Relationships among Soil Properties and Soil CO_2 Efflux in a Loblolly Pine-Switchgrass Intercropped System

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ABSTRACT

The components of soil CO_2 efflux are affected by many soil properties including temperature, moisture, microbial abundance and activity, and other soil physical and chemical properties. Changes in these factors can result in high spatial and temporal variability of total soil CO_2 efflux. Low molecular weight organic acids (LMWOAs), dissolved organic carbon (DOC) and dissolved organic nitrogen (DON), microbial biomass and activity were measured to evaluate the impact of intercropping switchgrass (*Panicum virgatum* L.) in a loblolly pine (*Pinus taeda* L.) plantation. Surface soil samples (0-15 cm) were collected on the bed (PSG-B), interbed (PSG-I) and edge (PSG-E) of pine-switchgrass intercropped treatments, as well as pine only (P-B) and switchgrass only (SG-I) treatments. Differences in most soil properties and processes of intercropped treatments were sporadic and most did not show clear trends. However, significant correlations between DOC, soil temperature, oxalic and acetic acids and soil CO_2 efflux were present. In an multiple regression model these factors explained 57% of the variance in total soil CO_2 efflux. Therefore we think that LMWOAs, as a labile component of DOC, are influencing total CO_2 efflux because they are being consumed by microbial community, increasing heterotrophic respiration and as a result overall total CO_2 efflux. The amount and distribution of labile C controls microbial community dynamics, heterotrophic respiration as well as the stabilization of soil C.

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Chapter 1
Introduction

1.1 Context and Justification

Soil carbon (C) is the largest terrestrial C pool and CO_2 efflux from the soil surface is the second largest C flux in terrestrial ecosystems (Raich and Schlesinger 1992; Yuste 2007). Microbially mediated decomposition of soil organic matter (heterotrophic respiration) and root respiration (autotrophic respiration) are two major pathways in which soil C is lost into the atmosphere as CO_2 (Raich and Schlesinger 1992). These components of soil respiration are affected by temperature, moisture, root and microbial abundance and activity, and other soil physical and chemical properties (Tang 2005; Yuste 2007). Spatial and temporal variations in soil CO_2 efflux or soil respiration also occur due to differences in soil and site factors (Raich and Nadelhoffer 1989; Hanson et al. 1993; Norman et al. 1997). Soil temperature and moisture have been shown to be the most important factors that influence soil CO_2 efflux rates (Raich and Schlesinger 1992; Raich and Potter 1995; Davidson et al. 1998; Suseela 2012; Buchmann 2000). However, the amount and quality of C stored in the soil, N concentrations, photosynthetic activities and pH have also been shown to have an effect (Grant et al. 1994; Randerson et al. 1996; Boone et al. 1998; Pregitzer et al. 1998). Additionally, soil physical and chemical properties (Borken et al. 2002) and stand age (Irvine and Law 2002; Litvak 2003; Saiz et al. 2006) can influence soil CO_2 efflux rates.

Agroforestry systems are intensively managed and deliberately designed to optimize the use of space, time, and nutrient retention while minimizing competition among the components of the system (Jose et al. 2000). Alley cropping, a form of agroforestry, optimizes these resources because agricultural crops are combined with perennial woody species. With different growth cycles, plant biomass production is distributed over the course of the year, enabling both species to flourish (Nair 1993). Combining perennial woody species with agriculture crops can provide several benefits

including increased soil organic matter (SOM) with depth, enhanced physical properties and nutrient use. Understanding how soil processes are influenced by alley cropping is essential to the success and long-term viability of agroforestry in temperate ecosystems. Therefore investigations on SOM, nutrient cycling and microbially driven processes in agroforestry systems, and their relationship to soil respiration, are justified.

An intensively managed loblolly pine (*Pinus taeda* L.) plantation with intercropped switchgrass (*Panicum virgatum* L.) was evaluated to determine relationships between various soil properties and processes. Our overall objective was to determine the relationship between dissolved C and N, microbial biomass and activity, soil temperature and moisture, and soil CO_2 efflux. Subsequent objectives were developed to further investigate differences between soil properties in intensively managed loblolly pine plantations intercropped with switchgrass versus monoculture treatments of switchgrass and traditional pine.

1.2 Main Objectives

The specific objectives of this research were to:

1. Quantify changes due to intercropping switchgrass in loblolly pine plantations on soil properties such as low molecular weight organic acids (LMWOAs), dissolved organic nitrogen (DON), dissolved organic carbon (DOC), microbial community biomass and activity, and soil nutrients [i.e., C, N, and phosphorus (P)].

2. Identify mechanistic relationships between soil properties and soil CO_2 efflux.

1.3 Research Hypotheses

Based on the above objectives the following null hypotheses will be tested:

H_o. The presence of switchgrass in intercropped treatments does not alter microbial activity, DOC, DON and LMWOAs of the pine.

H_o. The presence of pine in intercropped treatments does not alter microbial activity, DOC, DON and LMWOAs of switchgrass.

H_o. There is no additive effect in combined area (PSG-E) verses bed (PSG-B) and interbed (PSG-I) treatments.

H_o. There is no correlation between DOC, individual LMWOAs, PO_4^{3-}, inorganic and organic forms of nitrogen and soil CO_2 efflux.

1.4 References

Boone, R.D., Nadelhoer, K.J., Canary, J.D., Kaye, J.P., 1998. Roots exert a strong influence on the temperature sensitivity of soil respiration. Nature 396: 570±572.

Borken, W., Xu, Y.J., Davidson, E.A., Beese, F., 2002. Site and temporal variation of soil respiration in European beech, Norway spruce, and Scots pine forests. Global Change Biology 8: 1205–1216.

Buchmann N., 2000. Biotic and abiotic factors controlling soil respiration rates in Picea abies stands. Soil Biology & Biochemistry 32: 1625-1635.

Davidson, E.A., Belk, E., Boone, R.D., 1998. Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. Global Change Biology 4: 217-227.

Grant, R.F., Rochette, P., 1994. Soil microbial respiration at different water potentials and temperatures: theory and mathematical modeling. Soil Science Society of America Journal 58: 1681-1690.

Hanson, P.J., Wullschleger, S.D., Bohlman, S.A., Todd, D.E., 1993. Seasonal and topographic patterns of forest floor CO2 efflux from an upland oak forest. Tree Physiology 13: 1-15.

Jose, S., Gillespie, A.R., Seifert, J.R. and Biehle, D.J., 2000. Defining competition vectors in a temperate alley cropping system in the mid-western USA. 2. Competition for water. Agroforestry Systems 48: 41–59.

Irvine, J., Law, B.E., 2002. Contrasting soil respiration in young and old growth ponderosa pine forests. Global Change Biology 8: 1183–1194.

Litvak, M., Effect of stand age on whole ecosystem CO_2 exchange in the Canadian boreal forest. Journal of Geophysical Research: Atmospheres 108: D3 16.

Nair, P.K. Ramachandran. 1993. An Introduction to Agroforestry. Klgwer Academic Publishers. S494.5.A45N3543 634.9'9—dc20.

Norman, J.M., Kucharik, C.J., 1997. A comparison of six methods for measuring soil-surface carbon dioxide fluxes. Geophysical Research 102: 28773-28777.

Pregitzer, K.S., Laskowski, M.J., Burton, A.J., Lessard, V.C., Zak, D.R., 1998. Variation in sugar maple root respiration with root diameter and soil depth. Tree Physiology 18: 665-670.

Raich, J.W., Potter, C.S., 1995. Global patterns of carbon dioxide emissions from soils. Global Biogeochemical Cycles 9: 23-36.

Raich, J.W., Nadelholer, K.J., 1989. Belowground carbon allocation in forest ecosystems: global trends. Ecology 70: 1346-1354.

Raich J.W., Schlesinger W.H., 1992. The global carbon-dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus Series B-Chemical and Physical Meteorology 44: 81–99.

Randerson, J.T., Thompson, M.V., Malmstrom, C.M., 1996. Substrate limitations for heterotrophs: implications for models that estimate the seasonal cycle of atmospheric CO_2. Global Biogeochemical Cycles 10: 585-602.

Siaz G., Black K., Reidy B., Lopez S., Farrell, E.P., 2007. Assessment of soil CO_2 efflux and its components using a process-based model in a young temperate forest site. Geoderma 139: 79- 89.

Suseela, V., Conant, R. T., Wallenstein, M. D., & Dukes, J. S., 2012. Effects of soil moisture on the temperature sensitivity of heterotrophic respiration vary seasonally in an old-field climate change experiment. Global Change Biology 18: 336-348.

Tang J.W., Qi Y., Xu M., Misson L., Goldstein A.H., 2005. Forest thinning and soil respiration in a ponderosa pine plantation in the Sierra Nevada. Tree Physiology 25: 57–66.

Yuste J.C., Baldocchi, D.D., 2007. Microbial soil respiration and its dependency on carbon inputs, soil temperature and moisture. Global Change Biology 13: 1–18.

Chapter 2
Literature Review

2.1 Biomass for Biofuels and Switchgrass

The global energy demand is projected to grow by more than 50% by 2025 with most of the demand coming from rapidly growing developing nations. In order to meet these increasing demands and shift society's dependence for finite nonrenewable resources a multidimensional approach is needed that includes solar, wind, biofuels, and other renewable energy sources. The utilization of biomass as a biofuel is viewed as a major contributor in this shift away from nonrenewable resources (Ragauskas 2006). Biomass fuels provided about 4% of the energy used in the United States in 2011. Of this, about 45% was from wood and wood-derived biomass, 44% from biofuels (mainly ethanol), and about 11% from municipal waste (http://www.eia.gov/). Renewable energy from biomass has the potential to reduce the dependency on fossil fuels, though it is unlikely to completely replace them. Realizing the potential of biomass requires more research on the development of high yielding biomass production systems that can efficiently convert biomass into usable forms of energy (McLanghin 1999).

Switchgrass is a warm season perennial and a potential biofuel because of its high productivity and ability to be grown under a wide range of climatic conditions. Switchgrass is a C4 species (i.e., the first product of photosynthesis is a four C compound) that breaks winter dormancy in late April and grows the most between June and August in the south (McLaughlin 1999, 2005; Wolf 2009). Because switchgrass is a C4 species it has a high water use efficiency when compared to a C3 species and in general fixes 30% more C per unit water (Samson et al. 1994; McLaughlin 1999). It also provides excellent erosion control and protection for wildlife (Wolf 2009). It is resilient to handling and transportation and stores as both wet and dry feedstock. In the Southeastern

United States, switchgrass can grow more than 3 m in height and develops a deep vigorous root system that can extend more than 3.5 m (McLaughlin 1999). Typically it takes three years to reach its full maturity and after establishment there are only minor disease problems and no known insect pests (Parrish 2008; Sokhansanj 2009; Wolf 2009). Switchgrass is also a good candidate for genetic improvement research due to the reproductive characteristics of the plant and its large genetic variability (Parrish 2008; Sokhansanj 2009).

The allocation of C fixed during photosynthesis to the rooting system of a plant is an important aspect that affects potential yields, ecological adaptability, and long-term C dynamics. Switchgrass has an extensive rooting system and belowground biomass (Parker 1996; McLaughlin 1998; Bowden 2010). An extensive rooting systems increases turnover of soil C, and nutrient use efficiency after fertilizer application (Kramer 1995). As with most plant systems, switchgrass roots are predominantly in the surface horizons. In one study, approximately 87% of switchgrass roots were located in the top 30 cm in a sandy loam soil (Bransby et al. 1998). Studies done on switchgrass have estimated soil C gains in the surface horizons were from 1.1 Mg C ha^{-1} $year^{-1}$ to 2.9 Mg C ha^{-1} $year^{-1}$ (Gebhart et al. 1994; Liebig et al. 2008). Increases in soil C, affect both water and nutrient retention, promote aeration, and result in increased root growth (Reeves 1997).

Research done by Wullschleger (2010) shows that total switchgrass yields are highly variable across the United States but this data shows that all varieties have a clear response to Nitrogen (N) fertilization. Average yields following N fertilization increased, reaching an optimum at 100 kg ha^{-1} of N. N is considered the most limiting nutrient for switchgrass production but relative to other cultivated crops switchgrass requires less N (Bransby et al. 1998; Parrish 2005). N is translocated out of the aboveground biomass prior to senescence allowing switchgrass to efficiently utilize limiting N resources (Lemus 2008). Wullschleger (2010) also concluded that soils, climate

(temperature and precipitation), management practices, and geographic location influence switchgrass yields (Wullschleger 2010).

2.2 Intercropping Loblolly Pine and Switchgrass

Agroforestry systems are intensively managed and deliberately designed to optimize the use of space, time and nutrient retention while minimizing competition among the components of the system (Jose et al. 2000). According to the *resource-ratio hypothesis* proposed by Tilman (1985) coexistence occurs where resource requirements differ among the species allowing for productive agroforestry systems to exist (Tilman 1985). The combination of the right species mixture allows for greater utilization of both non-limiting and limiting resources and the system as a whole can achieve greater total biomass production on an equivalent land area (Jose 2004). Agroforestry systems that combine agricultural crops with perennial woody species optimize spatial and temporal resources because the two species can have different growth cycles that distribute biomass production over the course of the year. Plant biomass production is distributed over the course of the year if selected species have different growth cycles. In traditional monoculture systems there is more competition between plants because they are genetically similar and have the same resource requirements (Nair 1993).

Alley cropping, a form of agroforestry, is where agricultural crops are grown in between trees. Some of the potential benefits of alley cropping include increased cash flow, shorter return on investment, improved efficiency of N cycling and increased biodiversity. Other benefits include the potential for greater C sequestration relative to a monoculture cropping system due to different sources of C inputs into the system (Udawatta 2011).

Currently, land for new biofuel production is often created by switching from one crop to another or diverting food crops into biofuel production. Intercropping biofuels in already intensively managed systems, like loblolly pine plantations can reduce the total land area used for fuel production and reduce the impacts resulting from land-use change (Fargione 2010). Very few studies have been done on productivity of alley cropping practices but the limited data that does exist suggests an above ground biomass production in alley cropping system as 26.8 Mg C ha^{-1} per year for younger systems (i.e.,< 13 years old) (Udawatta, 2011). Even less work has been done on the effects on biodiversity and sustainability from intercropping switchgrass in intensively managed loblolly pine plantations. Intercropping switchgrass would likely alter surface and subsurface soil horizons in these pine stands but also provides good ground cover. The potential competition and shading between pine and switchgrass has yet to be determined. Despite the uncertainty of intercropping switchgrass with pine, it offers an opportunity to produce traditional forest products and cellulosic bioenergy crops on the same land base. (Dale 2010; Riffle 2011). More work still needs to be done to evaluate the competition between the two species and effects on sustainability of these species interactions. In 2008, Catchlight Energy LLC, a Chevron/Weyerhaeuser Joint Venture, was formed to assess the large-scale viability of this type of system and currently research is being done to answer some of these questions (http://www.catchlightenergy.com/). Spatial variations, including planted space and rooting requirements between the loblolly pine and switchgrass also potentially allow for reduced competition between the two species.

Despite limited data an economic model developed by Susaeta (2011) evaluated intercropping switchgrass in loblolly pine plantations. This model determined that there would be increased competition between the two crops and low stumpage and switchgrass prices would greatly reduce the overall profitability of intercropping. When comparing systems, the loblolly pine

monoculture would be the most profitable option compared to intercropping systems if the price for switchgrass is below \$30 Mg^{-1}. If the price for switchgrass exceeded \$30 Mg^{-1}, adopting intercropping system would be beneficial and the decrease in growth due to competitive interaction between the crops would be worth the costs of adopting this system. Findings from this study suggested that the optimal system would depend mainly on the competitive interactions and expected values of the two crops (Susaeta 2011).

