus sp, Propionibacteria,* while the control outlier contained *Ralstonia, Desulfobacca, Rosomanas,* and *Actinomyces.*

Many of the taxa that were found within the samples of the initial time period were facultative and obligate anaerobes. Groundwater is likely to contain low dissolved oxygen and low concentrations of organic carbon sources. This anaerobic environment would promote the existence of these anaerobic taxa like those found at the initial period (*Desulfovibria, Lactobacillus, Clostridia,* and *Citrobacter*). Another alternative is that the source of the bacteria found at the initial sampling time period may be from the leaves themselves. Culture based studies have demonstrated that bacteria are the most numerous and diverse colonists of leaves (Whipps et al., 2008). Furthermore, analysis of 16s rDNA cloned directly from leaf samples has demonstrated that Proteobacteria are the
most dominate group found on leaves, (Whipps et al., 2008). Proteobacteria were found in all samples at the initial sampling time period in this study, suggesting that the source of bacteria found may be from both the leaves and groundwater.

Bacterial community sampling at the 48 hour time period when Chinese tallow treatments caused dissolved oxygen to drop to near hypoxic levels shows that despite differences in mean dissolved oxygen among treatments, there were no differences in bacterial diversity among treatments (Table 9). Differences in pH and turbidity seen among treatments also did not seem to have an effect on bacterial diversity. However, bacterial diversity, including richness and evenness, declined in all treatments from the initial sampling period. Although no differences in bacterial diversity were observed at the 48 hour period, the NMDS scatter plot of the 48 hour period (Figure 10) suggests that litter treatments were beginning to have an influence on composition of bacterial communities after 48 hours of decomposition when compared to control treatments.

Table 9. Results and summary of One-Way ANOVA and post-hoc Tukey-Kramer test comparing Chinese tallow, water oak, loblolly pine and control treatment means of the Shannon-Wiener Diversity Index values of bacteria at the 48 hour time period.

<table>
<thead>
<tr>
<th>Shannon-Wiener Index</th>
<th>One-Way ANOVA Results</th>
<th>Treatment Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>DF</td>
</tr>
<tr>
<td>Richness (S)</td>
<td>33</td>
<td>3,29</td>
</tr>
<tr>
<td>Evenness (E)</td>
<td>33</td>
<td>3,29</td>
</tr>
<tr>
<td>Diversity (H)</td>
<td>33</td>
<td>3,29</td>
</tr>
</tbody>
</table>

Note: Means with the same letter in a row are not significantly different.
Figure 12. Non-metric Multidimensional Scaling (NMDS) ordination scatter plot of Chinese tallow, water oak, loblolly pine, and control treatment samples at the 48 hour time period (n=33; C = 7, O = 9, P = 7, T = 10) (p = 0.001; r² = 0.4237). Each sample represents is own bacterial community. Groups of samples are based on bacterial community similarities (α = 0.05) at the phylogenetic level.
Not only did the bacterial communities change from the initial period in litter treatments, all litter treatments clearly show differences in microbial community composition from that of control treatment samples. The NMDS ordination scatter plot (Figure 10) results shows that all litter treatments produced similar bacterial communities with similar composition at the 48 hour time period. The bacterial community composition of two of the control treatment outliers were similar to both oak and tallow treatments, while the other five control treatment samples did not change.

These five samples looked more similar (based on community composition) to the initial time period with *Deltaproteobacteria*, *Betaproteobacteria*, and *Burholderiales* dominating the community with a relative abundance of these two taxa ranging from 20 to 72%, while *Desulfobacterales*, *Chloroflexi*, *Citrobacter*, *Geobacter*, *Pelomonas* and *Bradyrhizobium sp* and many more made up the rest of the bacterial community with low relative abundance. This may suggest that nutrients from all litter types, (despite differences in litter quality and water soluble elements are contributing to the composition of the bacterial communities found at this time period.