Loblolly pine is an important commercial species and with slash pine occupies roughly 32 million acres of pine plantations in the south (Schultz 1997; Wear and Greis 2002; Fox 2007). Intensively managed plantations are potentially some of the most productive forests in the United States and also have the great potential to sequester atmospheric C (Johnsen et al. 2001; Maier 2004). In forested ecosystems, belowground C may account for over 70% of total C with soil C being the largest component (Schlesinger et al. 2001). Rapidly growing young forests sequester C at very high rates, in contrast to older forests, which store large amounts of C but sequester it more slowly (Birdsey 1992). The ability to sequester C depends on rotation length, silvicultural applications (e.g., fertilization, stocking rate) and genetic improvement, (Jayawickrama 2001; Oren et al. 2001).

Most forests occur on soils that are less fertile than soils used for agricultural crops. In the southeastern U.S. the major nutrient deficiencies that limit growth of forested systems are N and P (Fox 2007; Stape et al. 2006). Therefore, N and P fertilization is needed in many forests to produce higher yields and increase productivity (Fox 2007; Stape et al. 2006; Trichet et al. 2009). In a review of elevated atmospheric CO_2 experiments across a range of ecosystem types, De Graaff et al. (2006) concluded that significant C accumulation in soils required rates of N supply above typical inputs derived from atmospheric deposition and biological N fixation. This result is consistent with studies

of ecosystem recovery following decades of agricultural soil organic matter (SOM) depletion, showing that soil C accumulation is controlled by the availability of N from other sources (Knops and Tilman 2000). Sequestration of C in soils is likely to be constrained by the availability of sources of N (Hungate et al. 2003). Combining the right tree and crop species will allow for greater utilization of site resources while accumulating SOM and potentially turning the site into a sink for C while achieving greater aboveground biomass on an equivalent land area (Jose 2004).

2.3 CO_2 Efflux in Agroforestry Systems

Total soil CO_2 efflux at the soil surface, or soil respiration (R_s), is predominately a process that includes root, microbial and faunal respiration. Chemical processes including oxidation of some soil minerals also contribute to soil CO_2 efflux. Soil CO_2 efflux depends on diffusion and transport of CO_2 to the soil surface. Biotic processes, the largest factor influencing soil respiration can further be subdivided into heterotrophic (microbial and faunal respiration) and autotrophic (root respiration) both of which are influenced by various soil parameters (Buchmann 2000; Raich and Schlesinger 1992). Soil temperature and soil moisture are in most cases the most important factors that regulate soil CO_2 efflux (Raich and Schlesinger 1992; Davidson et al. 1998; Lin 1999). Soil temperature has been found to be the principle factor in regulating soil respiration but is often coupled with soil moisture. Elevated soil temperatures and moistures provide favorable conditions for decomposition of organic matter. Other soil parameters that affect soil respiration include substrate quality and quantity, texture, pH, vegetation type and activity along with nutrient concentrations in the rhizosphere (Buchmann 2000; Reth 2005). Intercropping species will change the litter quality because of the various types of litter inputs incorporated from tree and intercropped components. This change can lead to microbial diversity and root dynamics leading to changes in soil CO_2 efflux

(Wardle and Lavelle 1997; Mungai 2005; Lee 2003). The presence of trees in alley cropping systems can cause variations in soil temperature and water content creating microclimate differences in soil CO_2 efflux (Mungai 2005). The majority of the literature on the subject has shown increases in soil respiration due to intercropping crop and tree species in older systems (Table 2.1). There was one study in which researchers observed a decrease in soil respiration, but the crop species experienced tillage and frequent disturbance (Bae 2013).

2.4 Soil Carbon and Microbial Communities in Agroforestry Systems

Total SOM content of soil changes relatively slowly because it is the largest terrestrial pool of C and is difficult to accurately measure. However, soil microbial biomass, defined as the living microbial component of soils, responds more rapidly to soil inputs and changes to the total SOM pool. Therefore changes in microbial biomass that can be easily measured over a shorter time period can provide insight into changes occurring in the total SOM pool (Polwson 1987; Sparling 1992) Microbial biomass is predominately made up of bacteria and fungi, and most of the microbial population can be found in the surface of the soil profile due to a greater availability of more easily degraded organic compounds (Murphy 1998). However, bacteria and fungi vary in their ability to store and use C and measures of total microbial biomass do not accurately measure the active portion of the population. Total microbial populations and their activity are affected by many factors including variations in temperature, moisture, management practices and soil type.

In agroforestry systems, differences in litter quantity and quality between tree and crop components can greatly alter microbial populations and diversity (Mungai 2005). Of the literature found on microbial biomass and soil C, most observed higher microbial biomass and SOM due to intercropping in both young and old systems (Table 2.2).

2.5 Soil Nitrogen

Nitrogen is the nutrient that controls net primary productivity in most ecosystems (Jansson and Persson 1982; Jones 2004). Organic matter decomposition and mineralization of N are the means by which organic forms of N are released into the soil as inorganic forms. Inorganic N released in this way is then available for utilization by plants or microorganisms or is lost from the system. Mineralization is the transformation process where ammonium (NH_4^+) is released by the microbial community as they utilize organic C containing compounds containing N as an energy source (Jansson and Persson 1982; Jarvis 1996). Ammonium (NH_4^+) is rapidly converted to nitrate (NO_3^-), another inorganic form of N that can be lost from the system due to its mobility. Mineralization of organic N to NH_4^+ before microbial integration is considered the dominant pathway of soil-derived N supply (Jarvis 1996; Jones 2004). DON is an extremely labile N pool and is a source of N for microorganisms that can also be directly utilized by some plants (Jones 2004).

2.6 Definition and Role of Low Molecular Weight Organic Acids (LMWOAs)

Soil C is important factor in maintaining soil quality, nutrient cycling and soil biological activity (Raich and Schlesinger 1992; Murphy 1998). Carbon allocation belowground by a plant in the form of root exudates can release 5 to 21% of all photosynthetically fixed C in the soil-root interface (Marschner 1995; Walker 2003). This increases readily available labile C sources may regulate soil microbial communities, create symbiotic relationships and change the chemical and physical properties of the soil in the rhizosphere (Nardi 2000; Walker 2003; Phillips 2011). Root exudates have traditionally been grouped into low and high molecular weight compounds. The majority of root exudates are the low molecular weight compounds such as organic acids, amino acids, sugars and phenolics; whereas high molecular weight compounds primarily include

polysaccharides and proteins (Aiken 1992; Walker 2003). LMWOAs are water-soluble, hydrogen and oxygen containing compounds, which are characterized as having of one or more carboxyl groups. These can be further classified into hydrophilic and hydrophobic factions. The hydrophobic acid fraction contains some aliphatic carboxylic acids, one- to two-ring phenols, and one- to two-ring aromatic carboxylic acids. The hydrophilic acid fraction contains organic acids and aliphatic acids (Aiken 1992). Roots typically exude many types of organic acids with varying chain lengths; some of the aliphatic acids include acetate, citrate, formate, fumarate, lactate, malate, oxalate and succinate (Marschner, 1995; Neumann, 2001). Some of the aromatic acids include caffeic, ferulic, gallic, gentisic, p-hydroxbenzoic and salicylic (Neumann 2001). Root exudates are also involved with many other metabolic processes including the uptake of C and N, the regulation of osmotic potential, and balancing charge during excess cation uptake (Marschner 1995; Jones 1998; Ryan 2001; Strobel 2001a). Nutrient deficiencies (particularly P), exposure to toxic cations (e.g., Al^{3+}), and anoxia have been associated with enhanced organic anion exudation from roots (Marschner 1995; Jones 1998). LMWOAs that make up part of the dissolved organic C pool have also been shown to increase the availability of nutrients in the rhizosphere (Giesler 2007). Understanding the function of these LMWOAs in the rhizosphere requires knowledge of the main reactions they participate in and their concentration in the rhizosphere (Bar-Yosef 1991; Bolan 1994; Hue 1986; Ryan 2001). When LMWOAs compounds are released into the soil by plant roots, or from decomposition, the microbial community uses them as an energy source (Qualls and Haines 1992; Jones 1996; Jones and Darrah 1994; van Hees 2005). Root exudation largely determines organic anion concentrations in the rhizosphere is also influenced by soil solid phase reactions (sorption and desorption), losses from leaching, degradation by soil microorganisms, and complexation and precipitation reactions (Jones 1998, Strahm and Harrison 2006).

Research shows that LMWOAs comprise less than 10% of total DOC in most soil solutions (Fox and Comerford 1990; van Hees et al. 1996; Strobel et al. 1999, 2001b; Neff 2001). DOC is defined as the fraction of organic compounds in soil solution that pass a 0.45 μm membrane filter (Herbert and Bertsch 1995; Neff 2001). The movement of DOC in soil solution is an important part in the formation of SOM and is therefore important for understanding the distribution and stabilization of soil C as well as the controls over the activity of microorganisms within the soil (Trumbore 1993; Neff 2001). Dissolved organic matter (DOM) is a pathway for the loss of C, N, and P from ecosystems. Over long time scales, small but constant losses of DOM, containing limiting or essential nutrients can reduce the productive capacity of ecosystems (Hedin 1995; Vitousek 1998). For these reasons, DOM fluxes (including LMWOAs and nutrients) are an important component of the biogeochemistry of terrestrial ecosystems (Neff 2001).

Table 2.3 lists the types and concentrations of LMWOAs found under forested and cultivated crop (wheat) systems found in the literature. Data on root exudates from switchgrass was not found in the literature. The data displayed shows the concentration range for the surface mineral horizon in these systems. Both the types and ranges of aliphatic LMWOAs are not consistent.

2.7 Relationship Between Microbial Biomass and Root Exudates

Microbial biomass is also a sensitive measure of changes in the status of SOM because of rapid turnover rates (Powlson 1987; Sparling 1990, 1992). Microbially mediated degradation is important in regulating the amount of LMWOAs in soil solution (van Hees 2005). Typically, 60% of the organic acids are mineralized to CO_2 and 40% incorporated into new cell biomass during decomposition (Jones 1994, 1996, 1998). Roots exudates provide a C energy source for the microbial community they may have a major influence on both the structure and function of these

communities and ultimately the soil-root interface (Jones 1998; Marschner 1995; Shengjing 2011). While microbes consume root exudates, they can also produce a wide range of organic acids especially in nutrient limiting situations (Jones 1998). In soils with a high C:N ratio (>30:1) decomposition is slower and microorganisms will readily deplete the limiting N (Cleveland 2007). Having a concept of total C and N and nitrogen cycling is also crucial in understanding the quantity and activity of the microbial community and its role in understanding the soil system itself.

Table 2.1: Summary of studies examining CO_2 efflux rates in response to intercropping various species sampled during different time intervals and frequencies.

Intercropped Species						
Species 1	**Species 2**	**Age (yrs.)**	**Duration/ Frequency**	**Response to intercropping**	**Potential Reasons**	**Source**
Hybrid Poplar/Norway Spruce	Barley	13	7 1 day measurements	Elevated soil respiration	Tree root respiration or higher microbial respiration	(Peichl 2006)
Gliricidia	Maize	7/10	Weekly over 5 months	Elevated soil respiration	Root respiration or applied OM	(Makumba 2007)
Pecan	Cotton	3/47	7 months	Elevated soil respiration in oldest system	Tree root respiration	(Lee 2003)
Gamhar	Cacao/ Coffee/Zinger	22	5 times in dry /5 times in wet seasons	Reduced soil respiration	Less disturbance in forested system/reduced fine root & microbial biomass	(Bae 2013)
Dipterocarps	Cacao/ Fern	22	5 times in dry /5 times in wet seasons	Reduced soil respiration	Less disturbance in forested system/reduced fine root & microbial biomass	(Bae 2013)

Table 2.2: Summary of studies examining organic carbon and/or microbial biomass carbon in response to intercropping various species sampled during different time intervals and frequencies.

Intercropped Species						
Species 1	**Species 2**	**Age (yrs.)**	**Duration/ Frequency**	**Parameter measured**	**Response to intercropping**	**Source**
Hybrid Poplar/ Norway spruce	Barley	13	1 sampling period (3 reps.)	Total soil C	Increase due to intercropping	(Peichl 2006)
Gliricidia	Maize	7/10	1 sampling period (3 reps.)	SOC (heated potassium dichromate oxidation)	Increase due to intercropping	(Makumba 2007)
Pecan	Cotton	3/47	1 sampling period (2 reps.)	SOC/microbial biomass	MBC highest in 47 yr. pecan monoculture/ SOC highest in 47 intercropped	(Lee 2003)
Pecan	Bluegrass	22	1 sampling dates (5 reps.)	SOC (heated potassium dichromate oxidation)	No difference	(Mungai 2005)
Maple	Maize	12	2 sampling dates (5 reps.)	SOC (heated potassium dichromate oxidation)	Higher in tree row due to intercropping	(Mungai 2005)
Gamhar	Cacao/ Coffee/Zinger	22	5 times in dry /5 times in wet seasons	SOC	Higher SOC	(Bae 2013)
Dipterocarps	Cacao/fern	22	5 times in dry /5 times in wet seasons	SOC	Lower SOC	(Bae 2013)
D. Sissoo	Wheat/cowpea	3	1 sampling period (10 reps.)	SOC (heated potassium dichromate oxidation)	Increased with narrow spacing of intercropped species	(Chander 1998)
Hybrid Poplar	Soybean/maize	8	Annual sampling over 3 years ('93-'95)	Total soil carbon	Higher near tree row of intercropped	(Thevathasan 2004)
Sugarcane	Wheat/Maize/ Cowpea/Lentil/ Mustard/Potato	No age listed	1 sampling period (5 reps.)	SOC (heated potassium dichromate oxidation)	SOC increased with intercropping/ highest with maize	(Suman 2006)

Cupuacu/Peach palm/Brazil Nut/ Orleans tree	Cupuacu/Peach palm/Brazil Nut/Orleans tree	10	2 sampling dates (8 reps)	Microbial biomass C	Higher MBC in peach palm monoculture	(Kurzatkowski 2004)
Eucalyptus/Acacia/P opulus	Rice-Berseem	5	2 sampling dates (5 reps.)	Microbial biomass C	Highest MBC in Acacia + rice-Berseem	(Kaur 2000)
Radiata Pine	Mixed grassland	25	1 sampling period (25 reps.)	Microbial biomass C	Highest MBC in grassland monoculture	(Saggar 2001)

Table 2.3: Summary of studies examining types and concentrations of LMWOAs determined in monoculture forested and cultivated crop systems.

Source	System	Soil Horizon	Units	Acetic	Citric	Formic	Fumaric	Lactic	Malic	Oxalic	Succinic	Propionic
(Hue 1986)	Forest	E	µM		5-12	90		12-52	101-137	3-22	125-282	
(Fox &Comerford 1990)	Forest	A	µM	tr	tr	tr-151			tr	98-293	tr	
(Grierson 1992)	Forest	A	µg mL-1		0.15-0.24		0.61-0.09		0.09-0.14			
(van Hees 2002)	Forest	A	µM	2	0.1	0.7		0.7		3.1		<0.1
(van Hees 2005)	Forest	A	µM	<1-375	6-350				0-2500	<1-375		6-350
(Cieslinski 1998)	Cultivated crop	A	nmol g−1	42.8-2898.5	1.6-6.0		1.5-12.1		1.8-39.8	24.6-43.2	2.0-1941.2	6.8-40.0
(Strobel 1999)	Cultivated crop	A	µM	0-15.5		9.3-12.7		0.9-26.5	0-0.8	0-3.7	0-1.1	
(Lucas Garcia 2000)	Cultivated crop	A	µg/g	319.8-1542	87.14-1806.3	76.43-373.8	0.1-3.48	40.19-269.42	10.67-627	6.21-38.45	151.41-483.82	

Notes: tr = trace amounts (Aconitic, Fumaric, Malonic were tested but nothing was found)

2.8 References

Aiken, G. R. McKnight D.M., Wershaw, R.L., MacCarthy, P., 1985. Humic Substances in Soil, Sediment, and Water: geochemistry, isolation and characterization. Wiley-Interscience: New York. 692.

Bae, K. Lee, K.L., 2013. Seasonal variation of soil respiration rates in a secondary forest and agroforestry systems. Agroforestry Systems 10.1007/s10456-012-9530-8.

Bar-Yosef B. 1991.Root excretions and their environmental effects. Influence on availability of phosphorus. In *Plant Roots. The Hidden Half*, ed. Y Waisel, AEshel, U Kafkafi, 529–57. New York: Marcel Dekker 948.

Birdsey, R.A., 1992. Carbon storage and accumulation in United States forest ecosystems. Gen. Tech. Rep. WO-59. Washington D.C.: U.S. Department of Agriculture, Forest Service, Washington Office 51.

Bolan N.S., Naidu R., 1994.Influence of low molecular-weight organic acids on solubilization of phosphates. Biology and Fertility of Soils 18: 311–19.

Bowden, R.D. 2010. Aboveground and Belowground Biomass, Carbon, and Nitrogen Content of Two Strains of Switchgrass in Northwestern Pennsylvania. Allegheny College Center for Environmental and Economic Development Publication CEED-2010-1.

Bransby, D.I., Mclaughlin, S.B., Parrish, D.J., 1998. A review of carbon and nitrogen balances in switchgrass grown for energy. Biomass and Bioenergy 14: 379-384.

Buchmann, N. 2000. Biotic and abiotic factors controlling soil respiration rates in *Piceaabies* stands. Soil Biology & Biochemistry 32: 1625-1635.

Cieslinski G., Van Rees K.C.J., 1998. Low-molecular-weight organic acids in rhizosphere soils of durum wheat and their effect on cadmium bioaccumulation. Plant Soil 203: 109–17.

Chander K., Goyal S., Nandal D.P., Kapoor. K., 1998. Soil organic matter, microbial biomass and enzyme activities in a tropical agroforestry system. Biology and Fertility of Soils 27: 168–172.

Cleveland, C.C., Liptzin, D. 2007. C:N:P stoichiometry in soil: is there a 'Redfield ratio' for the microbial biomass? Biogeochemistry 85: 235–252.

Dale V., Kline K., 2010. Biofuels: implications for land use and biodiversity, Biofuels and Sustainability Reports 1–13.

Davidson, E.A., Belk, E., Boone, R.D. 1998. Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. Global Change Biology 4: 217-227.

De Graaff M.A., Van Groenigen K.J., Six J, Hungate B., van Kessel C., 2006. Interactions between plant growth and soil nutrient cycling under elevated CO_2: a meta-analysis. Global Change Biology 12: 2077–2091.

Fargione, J.E., 2010. The Ecological Impact of Biofuels. Annual Review Ecology Evolution, and Systematics 41: 351–77.

Fox, T.R., Comerford, N.B., 1990. Low-molecular-weight organic- acids in selected forest soils of the Southeastern USA. Soil Science Society America Journal 54: 1139–1144.

Fox, T.R., Allen, H.L., Albaugh, T.J., Rubular R., Carlson. C.A., 2007. Tree nutrition and forest fertilization of pine plantations in the southern United States. Southern Journal of Applied Forestry 31: 5–11.

Gebhart, D.L., Johnson, H.B., Mayeux, H.S., Polley, H.W., 1994. CRP increases soil organic carbon. Journal of Soil Water Conservation 49: 488–492.

Giesler, R Hogberg M.N., Bjarne, W., Richter, A., Nordgren, A., 2007. Production of dissolved

organic carbon and low-molecular weight organic acids in soil solution driven by recent tree photosynthate. Biogeochemistry 84: 1-12.

Grierson P.F., 1992. Organic-acids in the rhizosphere of *Banksiaintegrifolia* L. Plant Soil 144: 259-265.

Hedin L.O., Armesto J.J., Johnson A.H., 1995. Patterns of nutrient loss from unpolluted old-growth temperate forest: evaluation of biogeochemical theory. Ecology 76: 493–509.

Herbert B.E., Bertsch P.M., 1995. Characterization of dissolved and colloidal organic matter in soil solution: a review. In: McFee WW, Kelly JM (eds.) Carbon forms and functions. Soil Science Society of America Journal 63–88.

Hue, N.V., Craddock, G.R., Adams, F., 1986.Effect of organic acids on aluminum toxicity in subsoils. Soil Science Society of America Journal 50: 28–34.

Hungate B.A., Dukes J.S., Shaw M.R., Luo Y., Field C.B., 2003. Nitrogen and climate change. Science 302: 1512–1513.

Jansson S.L., Persson J., 1982. Mineralization and immobilization of soil nitrogen. In: Stevenson FJ (ed.) Nitrogen in agricultural soils. Monogr 22, American Society of Agronomy 229-252.

Jarvis S.C., Stockdale, E.A., Shepherd, M.A., Powlson, D.S., 1996. Nitrogen mineralization in temperate agricultural soils: Process and measurement. Advanced Agronomy 57: 187–235.

Jayawickrama, K. 2001. Potential genetic gains for carbon sequestration: a preliminary study on radiata pine plantations in New Zealand. Forest Ecology and Management 152: 313–322.

Johnsen K.H., Wear D., Oren R., 2001. Meeting global policy commitments: carbon sequestration and southern pine forests. Journal of Forestry 99: 14–21.

Jones, D.L., 1998. Organic acids in the rhizosphere-a critical review. Plant and Soil 205: 25–44.

Jones, D.L., Darrah, P.R., 1994. Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. Plant and Soil 166: 247–257.

Jones D.L., Darrah P.R., Kochian L.V., 1996. Critical-evaluation of organic-acid mediated iron dissolution in the rhizosphere and its potential role in root iron uptake. Plant Soil 180: 57–66.

Jones D.L., Hodge A., Kuzyakov Y., 2004. Plant and mycorrhizal regulation of rhizodeposition. New Phytologist 163: 459–480.

Jose, S., Gillespie, A.R., Seifert, J.R., and Biehle, D.J., 2000. Defining competition vectors in a temperate alley cropping system in the mid-western USA. 2. Competition for water. Agroforestry Systems 48: 41–59.

Jose, S., Gillespie, A.R. and Pallardy, S.G. 2004.Interspecific interactions in temperate agroforestry. Agroforestry Systems 61: 237–255.

Kaur, B., Gupta, S.R., Singh, G., 2000. Soil carbon, microbial activity and nitrogen availability in agroforestry systems on moderately alkaline soils in northern India. Applied Soil Ecology 15: 283–294.

Knops, J.M.H., Tilman, D., 2000.Dynamics of soil nitrogen and carbon accumulation for 61 years after agricultural abandonment, Ecology 81: 88–98.

Kramer, P.J., Boyer, J.S., 1995. Water relations of plants and soils. Academic, New York.

Kurzatkowski, D., Martius, C., Garica, M. V. B., 2004. Litter decomposition, microbial biomass and activity of soil organisms in three agroforestry sites in central Amazonia. Nutrient Cycling in Agroecosystems 69: 257– 267.

Lee K.H., Jose S., 2003. Soil respiration and microbial biomass in a pecan–cotton alley cropping system in southern USA. Agroforestry Systems 58: 45–54.

Lemus, R., Parrish, D.J., Abaye, O., 2008. Nitrogen-use dynamics in switchgrass grown for

biomass. Bioenergy Research 153–162.

Liebig, M.A., Schmer, M.R., Vogel K.P., Mitchell. R.B., 2008.Soil carbon storage by switchgrass grown for bioenergy. BioEnergy Research 1: 215-222.

Lin, G., Ehleringer J.R., 1999. Elevated CO_2 and temperature impacts in different components of soil CO_2 efflux in Douglas-Firtterracosms. Global Change Biology 5: 157-168.

Lucas Garcia J.A., Barbas, C., Probanza, A,. Barrientos, M.L., Gutierrez Manero, F.J., 2001. Low molecular weight organic acids and fatty acids in root exudates of two Lupinus cultivars at flowering and fruiting stages. Phytochemical Analysis 12: 305–311.

Makumba, W., Akinnifesi, F. K., Janssen, B., Oenema, O., 2007.Long-term impact of a gliricidia-maize intercropping system on carbon sequestration in southern Malawi. Agriculture Ecosystems and Environment 118: 237–243.

Maier, C.A., Albaugh, T.J., Allen, J.L. et al. 2004. Respiratory carbon use and carbon storage in mid-rotation loblolly pine (*Pinus taeda* L.) plantations: the effect of site resources on the stand carbon balance. Global Change Biology 10: 1335–1350.

Marschncr, H., 1995. Mineral Nutrition of Higher Plants, 2nd Ed.; Academic Press: London.

McLaughlin, S. B., & Walsh, M. E., 1998. Evaluating Environmental Consequences of Producing Herbaceous Crops for Bioenergy. Biomass and Bioenergy 317-324.

McLaughlin, S.B., Bouton, J.H., 1999. Developing switchgrass as a bioenergy crop. Alexandria, VA: ASHS Press 282–99.

McLaughlin, S.B., Kszos, L.A. 2005. Development of switchgrass (Panicum virgatum) as a bioenergy feedstock in the United States. Biomass and Bioenergy 28: 515–535.

Mungai, N.W., Motavalli, P.P., Kremer, R.J., Nelson K.A., 2005. Spatial variation of soil enzyme activities and microbial functional diversity in temperate alley cropping systems

Biology and Fertility of Soils 42: 129–136.

Murphy D.V., Sparling G.P., and Fillery I.R.P., 1998. Stratification of microbial biomass C and N and gross N mineralization with soil depth in two contrasting Western Australian soils. Australian Journal of Soil Research 36: 45-55.

Nair, P.K. Ramachandran. 1993. An Introduction to Agroforestry. Klgwer Academic Publishers.S494.5.A45N3543 634.9'9—dc20.

Nardi S., Concheri G., Pizzeghello D., Sturaro A., Rella R., Parvoli G., 2000. Soil organic matter mobilization by root exudates. Chemosphere 5: 653–658

Neff, J.C., Asner, G.P., 2000. Dissolved organic carbon in terrestrial ecosystems: synthesis and a model. Ecosystems 4: 29-48.

Neumann, G., Romheld, V., 1999. Root excretion of carboxylic acids and protons in phosphorus-deficient plants. Plant and Soil 211: 121–130.

Neumann, G., Romheld, V., 2001. The release of root exudates as affected by the plant's physiological status The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface. Marcel Dekker, New York 41–93.

Oren, R., Ellsworth, D.S., Johnsen K.H., Phillips N., Ewers, B.E., Maier C., Schafer, K.V. McCarthy, H., Hendrey, G., 2001. Soil fertility limits carbon sequestration by forest ecosystems in a CO_2 - enriched atmosphere. Nature 411: 469–472.

Parker, M.M., Van Lear, D.H., 1996. Soil heterogeneity and root distribution of mature loblolly pine stands in Piedmont soils. Soil Science Society of America Journal 60: 1920–1925.

Parrish, D.J., Fike, J.H., 2005. The biology and agronomy of switchgrass for biofuels. Crit. Rev. Plant Science 24: 423–459.

Parrish, D. J., Fike, J. H., Bransby, D. I., Samson, R., 2008. Establishing and managing

switchgrass as an energy crop. Online. Forage and Grazing lands, doi:10.1094/FG-2008-0220-01-RV.

Peichl, M., Thevathasan N.V., Gordon A.M., Huss J., Abohassan R.A., 2006. Carbon sequestration potentials in temperate tree-based intercropping systems, Southern Ontario, Canada. Agroforestry Systems 66: 243–257.

Phillips, R.P., Finzi, A.C., Bernhardt, E.S., 2011. Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. Ecology Letters 14: 187-194.

Powlson, D.S., Brookes P.C., and Christensen, B.T., 1987. Measurement of microbial biomass provides an early indication of changes in total soil organic matter due to the straw incorporation. Soil Biology and Biochemistry 19: 159-164.

Qualls R.G. and Haines B.L., 1992. Biodegradability of dissolved organic matter in forest throughfall, soil solution, and stream water. Soil Science Society America Journal 56: 578-586.

Raich, J.W. and Schlesinger, W.H. 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus 44: 81-99.

Ragauskas, A.J., Williams, C.K., Davidson B.H., Britovsek, G., Cairney, J., Eckert, C.A., Frederick Jr. W.J., Hallett, J.P., Leak, D.J., Liotta, C.L., Mielenz, J.R., Murphy, R., Templer, R., Tschaplinski, T., 2006. The path forward for biofuels and biomaterials. Science 311: 484-489.

Reth, S., Reichstein, M., Fagle, E. 2005. Modeling temperature and moisture effects on C-N transformations in soils: Comparison of nine models. Ecological Modeling 102: 325-329.

Reeves, D.W., 1997. The role of soil organic matter in maintaining soil quality in continuous cropping systems. Soil and Tillage Research 43: 131–167.

Riffell, S. 2012. Potential biodiversity response to intercropping herbaceous biomass crops on forest lands. Journal of Forestry 110: 42–47.

Ryan, P.R., Delhaize, E., 2001. Function and mechanism of organic anion exudation from plant roots. Annual Reviews in Plant Physiology and Plant Molecular Biology 52: 527–560.

Saggar S., Hedley, C.B., Salt G.J., 2001. Soil microbial biomass, metabolic quotient and carbon and nitrogen mineralization in 25-year old Pinus radiata agroforestry regimes. Australian Journal of Soil Research 39: 491–504.

Samson, F., Knopf, F., 1994.Prairie conservation in North America. Bioscience 44: 418–421.

Schlesinger W., Lichter J., 2001. Limited carbon storage in soil and litter of experimental forest plots under increased atmospheric CO_2. Nature 411: 466–469.

Schultz, R.P. 1997. The ecology and culture of loblolly pine (*Pinus taeda* L.). USDA Agric. Handbook, U.S. Gov. Print. Off., Washington, DC.

Shengjing, S. 2011. In situ sampling of low molecular weight organic anions from rhizosphere of radiata pine (Pinus radiata) grown in a rhizotron system. Environmental and Experimental Botany 70: 131-142.

Sokhansanj, S., Mani, S. Turhollow, A., Kumar, A., Bransby, D., Lynd, L. 2009.Large Scale Production, Harvest and Transport of a Switchgrass (Panicum virgatum L.) - Current Technology and Visioning a Mature Technology Biofuels, Bioprod. Bioref. 3: 124–141.

Sparling, G.P., 1990. Importance of soil water content when estimating soil microbial C, N, and P by the fumigation extraction method. Soil Biology and Biochemistry 21: 245-253.

Sparling G. P. 1992. Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soil organic matter. Australian Journal of Soil Research 30: 195-207.

Stape, J.L., Binkley, D., Jacob, W.S., Ernesto, N.T., 2006. A twin-plot approach to determine

nutrient limitation and potential productivity in Eucalyptus plantations at landscape scales in Brazil. Forest Ecology and Management 223: 358–362.

Strahm, B.D., Harrison R.B., 2006. Nitrate Sorption in a Variable-Charge Forest Soil of the Pacific Northwest. Lippincott Williams & Wilkins, Inc. 171: 313–321.

Strobel, B.W., Bernhoft, I., and Borggaard, O.K., 1999. Low-molecular-weight aliphatic carboxylic acids in soil solutions under different vegetations determined by capillary zone electrophoresis. Plant Soil 212: 115–121.

Strobel B.W. 2001a.Influence of vegetation on low-molecular-weight carboxylic acids in soil solution – a review. Geoderma 99: 169–198.

Strobel, B.W., Hansen, H.C.B., Borggaard, O.K., Anderson, M.K., 2001b. Composition and reactivity of DOC in forest floor soil solutions in relation to tree species and soil type. Biogeochemistry 56: 1-26.