Chinese tallow, pine and oak litter treatments had a similar bacterial community composition, with tallow having more similarities with oak litter treatments. These bacterial communities were dominated by *Oxalobacterase*, *Hymenobacter sp*, and *Shingomonas*. These three taxa represented 30 to 60%
of the taxa found in these bacterial communities of these samples. All other taxa found in the tallow, oak, and pine litter treatments represented less than 10% of the bacterial community and were made up of many common and rare taxa. These included *Alphaproteobacteria*, *Roseococcus*, *Massilia sp*, *Legionella*, *Acetobacter*, and *Chryseobacterium*. Chinese tallow and oak treatment samples also contained a few communities that had *Desulfocurvus sp*, *Pedobacter sp*, *Aquabacterium sp*., and *Novosphingobium sp* present.

Bacterial community sampling from the 16 week time period indicates that differences in bacterial diversity among treatments were only seen in the oak litter treatment samples (Table 10). However, an important note to discuss is the fact that bacterial diversity recovered from the 48 hour period. Mean dissolved oxygen also recovered from relatively low concentrations seen at the 48 hour period. Increases in dissolved oxygen may have contributed to the increase in bacterial diversity seen in all litter treatments at the 16 week time period.

Table 10. Results and summary of One-Way ANOVA and post-hoc Tukey-Kramer test comparing Chinese tallow, water oak, loblolly pine and control treatment means of the Shannon-Wiener Diversity Index values of bacteria at the 16 week time period.

<table>
<thead>
<tr>
<th>Shannon-Wiener Index</th>
<th>One-Way ANOVA Results</th>
<th>Treatment Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>DF</td>
</tr>
<tr>
<td>Richness (S)</td>
<td>40</td>
<td>3, 36</td>
</tr>
<tr>
<td>Evenness (E)</td>
<td>40</td>
<td>3, 36</td>
</tr>
<tr>
<td>Diversity (H)</td>
<td>40</td>
<td>3, 36</td>
</tr>
</tbody>
</table>

Note: Means with the same letter in a row are not significantly different.
Overall temperature increases are another possible explanation of high bacterial diversity found at the 16 week sampling period. Aquatic pH at this time period was very alkaline, suggesting that high alkaline levels do not affect bacterial diversity. The NMDS ordination scatter plot of the 16 week time period (Figure 11) reveals differences in bacterial community composition in Chinese tallow treatment samples when compared to native litter treatment samples. Chinese tallow treatments had the highest pH and turbidity among all treatments. Perhaps the higher pH and turbidity contributed to the different bacterial community composition that was found in Chinese tallow litter treatment samples at the 16 week time period.

Chinese tallow treatment samples were difference from native litter treatment samples because they contained communities that were dominated by Rosoeccoccus, Hymenobacter, Desulfuromonas sp, Novosphingobium, and Chryseobacterium sp. These taxa represented 20 to 50% of the community, while the rest of the community was composed of several low relatively abundant taxa. Perhaps secondary chemicals and other leachate substances in Chinese tallow litter may have influenced the differences found in bacterial community composition among native litter treatment samples. Secondary chemicals play an active role in herbivory defense (Marquis, 1991). The phenolic compounds found in Chinese tallow litter (Leonard, 2008) may have had an influence on aquatic bacterial community composition found in this study.
Figure 13. Nonmetric Multidimensional Scaling (NMDS) ordination scatter plot of Chinese tallow, water oak, loblolly pine, and control treatment samples of at the 16 week time period (n=40; p = 0.001, $r^2 = 0.3333$). Each sample represents its own bacterial community. Groups of samples are based on bacterial community similarities ($\alpha = 0.05$) at the phylogenetic level.
The control, oak, and pine litter treatment samples were dominated by *Deltaproteobacterium, Alphaproteobacterium, Betaproteobacterium, Candidatus haliscome* and *Synechoccus sp*. These taxa made up 10 to 20% of the bacterial community in all control, oak, and pine litter treatment samples. The other 80 to 90% percent of taxa in these communities were of composed of many, low abundant taxa. Many of these samples had communities that also contained “No Hit” or unclassified taxa.