Suman A., Lal M., Singh A.K., Gaur A., 2006. Microbial biomass turnover in India subtropical soils under different sugarcane intercropping systems. Agronomy Journal 98: 698–794

Susaeta, A., 2011. Economics of intercropping loblolly pine and switchgrass for bioenergy markets in the southeastern United States. Agroforestry Systems; DOI: 10.1007/s10457-011-9475-3.

Thevathasan N.V., Gordon A.M., 2004. Ecology of tree intercropping systems in the north temperate region: experiences from southern Ontario. Can. Agroforestry Systems 61: 257–268.

Tilman D.J., 1985. The resource ratio hypothesis of plant succession. The American Naturalist. 125: 6.

Trichet P, Bakker, M.R., Aususto L., Alazard, P., 2009. Fifty years of fertilization experiments on Pinuspinaster in southwest France: the importance of phosphorus as a fertilizer. Forest Science 55: 390–402.

Trumbore, S.E., 1993. Comparison of carbon dynamics in tropical and temperate soils using radiocarbon measurements. Global Biogeochemical Cycles 7: 275–290.

Udawatta, R.P., Jose, S. 2011. Carbon sequestration potential of agroforestry practices in North American. Kumar B.M., and Nair P.K.R. (eds.). *Carbon Sequestration Potential of Agroforestry Systems: Opportunities and Challenges. Springer Science*. Advances in Agroforestry 8.

"U.S. Energy Information Administration - EIA - Independent Statistics and Analysis." *U.S. Energy Information Administration (EIA)*. Web. <http://www.eia.gov/>.

van Hees P.A.W., Andersson A-M.T., Lundstrom, U.S., 1996. Separation of organic low-molecular-weight aluminum complexes in soil solution by liquid- chromatography. Chemosphere 33: 1951–66.

van Hees P.A.W., Jones, D.L., Finlay, R., Godbold, D.L., 2005. The carbon we do not see? The impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. Soil Biology and Biochemistry 37: 1-13.

van Hees P.A.W., Jones, D.L., Godbold, D.L., 2002. Biodegradation of low molecular weight organic acids in coniferous forest podzolic soils. Soil Biol. Biochemistry 34: 1261-1272.

Vitousek P.M., 1998. Within-system element cycles, input–output budgets and nutrient limitation. In: Groffman PM, Pace ML, editors. Successes, limitations and frontiers in ecosystem science. New York: Springer 432–451.

Walker T.S., Bais H.P., Grotewold E., Vivanco J.M., 2003. Root exudation and rhizosphere biology. Plant Physiology 132: 44–51.

Wardle D.A., Lavelle P., 1997. Linkages between soil biota, plant litter quality and decomposition. In: Cadisch G, Giller KE (eds.) Driven by nature: plant quality and decomposition. CAB

International, Cambridge, UK, 107-124.

Wear, D., Gries J. 2002. Southern forest resource assessment. USDA Forest Service SRS-53, 635.

Wolf, D. D. 2009. Planting and Managing Switchgrass for Forage, Wildlife, and Conservation. Virginia Cooperative Extension Publication 418-013.

Wullscheleger S., Borsul D.E., Gunderson M.C., Lynd L., 2010. Biomass production in switchgrass across the United States: Database description and determinants of yield. Agronomy Journal 102: 1158–1168.

Chapter 3
Materials and Methods

3.1 Site Description & Experimental Design

In 2009, Catchlight Energy, LLC, a joint venture between Weyerhaeuser Company and Chevron Corporation, initiated a long-term study to investigate the effects of managing biomass for biofuels production in an existing 81 ha loblolly pine plantation in Lenoir County, NC (35.2506° N, 77.6306° W). Mean monthly temperatures range from a high of 24.1°C in July to a low of 10.4°C in January. Average annual precipitation is 125.98 cm (U.S. Climate Data). The elevation is 18.9 m and located on the Peedee geologic formation (LeGrand 1955). The study site was established following harvest of a loblolly pine plantation, planted in 1974, which had a site index of 70 at 25 years. Soil water table levels are lowered using linear ditches that run along forest edges and parallel each other through study site blocks (Figure 3.1). Site preparation comprising of biomass removal, v-shearing, raking of switchgrass plots, bedding, aerial herbicide application, and pre-planting fertilization (See Appendix B for details). Pine seedlings were planted 1-2 m apart on parallel raised beds spaced 6.1 m apart in December of 2008. Switchgrass (Alamo cultivar) was planted in June of 2009.

Within the previous stand a 72-ha research area was designated for the present study. This included a 38.4 ha reference unharvested stand and a 33.6 ha section divided into 28 experimental plots that were clear-cut harvested. The experimental design for this study was a randomized complete block with four replicates and five treatments per block. Treatments were randomly assigned to plots which ranged 0.8 ha in size. Of the five treatments installed, only the plots with biomass removed were used as part of this study (Figure 3.1). Treatments are depicted in Figure 3.2.

The three treatments used for this project are as follows:

- Pine (P): All residual material suitable for biofuel production was removed by an excavator and piled along the sides of the treatment area. Third generation loblolly pine seedlings were planted on beds at a density of 1,100 trees ha^{-1}. The remaining woody material was left as groundcover within the plot boundaries.

- Switchgrass (SG): A root-rake was utilized to pile all logging residuals along the edge of the treatment boundary. Switchgrass (Alamo cultivar) was planted at a rate of 9 kg $ha^{-1}$of pure live seed and is harvested once annually, during the fall.

- Pine intercropped with Switchgrass (PSG): All residual material feasible for biofuel production was removed by an excavator and piled along the sides of the treatment area. Pines were planted as described in the P treatment, and switchgrass (Alamo cultivar) was planted in-between pine rows as described in the SG treatment. The intercropped switchgrass was also harvested once annually in the fall.

3.2 Soils and Geology

The dominant soil types within the treatment areas were mapped as the Pantego (fine-loamy, siliceous, semiactive, thermic Umbric Paleaquults) and Rains (fine-loamy, siliceous, semiactive, thermic Typic Paleaquults) soil series. The parent materials for both soils are derived from marine deposits. Both soils are very poorly drained with medium to coarse textures (sandy loam to sandy clay loam). The diagnostic horizons of the Rains series include an ochric epipedon, argillic horizon. Ochric epipedons fail to meet the definition of any of the other eight epipedons and an argillic horizon is a subsurface horizon that has a significant accumulation of phyllosilicate clay. The

diagnostic horizons of the Pantego series are an umbric epipedon, and an argillic horizon. An umbric epipedon is similar to a mollic epipedon in color, organic carbon content and structure but has a base saturation of < 50%. Both soils have aquic features, are acid (pH = 3.5 to 5.5) and in relatively flat topographic positions (0 to 2 percent slope). These soils are widespread in the Lower, Middle and Upper Coastal Plain region mainly in depressional landscapes. Table 3.1 displays the inherit soil properties of the Pantego and Rains soil series (Soil Survey Staff 1999; 2006).

3.3 Field Measurements

Soil Sample Collection

Mineral soil (0-15 cm) was sampled roughly every six weeks with three subsamples averaged for each treatment plot, in all four blocks. Thus, there were 60 subsamples per sampling period, representing four samples per treatment. Soil samples were collected for microbial analysis to a depth of 20 cm using a 2.5 cm push tube and transferred into a plastic bag for transport. *In situ* soil samples were collected using constructed polyvinyl chloride (PVC) pipes (Figure 3.3) for the centrifuge drainage portion of the study using a 2.5 cm diameter x 15 cm length PVC pipe (Giesler and Lundstrom, 1993; Aiken, 1992). After collection all samples were immediately sealed in plastic bags and transported on ice to the laboratory for analysis. Until processing soil solutions were kept frozen at -4°C.

Soil CO_2 efflux rate, soil temperature, and moisture measurements

Every six weeks, soil CO_2 efflux rate was measured using a portable LI-6200 infrared gas analyzer equipped with a Li-Cor 6000-09S chamber (Li-Cor, LI-6200, Lincoln, NE, USA). The chamber volume was 926 cm^3 and covered 72 cm^2 of the soil surface. Prior to leaving for the field

the gas analyzer was zeroed and spanned with a 359 ppm CO_2 standard. Data was collected by block starting shortly after sunrise and continuing until late afternoon and three subsamples were taken from each treatment. The CO_2 concentration in the cuvette chamber was allowed to come to equilibrium with the ambient CO_2 concentration near the soil surface and the chamber was then placed on a vegetation free spot. Measurements began only when CO_2 concentrations steadily rose for at least a 30 second period.

At the same time, soil temperature and volumetric soil moisture were measured. Soil temperature was measured for each plot at a depth of 7.5 cm using a digital thermometer. Volumetric soil water content was measured across a 12 cm depth using a Hydrosense meter (Campbell Scientific, Australia).

3.4 Lab Analysis

Active Microbial Biomass C (Microbial Activity):

Substrate-induced respiration (SIR) is a measure of the active microbial biomass C. It is based on the respiration rates (CO_2 production) measured over a short-time period when the soil is given an excess supply of labile C (e.g., glucose). Respiration rates are proportional to the microbial activity in the given soil (Fierer 2003).

Soil used for SIR was analyzed within 48 hours of sample collection but was first quickly sieved to remove excess living plant and large woody biomass. Roughly 10 grams of sample were weighed into 50 mL incubation vials and a glucose solution that delivered 6 mg glucose per gram $soil^{-1}$ was added to each vial based on the method developed by Fierer (2003). The soil and glucose solution was mixed and caped with a septum for 20 min before initial CO_2 concentration measurements were taken from the headspace. Headspace gas was collected from each vial using a

100 µl syringe and injected into a closed loop design containing a septa of an infrared gas analyzer (Li-Cor 6200, Lincoln, NE). Samples were then shaken horizontally at 100 oscillations min^{-1} for 1 h before additional headspace gas sampling. Samples where then returned to the shaker and resampled every hour over a four-hour interval (Sparling 1995; Fierer 2003). A calibration curve to determine concentrations of unknowns was established using a 5000 ppm CO_2 standard where 50, 100, 250 µl of the standard was injected into the septa of the closed loop. A moisture-correction was done to correct for the percent water of the soil's weight. The moisture correction is the ratio of dry weight to the wet weight determined by placing 10 g of soil into an aluminum weight dish and placing the dish into a drying oven at 105°C for 24 hours before weighing.

Total Microbial Biomass C (Microbial Biomass):

Chloroform fumigation was used to estimate total microbial biomass C (Fierer 2003; Bailey 2002). Fumigation is used to lyse the microbial cells and the difference is compared to non-fumigated samples to access the C released by soil microbial biomass (Arias 2005).

Field moist, sieved soil used for chloroform fumigation was analyzed within 96 hours of collection based the methods developed by Vance (1987). Soils for fumigation (25 grams) were placed into 50 ml beakers and put into a vacuum desiccator with 80 ml of chloroform and some boiling chips. The vacuum was turned on for 4 minutes until the chloroform boiled and then the pressure was released and the desiccator allowed to vent. This step was repeated three more times and not allowed to vent the last time. The samples where left under vacuum for 24 h in a dark hood before the vacuum was released. Non-fumigated samples (25 grams) were weighted into 50 ml Nalgene bottles but not placed fumigated in a dessicator. For both fumigated and non-fumigated samples 50 ml of 0.5 M K_2SO_4 was added to the samples in Nalgene bottles and agitated on a

reciprocating shaker at a rate of 100 oscillations min^{-1} for 2 h and allowed to settle overnight after shaking. After settling, samples were passed through a pre-wetted (with 0.5 M K_2SO_4) Whatman #2 filter paper into scintillation vials and analyzed for a total organic carbon (TOC) on a Apollo 200 combustion TOC analyzer (Teleclyne, Ohio). The difference in TOC between chloroform-fumigation soils and the non-fumigated soils was calculated as microbial biomass C (Vance 1987). A moisture-correction was done to correct for differences in percent soil water. The moisture correction is the ratio of dry weight to the wet weight determined by placing 10 g of soil into an aluminum weight dish and placing the dish into a drying oven at 105°C for 24 hours before weighing.

Total Carbon & Nitrogen and Extractable Nutrients:

Total C and N and other extractable nutrient concentrations were analyzed for each grab sample collected. Once microbial biomass and activity samples were analyzed leftover mineral soil samples were removed from the freezer and dried at 65°C for 96 hours. After the samples were oven-dry they were then passed through a 2 mm sieve and ground using a mortar and pestle to break up the aggregates and analyzed for total C and N on a Vario MAX Dry Combustion CNS analyzer (Elementar, Hanau, Germany) Samples were analyzed four times over the course of this study in March 2012, June 2012, October 2012, and February 2013. Extractable P, K, Ca, Mg, Zn, Mn, Cu, Fe, and B were analyzed by using the Mehlich 1 extraction with an automatic pipetting machine (Sparks 1996). All elements are analyzed in the same extract by an ICP (inductively coupled plasma atomic emission spectrometer). A combination electrode was used to measure soil pH in a standard 2:1 water to soil suspension.

LMWOAs, DOC, Total Dissolved N, Ammonium and Nitrate:

The centrifuge drainage technique is based on the procedure by Giesler and Lundstrom (1993) where intact soil cores (constructed pipes) seen in Figure 3.3 were centrifuged for 30 min at 1,340 g within 36 h of sampling to collect entrained soil solution. The soil solution was passed through a 0.45 μm membrane filter and stored at -4°C until DOC, TDN, LMWOAs, NH_4^+-N and NO_3^--N could be analyzed (Aiken 1992; DiStefano 1986; Giesler and Lundstrom 1993).

LMWOAs were analyzed on a Dionex Ion Chromatography System (IC) (Dionex Corp., Sunnyvale, CA) using an anion exclusion conductivity detector. The Dionex ASRS* 300 Anion Self-Regenerating Suppressor and a KOH elutant generator were also used and delivered at a rate of 1 ml min^{-1}. Dionex IonPac AS17 guard and AS17 analytical columns were used to separate the organic anions. The Dionex IonPac AS17 columns are specifically designed for complex sample matrices of wastewater solutions that contain inorganic and organic acids using a hydroxide gradient. Ten species of LMWOA were analyzed including: acetate, citrate, formate, fumarate, lactate, malate, malonate, oxalate, tartrate, and succinate. Phosphate was also analyzed on the IC (Fox and Comerford 1990; Aiken 1992; Town 1993; Tani 2001). Citrate, fumarate, acetate, succinate and oxalate are some of the listed species that are commonly found in cultivated C4 plants. Determination of the chromatographic retention times for a single organic acid was made by running duplicate chromatograms over the course of 30 min with the individual acids. Individual retention times were determined before mixed standards were made. Linear calibration plots were generated by the analysis of the mixed organic acid standards. Mixed acid standards containing 0.25, 0.5 and 5 ppm of each of ten acids and phosphate were analyzed three times and standards were not kept more than five days. A conservative measure of the detection limit for an individual acid was estimated by extrapolation of the standard linear calibration to the low concentration standard. Results were

calculated using Camilion software (Dionex Corp., Sunnyvale, CA) and were converted to μM. Since limited sample volume could be obtained using the centrifuge drainage technique, only a 0.5 ml was used for the analysis and no replicates were possible. Accurately measuring LMWOA concentrations is extremely difficult and the centrifuge drainage technique used for this study only measures the easily accessible acids present in solution collected during the specific time the sample was taken and this method does not release significant amounts of the acids from live roots and microbes (Jones 1998).

Soil water from constructed pipes was analyzed for NH_4^+-N and NO_3^--N concentration using a TRAACS 2000 analytical console (Bran & Luebbe, Norderstedt, Germany). Mixed standards containing 0.25, 0.5, 2.5 and 5 ppm of NH_4^+-N and NO_3^--N were used to calibrate the TRAACS 2000 analytical console (Leenheer 2007).

DOC and total dissolved N (TDN) were also measured using the centrifuge drainage technique described above. Since soil water was limited, all samples were diluted to 7 ml before being analyzed using Apollo 200 combustion TOC analyzer (Teleclyne, Ohio) and numbers were corrected for moisture content in order to express values on a dry weight soil basis.