The results from the 16 week time period show that Chinese tallow litter can influence changes in bacterial community composition after 16 weeks of decomposition. Although these bacterial community composition alterations are yet to be linked with functional changes within the ecosystem, it is a possibility. Bacteria in particular are responsible for many functions within an ecosystem including nitrification, denitrification, and methanogenesis; iron, manganese and sulfate reduction/oxidation, the decomposition of organic matter, and the global carbon cycle. Many of these biogeochemical processes are species specific. Therefore, changes in bacterial community composition from a changing litter type may have an impact on native wetland functions and ecosystem processes.

Alterations in ecosystem function though changing microbial communities is well documented (Meler and Bowman, 2008; McGuire and Treseder, 2009; Stoler and Relyea, 2015). Chinese tallow treatments were all absent in the *Firmicutes, Proteobacteria, Chloroflexi*, and *Cyanobacteria* phyla. All of these taxa
are phototrophic, with many of their species also capable of nitrogen fixation (Barton Northup, 2011). Perhaps the high turbidity produced by tallow litter had an influence on phototrophic bacteria by reducing light penetration in the water column. The absence of bacterial species capable of nitrogen fixation in Chinese tallow treatments is noteworthy. Based on these results it is possible that the leaf litter from the invasive Chinese tallow can change bacterial community composition in wetland habitat where infestation is prevalent.

Wetlands are starting to receive more attention in terms of their functional ability. The creation of constructed wetlands for improving water quality is spreading worldwide. Many of these functions rely upon bacterial processes that must be monitored and controlled. The presence of Chinese tallow in and around the wetland environment creates potential to further complicate the management of nutrients that the constructed wetland receives. Changes in bacterial community composition brought upon by Chinese tallow litter may alter the functional ability of nutrient removal mediated by specific bacterial species.

Carbon nitrogen and phosphorous are three major constituents that are cycled through wetland systems (Mitsch and Gosselink, 2007). Faster decomposition rates such as those found in Chinese tallow litter may increase these nutrient pools, leading to alterations in the trophic state of the wetland. Furthermore, nutrient enrichment has been shown to affect microbial community functional activities in wetland systems (Bressler and Paul, 2015).
Fungi. Like the results from the bacterial taxa matrix data set, four, Non-metric Multidimensional Scaling (NMDS) ordination scatter plots were generated to visualize PERMANOVA and Bray-Curtis distance matrix results from fungal community samples. The first NMDS ordination was of all 120 fungal samples (all three time periods), while the other three NMDS ordination scatter plots are of each time period alone (Figures 13-15). Results from the Non-metric Multidimensional Scaling ordination scatter plot of all time periods (Figure 12) suggests that litter treatment type had an influence on the composition of fungal communities at the initial and 48 hour sampling time periods.

The NMDS ordination scatter plot of all samples shows samples from the initial and 48 hour time periods clustered together by treatment, with Chinese tallow samples clustering together in a tight arrangement. The samples of oak and pine replicate samples from the initial and 48 hour periods also show clustering based on treatment, although the clustering is not as tight as samples from Chinese tallow samples. Large fungal community composition variation was seen in the control treatment samples from the initial and 48 hour time periods.

All treatment samples from the 16 week sampling period cluster together, suggesting that fungal communities at this time period were similar in fungal community composition in all samples from the 16 week time period, but different from those found at the initial and 48 hour time periods.
Figure 14. Non-metric Multidimensional Scaling (NMDS) ordination scatter plot of 120 fungal samples from Chinese tallow, water oak, loblolly pine and control treatments, along with the aquatic chemistry parameters that influenced those communities at the initial, 48 hour and 16 week time period.
Perhaps changes in aquatic chemistry had an influence on the composition of the fungal communities found at respective time periods. Temperature, turbidity and pH were much higher at the 16 week time period. Shannon-Weiner results of the initial period (Table 11) indicate that Chinese tallow litter treatments contained a lower fungal diversity than native litter treatments. Fungal communities from Chinese tallow litter treatments were also very uneven. Looking at each time period alone tells a more detailed story.

Results of the NMDS ordination scatter plot of fungal community composition from the initial time period (Figure13) reveals that Chinese tallow litter treatments had fungal communities that were very similar among all Chinese tallow treatment samples from the initial sampling time period. Oak litter treatment samples also had a nicely grouped arrangement of samples, suggesting that replicate samples contained very similar fungal communities in terms of fungal composition. However, larger variation was seen in the pine and control treatments replicate samples.