DON was calculated using TDN and NH_4^+-N and NO_3^--N data using the following equation:

$$DON = TDN - (NH_4^+\text{-}N + NO_3\text{-}N)$$

3.5 Statistical Analysis

Treatment and time effects for DOC, TDN, DON, individual LMWOAs, CO_2 efflux rate, active and total microbial biomass carbon were determined with a repeated measures analysis using the PROC MIXED procedure at $\alpha = 0.05$ (Littell et al., 1998) (SAS Institute Inc., Cary, NC, USA). Replicated subsamples within treatments were averaged for the statistical analysis. Differences in

treatment means for each dependent variable were tested using Kramer-Tukey test (HSD) at $\alpha = 0.05$.

Multiple linear regression analysis conducted in JMP software (SAS Institute Inc., Cary, NC, USA) was used to examine significant relationships between the various soils parameters measured during the study. A seasonal model was developed to predict soil CO_2 efflux, microbial activity, DOC, and TDN. The data set for the entire sampling period was used for this analysis.

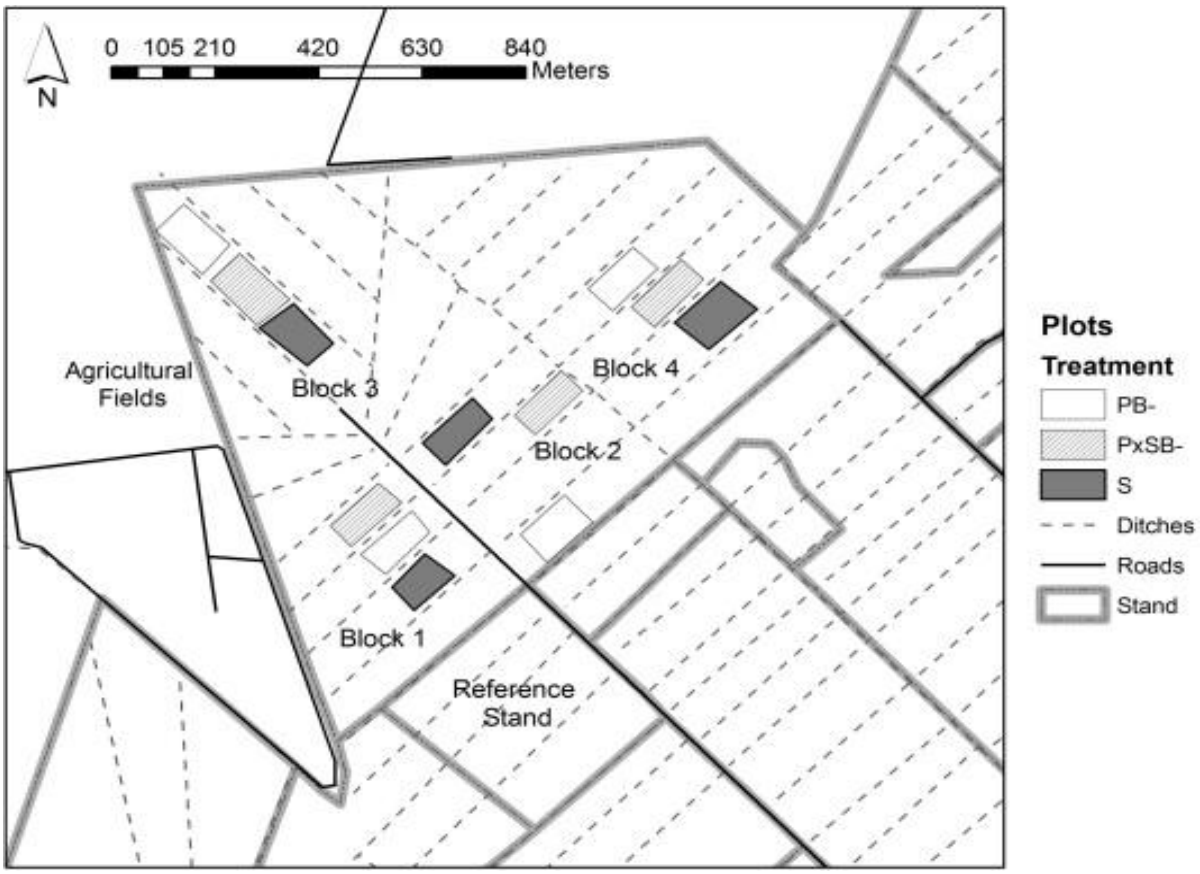
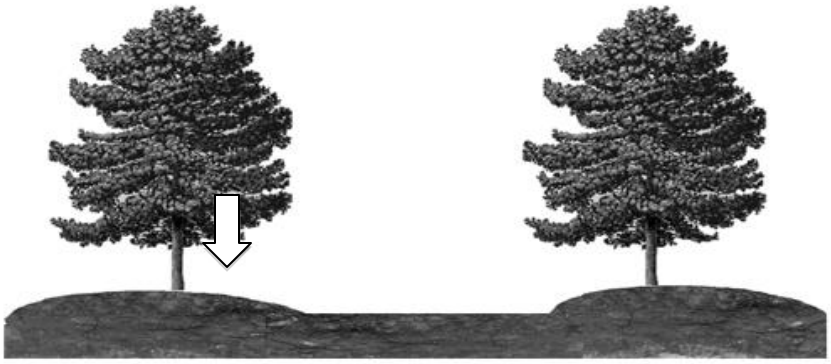
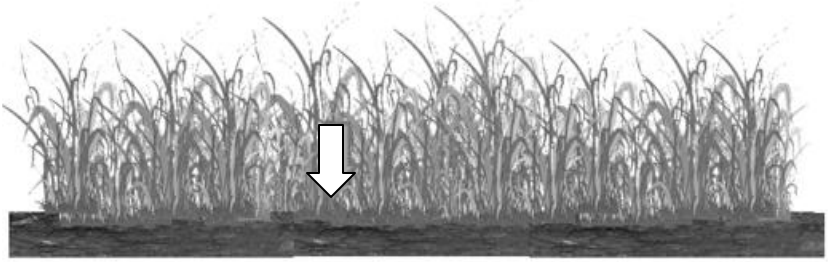


Figure 3.1: Lenior 1 site layout (Lenior County, NC) showing only plots sampled in each of the four blocks. Dashed lines indicate man-made linear ditches to maintain water levels.



Sampling on bed in pine only (PB) treatments indicated by the orange arrow.



Sampling of switchgrass (SG-I) only treatments that are flat planted.

Sampling on bed, boundary and interbed of pine-switchgrass (PSG) intercropped treatments.

Figure 3.2: Sampling location depicted (white arrow) in each of the treatments used as part of this study.

Figure 3.3: Sample device used to collect soil solution for the centrifuge drainage technique.

3.6 References

Aiken, G. R. McKnight D.M., Wershaw, R.L., MacCarthy, P., 1985. Humic Substances in Soil, Sediment, and Water: geochemistry, isolation and characterization. Wiley-Interscience: New York 692.

Arias, M.E., Gonzalez-Perez, J.A., Gonzalez-Vila, F.J., Ball, A.S., 2005. Soil health: A new challenge for microbiologists and chemists. Int. Microbial 8: 13–21.

Bailey, V.L., Peacock A.D., Smith, J.L., Bolton, H. Jr., 2002.Relationship between soil microbial biomass determined by chloroform fumigation-extraction, substrate-induced respiration, and phospholipid fatty acids analysis. Soil Biology and Biochemistry 34: 1385–1389.

DiStefano, J.F. and Gholz, H.L., 1986. A proposed use of ion exchange resins to measure nitrogen mineralization and nitrification in intact soil cores. Communications in Soil Science and Plant Analysis. American Society of Agronomy.

Fierer N., Schimel, J.P., Holden, P.A., 2003. Variations in microbial community composition through two soil depth profiles. Soil Biology & Biochemistry 35: 167–176.

Fox, T.R., Comerford, N.B., 1990. Low-molecular-weight organic- acids in selected forest soils of the Southeastern USA. Soil Science Society of America Journal 54: 1139–1144.

Giesler R., Lundström U.S., 1993. Soil solution chemistry - the effects of bulking soil samples and spatial variation. Soil Science Society of America Journal 57: 1283–1288.

Janssens, I.A., Kowalski, A.S., Longdoz, B., and Ceulemans, R., 2000. Assessing forest soil CO_2 efflux: an *in situ* comparison of four techniques. Tree Physiology 20: 23-32.

Jones, D.L., 1998. Organic acids in the rhizosphere-a critical review. Plant and Soil 205: 25–44.

Leenheer, Jerry A. 2007. Dissolved Organic Nitrogen Fractionation. Annuals of Environmental

Science 1: 45-56.

LeGrand H.E., Brown P.E., 1955. Guidebook of Excursion in Coastal Plain of North Carolina. October 8-9, 1955. Carolina Geological Society. US Geological Survey, Raleigh N.C.

Littell, R.C., Henry, P.R., and Ammerman, C.J., 1998. Statistical Analysis of Repeated Measures Data Using SAS Procedures. Journal of Animal Science 76: 1216-1231.

NOAA, National Climatic Data Center, Department of Commerce. Dover, North Carolina. http://www.ncdc.noaa.gov/.

Soil Survey Staff, Pantego Series.1999. Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. http://websoilsurvey.nrcs.usda.gov/.

Soil Survey Staff, Rains Series. 2006. Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. http://websoilsurvey.nrcs.usda.gov/.

Sparks, D. L., 1996. Methods of Soil Analysis, Part 3, Chemical Methods, Soil Science Society of America.

Sparling, G. P., 1995. The substrate induced respiration method, pp. 397–404. In K. Alef and P. Nannipieri (ed.), Methods in applied soil microbiology and biochemistry. Academic Press, London, United Kingdom.

Tani, M., 2001.Determination of water-soluble low-molecular-weight organic acids in soils by ion chromatography. Soil Science. Plant Nutrition 47 2: 387-397.

Town, R.M., Powell, H.K.J., 1993. Limitations of XAD resins for the isolation of the non-colloidal humic fraction in soil extracts and aquatic samples. Analytica Chimica Acta 271 2: 195-202.

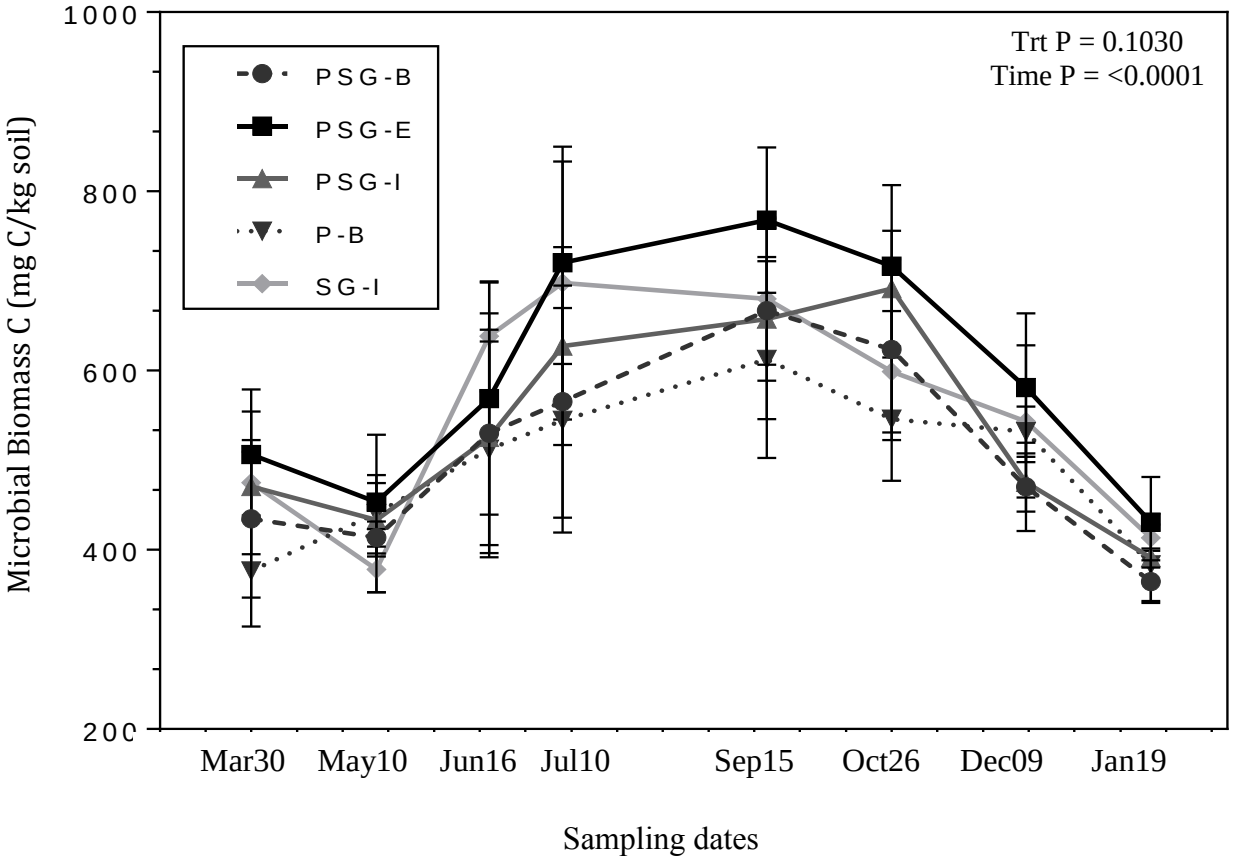
Vance E.D., Brookes P.C., Jenkinson D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19: 703–707.

Chapter 4
Results

4.1 Microbial Biomass and Activity

Microbial biomass did not differ significantly between treatments ($P = 0.1026$) for any of the eight sampling periods from March 2012 to January of 2013 (Figure 4.1). It is worth noting, however, that the mean microbial biomass for PSG-E was generally higher and P-B generally lower than the other treatments over the course of the year. The microbial biomass data did show seasonal patterns ($P<0.0001$). Microbial biomass increased during the growing season and peaked in September 2012 before declining to its lowest point for all treatments in January 2013 (Figure 4.1).