Table 11. Results and summary of One-Way ANOVA and post-hoc Tukey-Kramer test comparing Chinese tallow, water oak, loblolly pine and control treatment means of the Shannon-Wiener Diversity Index values for fungi at the initial time period.

<table>
<thead>
<tr>
<th>Shannon-Wiener Index</th>
<th>n</th>
<th>DF</th>
<th>MSE</th>
<th>F</th>
<th>P-Value</th>
<th>Treatment Mean</th>
<th>Tallow</th>
<th>Oak</th>
<th>Pine</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Richness (S)</td>
<td>40</td>
<td>3, 36</td>
<td>349.14</td>
<td>4.42</td>
<td>0.0096</td>
<td>52 a</td>
<td>67 b</td>
<td>76 b</td>
<td>50 a</td>
<td></td>
</tr>
<tr>
<td>Evenness (E)</td>
<td>40</td>
<td>3, 36</td>
<td>0.1833</td>
<td>14.595</td>
<td>2.22E-06</td>
<td>0.17 a</td>
<td>0.47 b</td>
<td>0.44 b</td>
<td>0.41 b</td>
<td></td>
</tr>
<tr>
<td>Diversity (H)</td>
<td>40</td>
<td>3, 36</td>
<td>0.2474</td>
<td>14.384</td>
<td>2.56E-06</td>
<td>0.69 a</td>
<td>1.97 b</td>
<td>1.92 b</td>
<td>1.59 b</td>
<td></td>
</tr>
</tbody>
</table>

Note: Means with the same letter in a row are not significantly different
Figure 15. Nonmetric Multidimensional Scaling (NMDS) ordination scatter plot of Chinese tallow, water oak, loblolly pine and control treatment samples at the initial time period (n=40; p = 0.001, $r^2 = 0.5324$). Each sample represents own fungal community. Groups of samples are based on fungal community similarities ($\alpha = 0.05$) at the phylogenetic level.
The community composition of fungi in Chinese tallow samples was dominated by a single species. The fungal species *Aureobasidium pullulans* made up 48 to 97% of each fungal community in Chinese tallow sample replicates. However, *Collectotrichum sp,* and *Hypoxylon* was also present in Chinese tallow samples. These species were not found in native or control treatments samples. All other fungal species in Chinese tallow treatments represented less than 10% of the community.

Oak litter treatments contained a community dominated by *Dicarpella sp.*, and *Gnomoniaceae* taxa while the rest of the community was made up of *Saccharomycetes pararoseus,* *Dioszegia,* and *Rhodosporidium.* One oak treatment was found to be dominated by *Epicoccum sorghi.* All of the dominate species in oak treatments represented 30% of the community, while all other species found made up the rest of the fungal community. While the community in oak litter treatment samples was more even than those found in tallow treatments, it is still quite low.

Fungal communities in the pine litter treatments at the initial time period were found to be a mix of species. A few of the fungal communities in pine replicate samples were dominated by *Aureobasidium pullulans,* while others were dominated by *Alternaria sp,* *Cladosporium cladosporiodes,* *Pleosporales,* *Fusarium,* and *Curreya* taxa. Additionally, many *Cryptococcus sp.* were found in
the fungal communities of pine treatment samples. All other fungal species represented less than 10% of the community.

The fungal community composition found in control treatments from the initial period were more variable and were characterized by *Cladosporium.cladosporioides*, *Cryptococcus mangus*, *Amphisphaeriaceae*, *Stereum.hirsutum*, *Fraxinus* and *Undeniomyces*. These species represented 30 to 60% of the community in these control treatment samples, while the rest of the community was made up of many low abundance taxa and species.

Variation in fungal community composition existed in the low abundant species that made up the rest of the fungal community in the control samples. One of the control samples was a major outlier. This control samples is located in the upper left hand corner of the NMDS. The distant from the rest of the control samples suggest that this control samples was very different from the rest of the control treatments replicate samples. This specific outlier was dominated by *Leucosporidium*. This species represented 99% of the fungal community in this sample. All other fungal species found represented less than 1% of the fungal community in this outlier.

Sampling of the initial time period indicates that variation of fungal community composition existed prior to any leaching of leaves or aquatic chemistry changes associated with leaf litter. This demonstrates that litter from specific tree species may promote a habitat that is favorable to specific fungal
species. While some research has shown that microbial communities of the phyllosphere (above ground parts of plants) are diverse, supporting numerous genera of both bacteria and fungi (Morris and Kinkel, 2002), compared to most other habitats, there has been relatively little examination of phyllosphere microbiology (Lindow and Brandl, 2003).

After 48 hours of decomposition, fungal diversity declined in all treatments except for pine litter treatments (Table 12). Chinese tallow treatments, again, had the lowest fungal diversity of all treatments. Chinese tallow litter treatments did cause dissolved oxygen to decline the most. It is reasonable to assume that the lower mean dissolved oxygen concentrations in Chinese tallow litter treatments was the cause of the diversity decline.

Table 12. Results and summary of One-Way ANOVA and post-hoc Tukey-Kramer test comparing Chinese tallow, water oak, loblolly pine and control treatment means of the Shannon-Wiener Diversity Index values for fungi at the 48 hour time period.

<table>
<thead>
<tr>
<th>Shannon-Wiener Index</th>
<th>One-Way ANOVA Results</th>
<th>Treatment Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>DF</td>
</tr>
<tr>
<td>Richness (S)</td>
<td>40</td>
<td>3, 36</td>
</tr>
<tr>
<td>Evenness (E)</td>
<td>40</td>
<td>3, 36</td>
</tr>
<tr>
<td>Diversity (H)</td>
<td>40</td>
<td>3, 36</td>
</tr>
</tbody>
</table>

Note: Means with the same letter in a row are not significantly different

Results from the 48 hour sampling time period also demonstrates that leaf litter from specific tree species can promote and foster those species found on the surface of the respective leaf types as those leaves decay. The NMDS
ordination scatter plot of the 48 hour time period (Figure 14) shows that fungal community composition is similar among respective litter treatment samples. Furthermore, Chinese tallow treatments caused differences in fungal community composition from that of native litter treatments. Chinese tallow sample replicates are grouped very tightly together in Figure 14, suggesting that there is little fungal community composition differences among the ten Chinese tallow fungal community sample replications. Oak and pine samples also cluster together in a very nice arrangement, suggesting that their respective fungal communities are very similar in composition when compared to other replicate samples from the same treatment.

What this reveals is that each leaf litter type continues to influence a specific composition of fungal communities found in the aquatic mesocosms after 48 hours of decomposition. All of the Chinese tallow treatment samples at the 48 hour period contained *Aureobasidium pullulans* as the dominate species that represented 80 to 95% of the fungal community in each sample. *Colletotrichum sp*, *Hypoxylon*, *Glomerella acutata*, and *Minimidochium sp* also represented other species found in the fungal community samples from Chinese tallow treatments that were not found in the other litter or control treatment fungal community samples. Furthermore, *Aureobasidium pullulans* was the same dominate fungal species found in Chinese tallow samples from the initial time period.
Figure 16. Non-metric Multidimensional Scaling (NMDS) ordination scatter plot of Chinese tallow, water oak, loblolly pine, and control treatment samples at the 48 hour time period (n=40; $p = 0.5857$, $r^2 = 0.0649$). Each sample represents its own fungal community. Groups of samples are based on fungal community similarities ($\alpha = 0.05$) at the phylogenic level.
All of the oak treatment samples also influence fungal communities that were similar to, but not exactly the same as that of Chinese tallow litter. Oak litter treatment samples were also grouped very tightly together indicating that oak litter treatment samples contained fungal communities that did not differ between each replicate sample. All of the oak litter treatments also contained *Aureobasidium pullulans* as the dominate species, but with a relative abundance of 50 to 75%. Differences in the rest of the community were defined by *Gnomoniaceae, Aspergillus, Diaporthales*, and *Cryptococcus aff amylolyticus*.