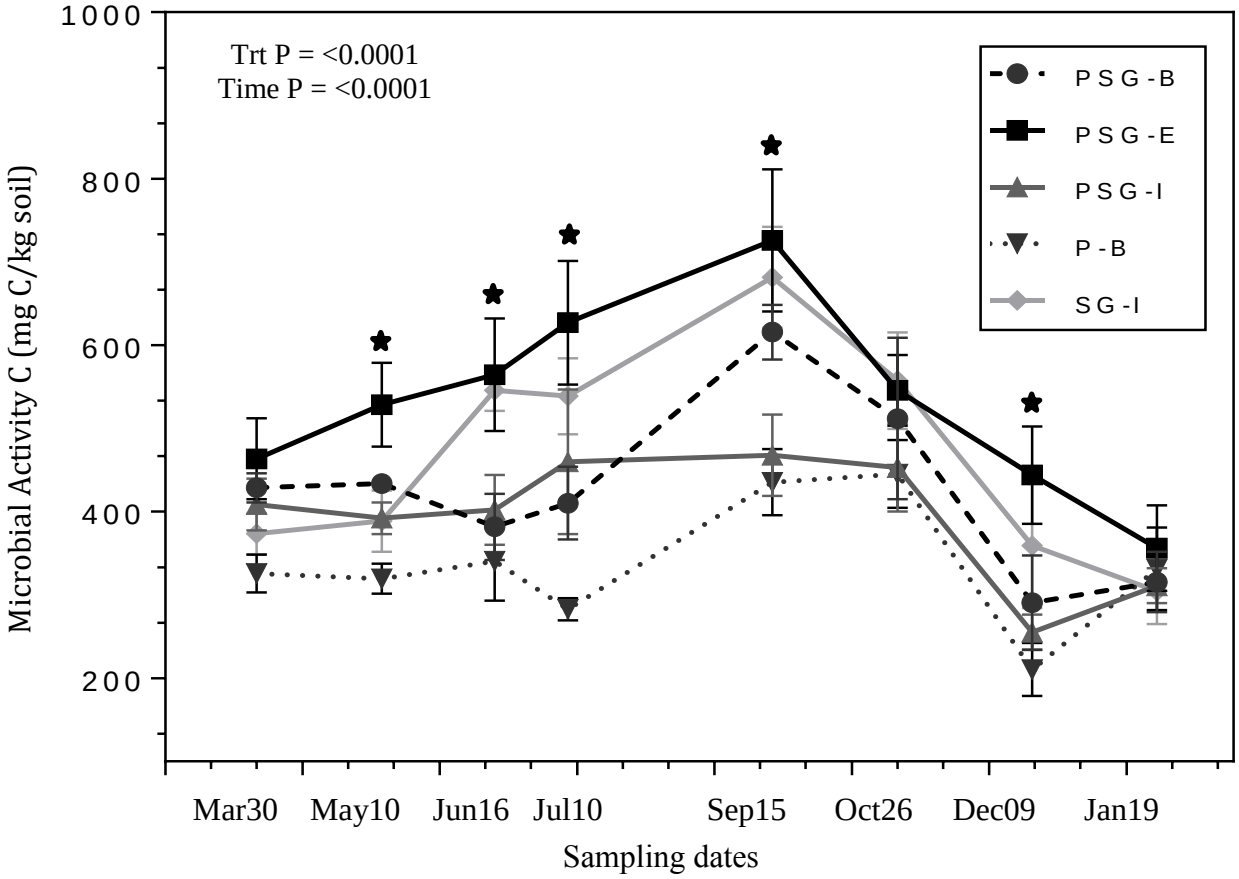
Microbial activity showed highly statistically significant differences for both treatment ($P<0.001$) and time ($P<0.001$) (Figure 4.2). Microbial activity, like microbial biomass, increased during the growing season and peaked in September 2012 before declining to its lowest point for all treatments in January 2013. PSG-E generally had the highest microbial activity of all treatments, while P-B generally had the lowest (Table 4.2).



	Mar 30	May 10	Jun 16	Jul 10	Sept 15	Oct 26	Dec 09	Jan 19
PSG-B	a[1]	a	a	a	a	a	a	a
P-B	a	a	a	a	a	a	a	a
PSG-E	a	a	a	a	a	a	a	a
PSG-I	a	a	a	a	a	a	a	a
SG-I	a	a	a	a	a	a	a	a

[1] Means with different letters show significant differences for treatments by each date using Tukey's HSD at the 0.05 level.

Figure 4.1: Statistical analysis of mean microbial biomass (mg C/kg soil) for pure pine (P-B); pine + switchgrass bed, edge and interbed (PSG-B, PSG-E, PSG-I); and pure switchgrass (SG-I) treatments. P-values in upper right corner indicate the test effects for treatment and time for the Proc Mixed analysis. Values represent the average of three subsamples and four blocks and error bars represent ± one standard error from the mean. A star on the graph indicates significant treatment differences.



	Mar 30	May 10	Jun 16	Jul 10	Sept 15	Oct 26	Dec 09	Jan 19
PSG-B	a[1]	ab	ab	bc	ab	a	ab	a
P-B	a	b	b	c	b	a	b	a
PSG-E	a	a	a	a	a	a	a	a
PSG-I	a	ab	ab	abc	b	a	ab	a
SG-I	a	ab	a	ab	a	a	ab	a

[1] Means with different letters show significant differences for treatments by each date using Tukey's HSD at the 0.05 level.

Figure 4.2: Statistical analysis of microbial activity (mg C/kg soil) for pure pine (P-B); pine + switchgrass bed, edge and interbed (PSG-B, PSG-E, PSG-I); and pure switchgrass (SG-I) treatments. P-values in upper right corner indicate the test effects for treatment and time for the Proc Mixed analysis. Values represent the average of three subsamples and four blocks and error bars represent ± one standard error from the mean. A star on the graph indicates significant treatment differences.

4.2 Total Carbon, Nitrogen and Extractable Nutrients

There were no treatment differences in total soil C, N, C/N ratio, pH, CEC, or any of the extractable nutrients (Table 4.1). Further, there were no clear trends within the data with respect to treatment.

Table 4.1: Statistical Analysis of basic soil nutrient levels for pure pine (P-B); pine + switchgrass bed, edge and interbed (PSG-B, PSG-E, PSG-I); and pure switchgrass (SG-I) treatments measured to 15 cm in a loblolly pine and switchgrass intercropped system. Collection dates in March, May, October, and December 2012.

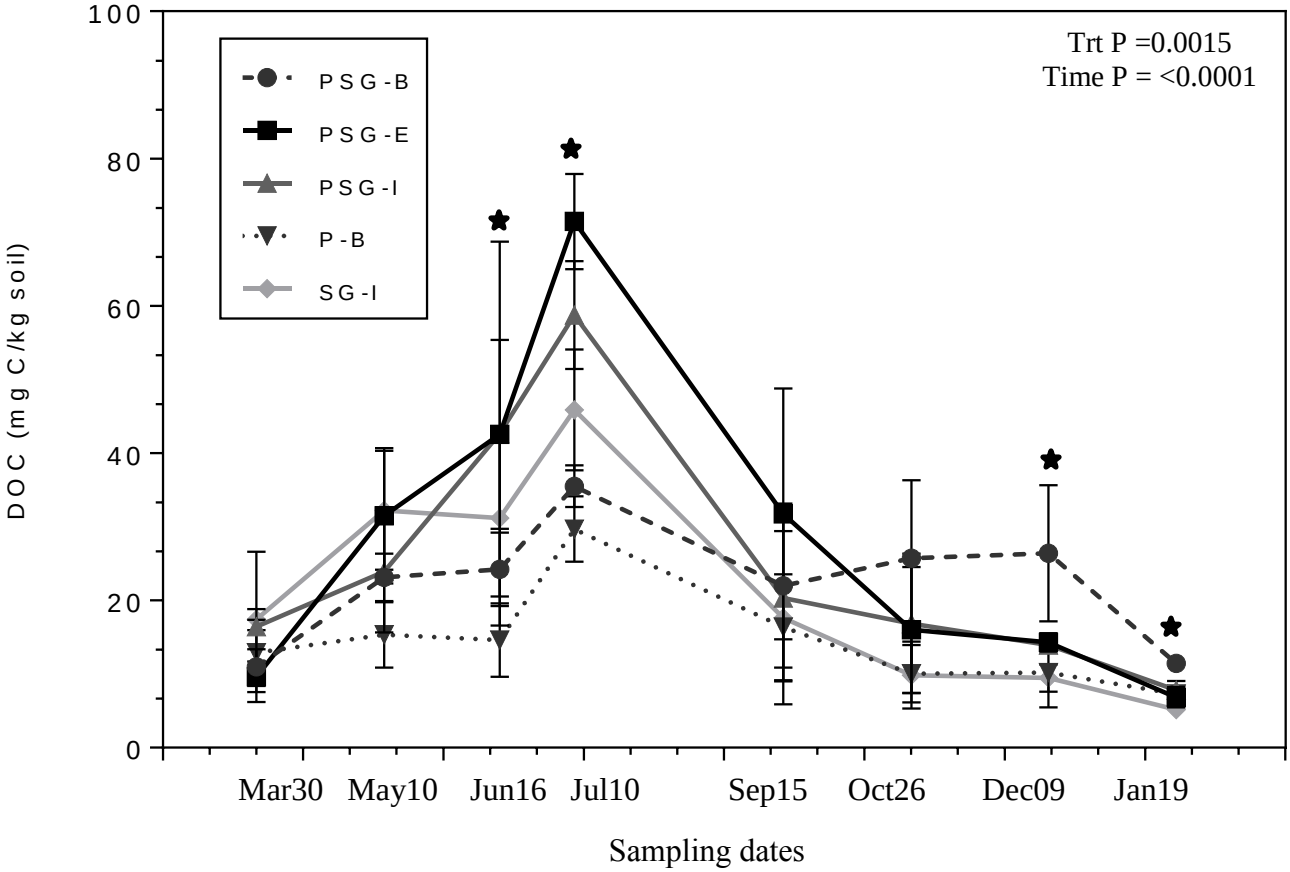
Mean Soil Nutrient Levels at 15 cm					
	Treatment				
	PSG-B	PB	PSG-I	SG-I	PSG-E
Soil C (%)	7.4±0.59(a)[1]	5.7±0.83(a)	6.2±0.14(a)	7.5±0.65(a)	7.3±0.86(a)
Soil N (%)	0.26±0.05(a)	0.30±0.02(a)	0.29±0.03(a)	0.29±0.04(a)	0.35±0.01(a)
C:N Ratio	28.5±1.64(a)	21.3±3.39(a)	21.5±3.00(a)	26.0±2.58(a)	20.9±3.21(a)
pH	3.8±0.07(a)	3.9±0.11(a)	3.6±0.06(a)	3.8±0.09(a)	3.9±0.04(a)
CEC ($cmol_c/kg$)	10.1±0.27(a)	10.3±0.55(a)	10.0±0.46(a)	11.1±0.40(a)	10.7±0.56(a)
Base Saturation (%)	11.0±1.18(a)	10.5±1.82(a)	6.5±0.60(a)	12.3±2.55(a)	9.2±1.02(a)
P (mg/kg)	8.3±0.48(a)	6.8±0.93(a)	14.8±1.01(a)	11.3±2.31(a)	9.3±0.63(a)
K (mg/kg)	25.3±1.11(a)	24.8±2.45(a)	27.0±2.16(a)	30.8±1.03(a)	25.8±1.93(a)
Ca (mg/kg)	148.0±11.03(a)	154.5±10.78(a)	78.8±4.78(a)	205.7±15.24(a)	121.5±8.19(a)
Mg (mg/kg)	34.5±1.01(a)	29±1.68(a)	21.5±0.28(a)	34.5±3.35(a)	34.0±3.18(a)
Zn (mg/kg)	0.78±0.12(a)	0.55±0.03(a)	0.52±0.05(a)	1.1±0.26(a)	1.0±0.10(a)
Mn (mg/kg)	2.0a±0.59(a)	1.2±0.30(a)	0.9±0.06(a)	2.0±0.50(a)	1.2±0.13(a)
Cu (mg/kg)	0.2±0.01(a)	0.2±0.01(a)	0.2±0.05(a)	0.2±0.03(a)	0.2±0.02(a)
Fe (mg/kg)	13.4±0.88(a)	12.2±0.86(a)	23.3±2.11(a)	13.1±1.77(a)	17.6±1.59(a)
B (mg/kg)	0.1±0.00(a)	0.13±0.02(a)	0.1±0.00(a)	0.15±0.01(a)	0.1±0.00(a)

[1] Means with different letters show significant differences for treatment by each date using Tukey's HSD at the 0.05 level.

4.3 Dissolved Carbon and Nitrogen in Soil Solution

Mean DOC in soil solution had significant treatment effects (P = 0.0015) and highly significant differences in concentration with time (P<0.0001) (Figure 4.3). Treatment differences were sporadic and as with microbial biomass and activity the PSG-E treatments generally had the highest DOC and P-B had the lowest DOC. This difference was significant on the June 16th and July 10th sampling dates. Overall all treatments increased as they moved into the growing season, peaking in the July sampling period and declined to the lowest point for all treatments in January (Figure 4.3).

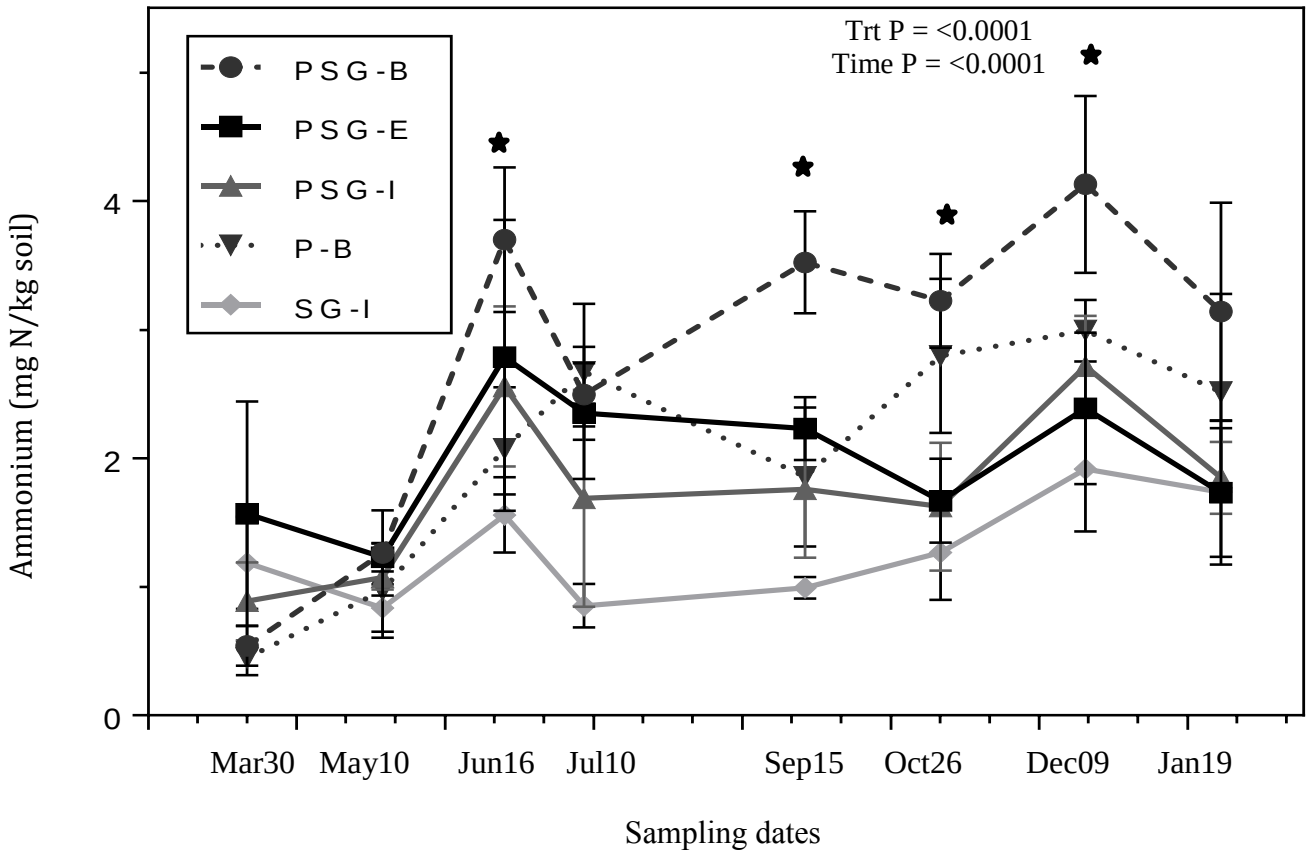
Although some significant treatment differences were present for NO_3^- (P = 0.0521), NH_4^+(P<0.0001), TDN (P<0.0001), and DON (P = 0.0122), there were no consistent or meaningful patterns among the treatments (Figures 4.4, 4.5, 4.6, 4.7). Significant differences in time (P<0.0001) were also evident. For the inorganic forms of N (NH_4^+ and NO_3^-), these differences occurred after N fertilization of the switchgrass. Fertilization occurred in April of 2012 in treatments where switchgrass was present (See timeline in Appendix B), and a spike in $NO_3^-$and NH_4^+can be seen in June 2012 in all treatments. After NH_4^+ concentrations peaked PSG-B, PSG-I, PSG-E and SG-I treatments continued to remain elevated above March 2012 data while P-B treatments, where no fertilization occurred, showed less change over time (Figure 4.4). NO_3^-concentrations remained relatively low when compared to NH_4^+. Like NH_4^+ a NO_3^- peak can be distinguished in June, but NO_3^- declined from 1.13 mg NO_3^--N kg^{-1} to 0.61 mg NO_3^--N kg^{-1}in September. There was a significant time lag between NH_4^+ and NO_3^- response and NO_3^- concentrations for most treatments continued to rise throughout the rest of the year (Figure 4.5).