Pine treatment samples, again, contained a more diverse community with Microbotryomycetes (family), *Polyscytalum algarvense*, Udeniomyces, *Saccharomyces cerevisiae*, and many *Cryptococcus sp*. Although grouped together, the cluster is not as tight as Chinese tallow or oak treatments sample clustering. What this indicates it that there was more variation within the fungal composition of pine litter treatment sample replicates than in the oak or Chinese tallow treatment samples.

The control treatments had the most variable results in fungal community composition within replicate samples at the 48 hour period. The control samples in the NMDS ordination scatter plot (Figure 14) show widespread plotting of control samples. This indicates that control samples are not only different from litter samples in terms of fungal community composition, but there are also differences in fungal community composition between the replicate control
treatments samples. *Leucosporidium, Cryptococcus mangus, Cladosporium cladosporiodes, Bulleromyces albu, Acermonium strictum, and Stereum hirsutum* dominated these treatments samples with these listed species making up 30 to 88% of the fungal communities found in the control samples. Many other species were also found. Among these are *Devriesia, Dioszegia, Trametes.versicolor, Ustilago.esculenta, Peniophora, Hyalodendriella*, and *Acremonium.strictum*.

This NMDS ordination scatter plot of the 48 hour period demonstrates that leaf litter may continue to contribute to specific fungal communities that are unique in fungal community composition due to the uniqueness of each litter type. The chemical composition, soluble nutrients and lignin content may be attributed to these differences in fungal community composition that has been found among litter types at the initial and 48 hour periods. Differences in aquatic chemistry did not seem to play a role in changing fungal community composition from the initial period, but it did seem to have an effect on richness and diversity.

The fact that the same species dominate Chinese tallow litter treatment samples (*Aureobasidium pullulans*) also suggests that Chinese tallow contributions to wetland fungal species may include a monoculture type. The dominance of *Aureobasidium pullulans* found in the fungal communities of Chinese tallow treatment samples in both the initial and 48 hour period suggest that Chinese tallow litter can contribute to a different fungal community.
composition than native species litter. This illustrates that differences in litter quality and litter leachate between native and non-native plants can contribute to different fungal community composition.

The reduced diversity of fungal species found in Chinese tallow litter should create concern over the presence of tallow in and around the wetland environment. As tallow continues to colonize riparian and wetland habitat the potential to change canopy composition and create a single, dominate, fungal species (*Aureobasidium pullulans*) is high. Additionally, native species replacement by Chinese tallow invasions may alter the functional ability of a fungal community to degrade lignin content when allochthonous litter reaches Chinese tallow infested areas.

This may have an overall impact on decomposition rates of native plant litter adjacent to Chinese tallow stands as they contribute to the growth and development of a single fungal species that may not have the enzymes necessary to break down lignin components. It is unknown how Chinese tallow litter can influence the decomposition rates of native litter species but it is clear that the diversity of fungal communities in Chinese tallow litter treatments are much lower than that of native species litter treatments. How this will affect fungus driven processes where Chinese tallow has replaced native species is yet to be discovered.
After 16 weeks of litter decomposition in the mesocosm experiments there were no differences in fungal richness, evenness or diversity among litter types (Table 13). Furthermore, fungal communities seemed to have declined over the 16 week experiment in all treatments. Richness, evenness, and diversity were lowest at this sampling time period in all treatments when compared to the initial and 48 hour sampling time periods.

Table 13. Results and summary of One-Way ANOVA and post-hoc Tukey-Kramer test comparing Chinese tallow, water oak, loblolly pine and control treatment means of the Shannon-Wiener Diversity Index values for fungi at the 16 week time period.