	Mar 30	May 10	Jun 16	Jul 10	Sept 15	Oct 26	Dec 09	Jan 19
PSG-B	a[1]	a	ab	bc	a	a	a	a
P-B	a	a	b	c	a	a	b	ab
PSG-E	a	a	a	a	a	a	b	ab
PSG-I	a	a	a	ab	a	a	b	ab
SG-I	a	a	ab	abc	a	a	b	a

[1] Means with different letters show significant differences for treatments by each date using Tukey's HSD at the 0.05 level.

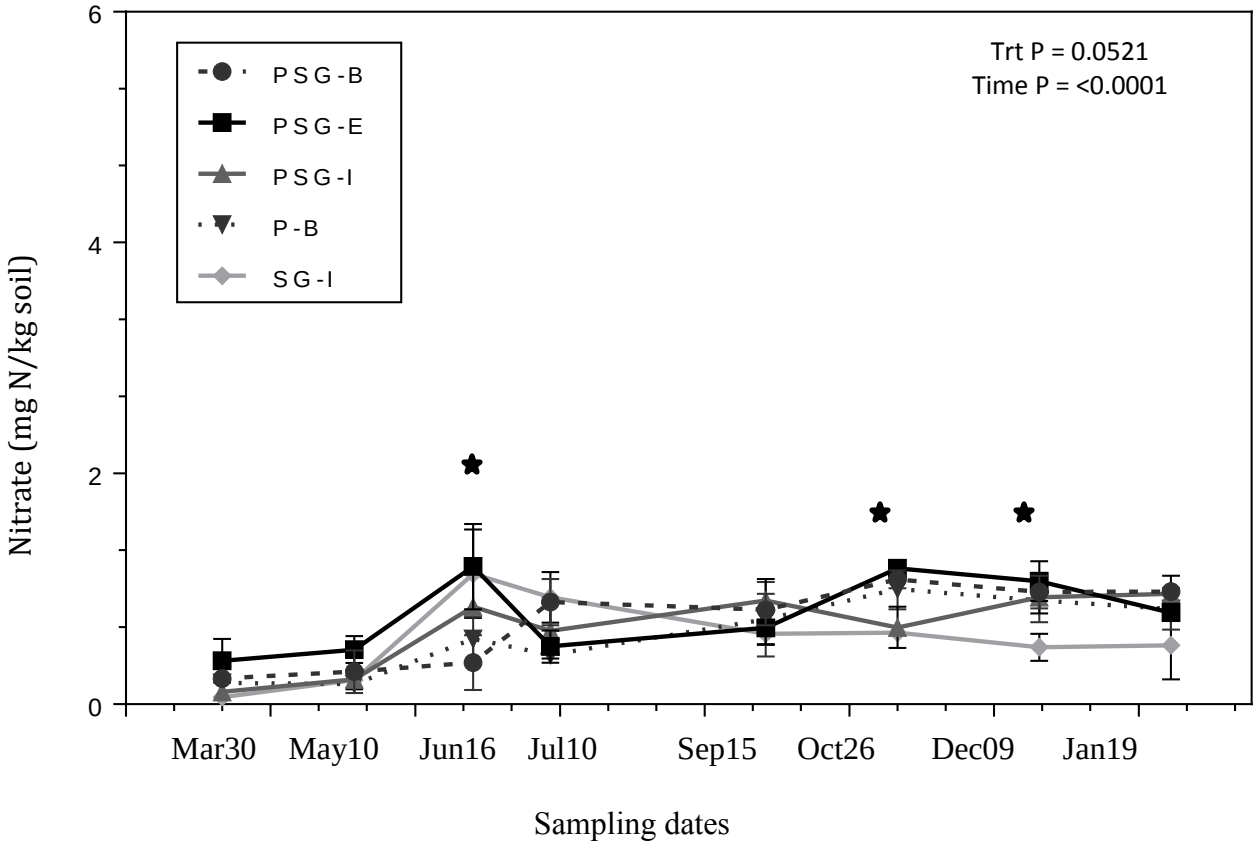
Figure 4.3: Statistical analysis of mean DOC (mg C/kg soil) for pure pine (P-B); pine + switchgrass bed, edge and interbed (PSG-B, PSG-E, PSG-I); and pure switchgrass (SG-I) treatments. P-values in upper right corner indicate the test effects for treatment and time for the Proc Mixed analysis. Values represent the average of three subsamples and four blocks and error bars represent ± one standard error from the mean. A star on the graph indicates significant treatment differences.



	Mar 30	May 10	Jun 16	Jul 10	Sept 15	Oct 26	Dec 09	Jan 19
PSG-B	a[1]	a	a	a	a	a	a	a
P-B	a	a	b	a	ab	ab	ab	a
PSG-E	a	a	ab	a	ab	ab	ab	a
PSG-I	a	a	a	a	b	ab	ab	a
SG-I	a	a	ab	a	b	b	b	a

[1] Means with different letters show significant differences for treatments by each date using Tukey's HSD at the 0.05 level.

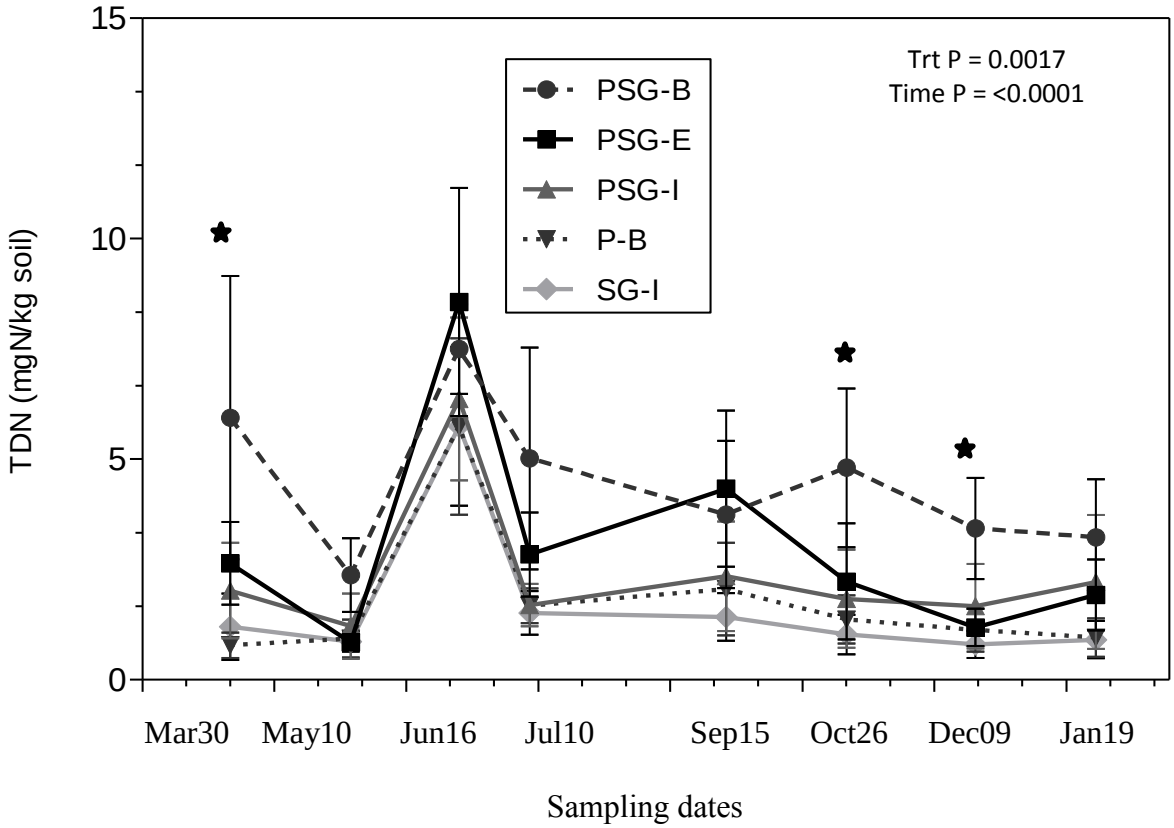
Figure 4.4: Statistical analysis of mean NH_4^+ (mg N/kg soil) for pure pine (P-B); pine + switchgrass bed, edge and interbed (PSG-B, PSG-E, PSG-I); and pure switchgrass (SG-I) treatments. P-values in upper right corner indicate the test effects for treatment and time for the Proc Mixed analysis. Values represent the average of three subsamples and four blocks and error bars represent ± one standard error from the mean. A star on the graph indicates significant treatment differences.



	Mar 30	May 10	Jun 16	Jul 10	Sept 15	Oct 26	Dec 09	Jan 19
PSG-B	a[1]	a	b	a	a	c	ab	a
P-B	a	a	ab	a	a	ab	ab	a
PSG-E	a	a	a	a	a	a	a	a
PSG-I	a	a	ab	a	a	ab	ab	a
SG-I	a	a	ab	a	a	b	b	a

[1] Means with different letters show significant differences for treatments by each date using Tukey's HSD at the 0.05 level.

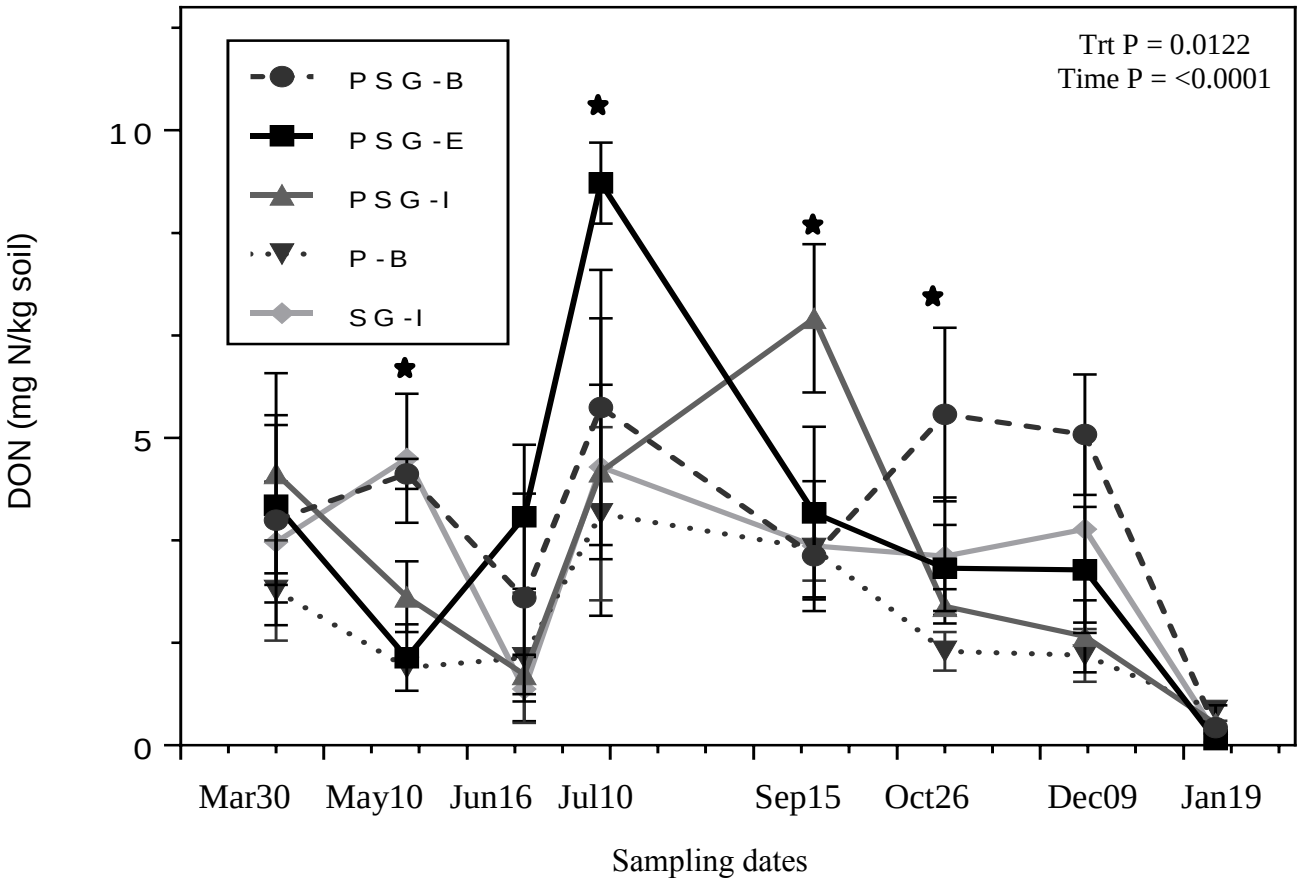
Figure 4.5: Statistical analysis of mean NO_3^- (mg N/kg soil) for pure pine (P-B); pine + switchgrass bed, edge and interbed (PSG-B, PSG-E, PSG-I); and pure switchgrass (SG-I) treatments. P-values in upper right corner indicate the test effects for treatment and time for the Proc Mixed analysis done in SAS. Values represent the average of three subsamples and four blocks and error bars represent ± one standard error from the mean. A star on the graph indicates significant treatment differences.



	Mar 30	May 10	Jun 16	Jul 10	Sept 15	Oct 26	Dec 09	Jan 19
PSG-B	a[1]	a	a	a	a	a	a	a
P-B	b	a	a	a	a	b	ab	a
PSG-E	b	a	a	a	a	ab	ab	a
PSG-I	b	a	a	a	a	ab	ab	a
SG-I	b	a	a	a	a	b	b	a

[1] Means with different letters show significant differences for treatments by each date using Tukey's HSD at the 0.05 level.

Figure 4.6: Statistical analysis of mean TDN (mg N/kg soil) for pure pine (P-B); pine + switchgrass bed, edge and interbed (PSG-B, PSG-E, PSG-I); and pure switchgrass (SG-I) treatments. P-values in upper right corner indicate the test effects for treatment and time for the Proc Mixed analysis. Values represent the average of three subsamples and four blocks and error bars represent ± one standard error from the mean. A star on the graph indicates significant treatment differences.



	Mar 30	May 10	Jun 16	Jul 10	Sept 15	Oct 26	Dec 09	Jan 19
PSG-B	a[1]	ab	a	b	b	a	a	a
P-B	a	b	a	b	b	b	a	a
PSG-E	a	b	a	a	b	ab	a	a
PSG-I	a	ab	a	b	a	ab	a	a
SG-I	a	a	a	b	b	ab	a	a

[1] Means with different letters show significant differences for treatments by each date using Tukey's HSD at the 0.05 level.

Figure 4.7: Statistical analysis of mean DON (mg N/kg soil) for pure pine (P-B); pine + switchgrass bed, edge and interbed (PSG-B, PSG-E, PSG-I); and pure switchgrass (SG-I) treatments. P-values in upper right corner indicate the test effects for treatment and time for the Proc Mixed analysis. Values represent the average of three subsamples and four blocks and error bars represent ± one standard error from the mean. A star on the graph indicates significant treatment differences.