<table>
<thead>
<tr>
<th>Shannon-Wiener Index</th>
<th>One-Way ANOVA Results</th>
<th>Treatment Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Richness (S)</td>
<td>n=40, DF=3, MSE=73.181, F=0.874, P=0.4635</td>
<td>Tallow: 16a, Oak: 16a, Pine: 20a, Control: 20a</td>
</tr>
<tr>
<td>Evenness (E)</td>
<td>n=40, DF=3, MSE=0.0068, F=1.4397, P=0.2473</td>
<td>Tallow: 0.08a, Oak: 0.04a, Pine: 0.11a, Control: 0.11a</td>
</tr>
<tr>
<td>Diversity (H)</td>
<td>n=40, DF=3, MSE=0.0851, F=1.4088, P=0.2561</td>
<td>Tallow: 0.23a, Oak: 0.13a, Pine: 0.36a, Control: 0.34a</td>
</tr>
</tbody>
</table>

Note: Means with the same letter in a row are not significantly different

The NMDS ordination scatter plot of fungal community composition from treatment samples taken at the 16 week period (Figure 15) show that fungal community composition among treatments samples are all very similar. All samples are grouped in a tight arrangement with only a few outliers. Chinese tallow litter treatments did not continue to promote *Aureobasidium pullulans* as the dominate species in Chinese tallow litter treatments. In fact, all but two of the treatment samples contained “No Hit” (unidentified) fungal species that made up 55 to 99% of the fungal communities found in most treatment samples.
The rest of the fungal species found in all of the treatment samples contained many species that were previously found at other time periods. *Alternia, Minimidochium, Udeniomyces, Dioszegia,* were all found at the initial and 48 hour time periods, just to name a few. The 16 week sampling time period also contained a few species that were exclusive of this sampling time period. These exclusive species included *Fusarium, Saccharomyces cerevisiae, Sporidiobolus pararoseus, Bulleromyces albus, Amphispaeriaceae, Tremellomycetes,* and *Thelephoracease.*

It is unclear why fungal communities from all treatment samples contained similar fungal community composition. Sampling outside and the transfer of the Quanta® Hydrolab water quality monitoring system from one mesocosm pool to the next may have cross contaminated the individual mesocosm pools. Much care was taken to decontaminate the Quanta® Hydrolab probe after each reading. Results from the bacterial community analysis show clear differences in bacterial community composition, suggesting that decontamination of the probe was effective at keeping cross contamination to a minimum.

The relatively high pH found at the 16 week period may have influenced the similarities in fungal community composition found among all treatments samples at the 16 week sampling time period. It is possible that fungal communities are sensitive to alkaline conditions. Unlike bacteria, fungal communities were not affected by leaf type after 16 weeks of decomposition.
Figure 17. Nonmetric Multidimensional Scaling (MNDS) ordination scatter plot of Chinese tallow, water oak, loblolly pine and control treatment samples at the 16 week time period (n=40; p = 0.5857, $r^2 = 0.0649$). Each sample represents its own fungal community. Clusters of samples are based on fungal community similarities ($\alpha = 0.05$) at the phylogenetic level.
This suggests that even though there were short terms changes in fungal communities based on leaf type, these communities eventually changed into fungal communities that were very similar to each other over time. Again, pH may be a driving factor that may have contributed to similar fungal community composition in samples from the 16 week time period. There were differences in dissolved oxygen, pH, and turbidity between litter treatments, but this did not contribute to differences in fungal community composition between litter treatments at the 16 week time period.

Mean dissolved oxygen in all of the mesocosms pools was sufficient to support aerobic respiration. Perhaps other chemical parameters had an influence on fungal richness, evenness, and diversity. Low concentrations of dissolved salts were found in all mesocosm pools at this time period. The low concentration of dissolved salts found in all mesocosm pools may have acted as a limiting resource for fungal communities, causing low richness, evenness, and diversity of fungal communities found at this time period. Salinity and specific conductance (a measurement of sodium, and all ions or disassociated salts, respectively) at this time period was at its lowest when compared to other time periods during the mesocosm experiment.

Based on the NMDS ordination scatter plot results of fungal communities from each time period it is reasonable to suspect that fungal species and possibly the entire community from each treatment sample came from the phyllosphere,
and not from the groundwater source. There were distinct fungal communities that were similar in community composition based on leaf type at the initial and 48 hour sampling time periods. All litter and treatment samples contained fungal communities that clustered in an arrangement based on litter treatment. This suggests that plant species may contain unique fungal communities that reside on the surface of the leaf of each respective plant species. To approach this idea, it is suggested that *in-situ* swab sampling of plant species be carried out.

A simple swab of the leaves themselves should provide enough DNA to make comparisons of microbial communities that inhabit the leaf surface. A similar swab approached was conducted by Lewis, (2015) in her attempt to identify skin microbiota on a particular frog species that was causing disease. She found that habitat type and environmental conditions played a role the microbiological species found on the skin of a particular frog species. Lewis (2015) also demonstrates that swab sampling can be an effective method for *in-situ* sampling of organisms. Swab sampling, followed by mass parallel DNA gene sequencing of microorganisms on the surface of plant leaves and litter may enhance knowledge on bacterial and fungal distribution based on plant community assembly. Based on this study, plant community composition may play a large role in the fungal species that are present at a site.
CONCLUSION

The invasion of non-native plants into southern forests continues to degrade forest productivity and biodiversity. Global climate change and anthropogenic activity seem to be a major contributor to these invasions. The invasive Chinese tallow tree has already colonized much of the southeastern forests of the United States where it has displaced native tree species and changed forest composition in a variety of habitat. The change in forest tree composition in wetland habitat will likely alter resource diversity and nutrient content that is a major driver of biodiversity in the wetland system.

This study demonstrates that Chinese tallow leaf litter is of different quality than native litter. Leaf litter quality can determine litter decomposition rates when all other factors (temperature, precipitation, lignin content) are held constant. The lack of lignin content in Chinese tallow leaf litter, relative to native species, is most likely contributing to a faster decomposition rate that that of native species. This faster decomposition rate is expected to release nutrients into ecosystems more quickly than native species in this study. How this will affect the biodiversity and function of native ecosystems over long periods of time is yet to be discovered. This study shows that the faster decomposition rate and different litter quality of Chinese tallow leaf litter can cause drastic changes in aquatic
chemistry when compared to native species in this study. Dissolved oxygen, pH, and turbidity are all affected when Chinese tallow leaf litter is present in water. Nutrients quickly released by Chinese tallow litter are stimulating microbial activity. This stimulated microbial activity is responsible for changes in dissolved oxygen when Chinese tallow litter is present. Although it is believed that stimulated microbial activity may partially be responsible for alterations in aquatic pH, other factors (secondary chemicals in Chinese tallow litter) are likely to be the driving force behind pH alterations.

This study also demonstrates that the difference in litter quality and faster decomposition rate of Chinese tallow leaf litter can contribute to different microbial communities than that of native litter. Microbial communities are responsible for many processes in the wetland environment. A changing litter source that alters the composition of microbial communities may alter the overall function of the ecosystem in which Chinese tallow tree invades. The results from mass parallel gene sequencing of fungal DNA suggests that plant species may contribute to the fungal species that were found in this study. Bacterial DNA gene sequencing of the samples from all three time periods strongly suggest that Chinese tallow litter can change bacterial community composition over longer periods of time.

Given the results of this study it is likely that the invasive Chinese tallow tree will continue to alter many components of the ecosystems they invade, from
forest tree composition, to aquatic chemistry, to microbial communities. The faster decomposition rate of Chinese tallow leaf litter and subsequent stimulation of microbial respiration may also contribute to the amount of carbon that can be stored in a Chinese tallow dominated forest. Carbon storage potential of Chinese tallow leaf litter may not be equivalent to native species litter. Furthermore, the rate at which carbon dioxide is released to the atmosphere may be much faster in the case of Chinese tallow litter when compared to native litter species. How this might affect atmospheric carbon concentrations and climate trends is yet to be studied and determined.

Based on this study it is recommended that swab sampling of living Chinese tallow tree leaves, followed by mass parallel gene sequencing, can reveal if microbial communities living on the leaf parts of plants differ between native and invasive tree species. It is realized that the native tree species in this study are not those found where Chinese tallow is a prevalent invader. To demonstrate a more realistic comparison of invasive versus native litter, it is recommended that tree species litter from similar habitat should be used to study the comparative effects of Chinese tallow on microbial communities in native terrestrial ecosystems.


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