4.4 Low Molecular Weight Organic Acids in Soil Solution

Of the nine LMWOAs analyzed only seven were found, including: acetic, citric, formic, lactic, malic, oxalic, and succinic acids. Fumarate, malonate and tartrate were not detected in this study. Analyses of the individual acids show significant time effects for all detected acids (Figure 4.8). Only malic and acetic acids showed significant treatment effects. Treatments with switchgrass present (PSG-I, SG-I) showed significantly higher concentrations of malic acid in June and July. Acetic acid only showed significant differences in March 2012 and January 2013 where bedded treatments (PSG-B and P-B) were significantly higher than treatments with switchgrass (PSG-I and SG-I). Despite the lack of treatment differences, some of the individual acids showed consistent patterns. Concentrations were lowest during the growing season and highest during the winter months for acetic, lactic, and formic acids. Malic and oxalic acids were the reverse with the highest values occurring in the summer months and lowest in the winter. Citric and succinic acids remained relatively low starting in the March 2012 and peaked in January of 2013 for most of the treatments. The total percent of all of the LMWOAs that contributed to measured DOC ranged from 5.8% to 12.5% calculated from adding the concentrations for all of the acids from DOC concentrations.

Phosphate (PO_4^{3-}) in soil solution showed slightly significant time effects ($P = 0.0127$) but not significant treatment effects ($P = 0.1147$) (Figure 4.9). PO_4^{3-} did not show any consistent patterns and standard errors were generally quite high.

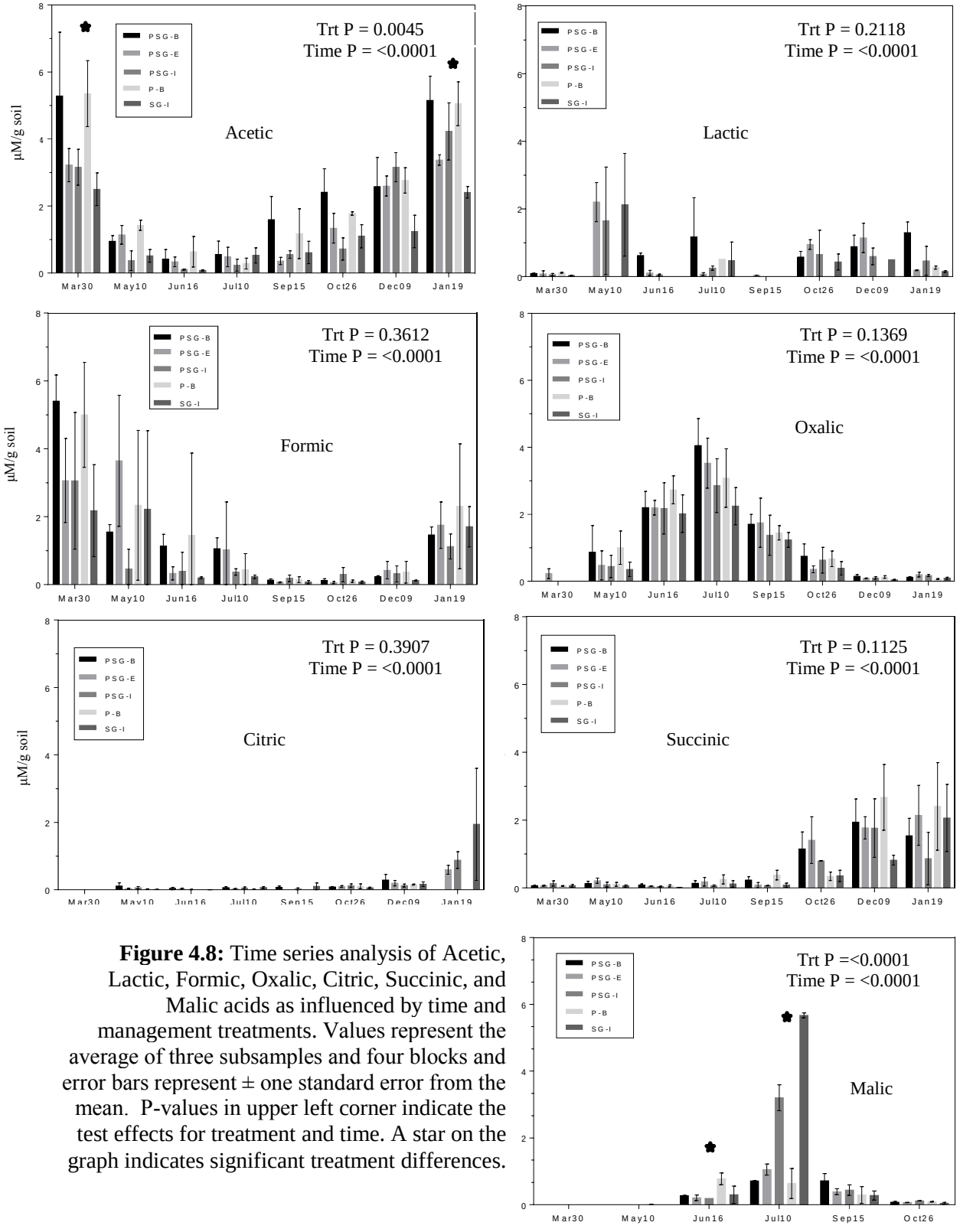
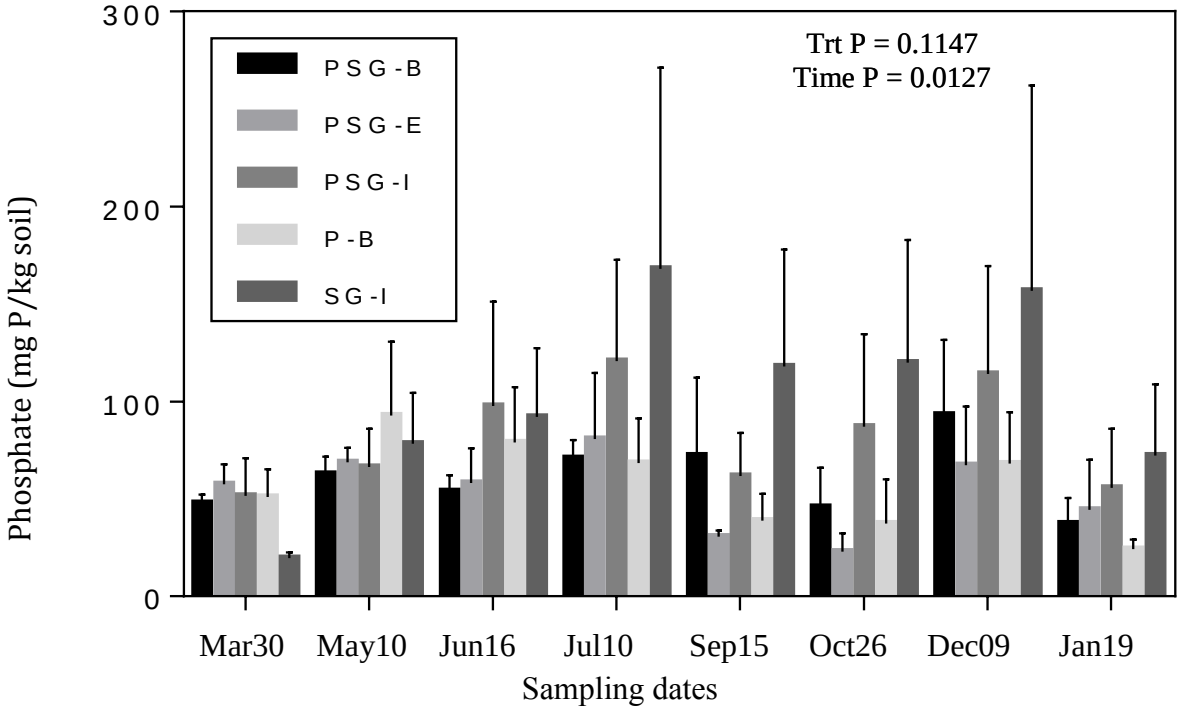


Figure 4.8: Time series analysis of Acetic, Lactic, Formic, Oxalic, Citric, Succinic, and Malic acids as influenced by time and management treatments. Values represent the average of three subsamples and four blocks and error bars represent ± one standard error from the mean. P-values in upper left corner indicate the test effects for treatment and time. A star on the graph indicates significant treatment differences.



	Mar 30	May 10	Jun 16	Jul 10	Sept 15	Oct 26	Dec 09	Jan 19
PSG-B	a[1]	a	a	a	a	a	a	a
P-B	a	a	a	a	a	a	a	a
PSG-E	a	a	a	a	a	a	a	a
PSG-I	a	a	a	a	a	a	a	a
SG-I	a	a	a	a	a	a	a	a

[1] Means with different letters show significant differences for treatments by each date using Tukey's HSD at the 0.05 level.

Figure 4.9: Statistical analysis of mean PO_4^{3-} (mg P/kg soil) for pure pine (P-B); pine + switchgrass bed, edge and interbed (PSG-B, PSG-E, PSG-I); and pure switchgrass (SG-I) treatments. P-values in upper right corner indicate the test effects for treatment and time for the Proc Mixed analysis. Values represent the average of three subsamples and four blocks and error bars represent ± one standard error from the mean. A star on the graph indicates significant treatment differences.

4.5 Soil Temperature and Moisture

Average soil temperature did not differ significantly between treatments from March 2012 to January 2013 ($P = 0.6885$) but temperatures differed with time ($P< 0.0001$) (Figure 5.0). There was not a significant treatment interaction between treatment and time for soil temperature throughout the study.

Average volumetric soil moisture showed highly significant differences between treatments ($P<0.0001$) and for time ($P<0.0001$). The largest effect was the result of the bedding treatments, where P-B and PSG-B treatments showed lower soil moisture than other treatments for all sampling periods (Figure 5.1).

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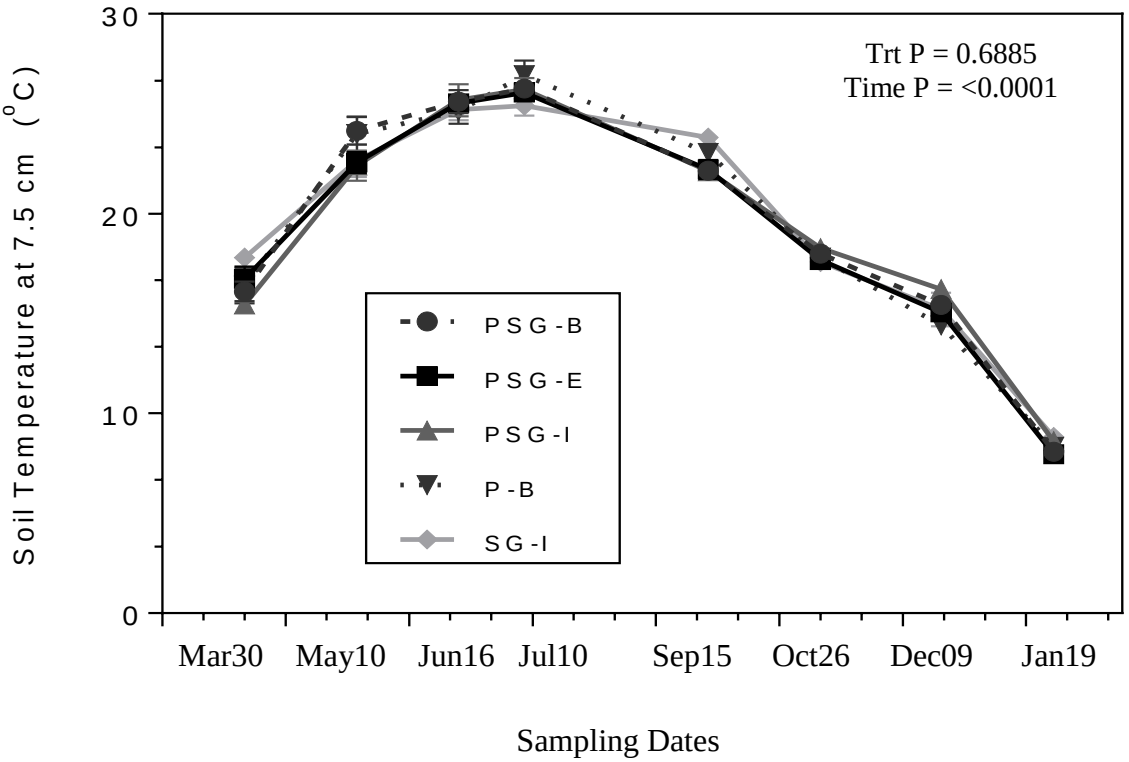
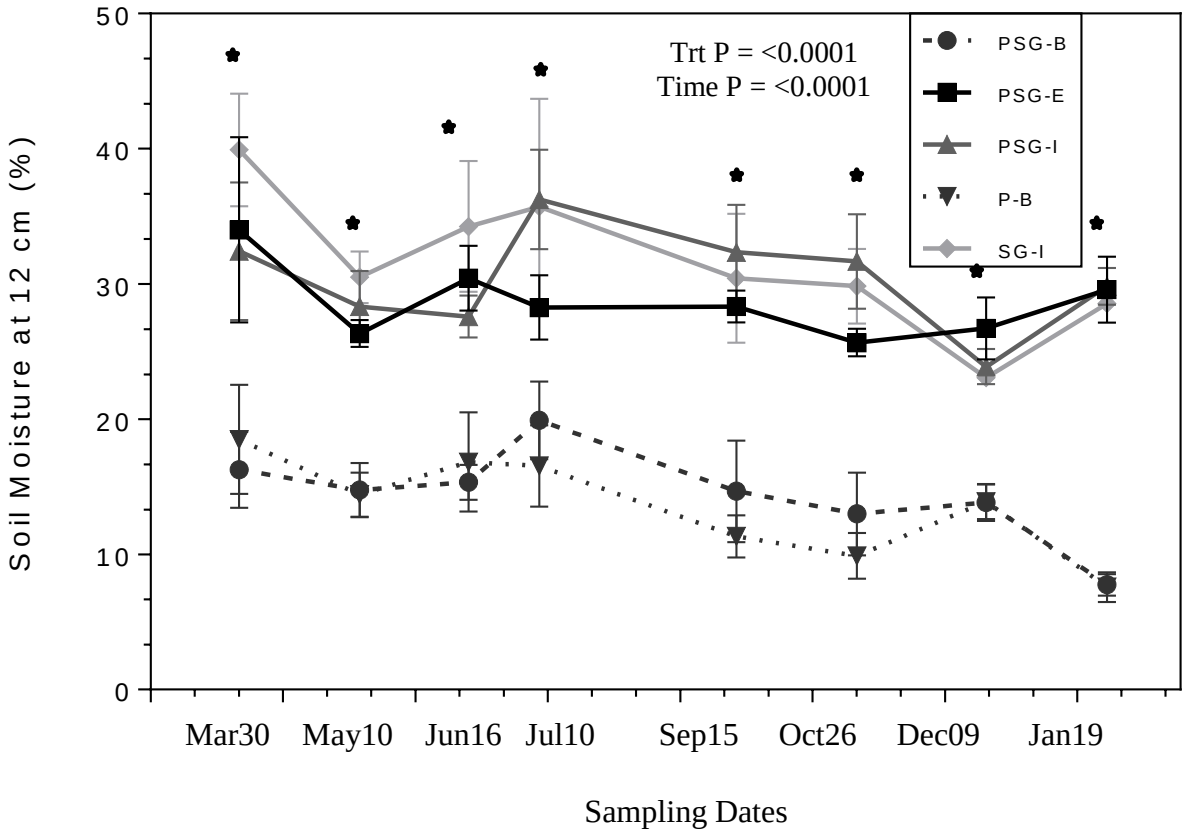


	Mar 30	May 10	Jun 16	Jul 10	Sept 15	Oct 26	Dec 09	Jan 19
PSG-B	a[1]	a	a	a	a	a	a	a
P-B	a	a	a	a	a	a	a	a
PSG-E	a	a	a	a	a	a	a	a
PSG-I	a	a	a	a	a	a	a	a
SG-I	a	a	a	a	a	a	a	a

[1] Means with different letters show significant differences for treatments by each date using Tukey's HSD at the 0.05 level.

Figure 5.0: Statistical analysis of mean soil temperature (°C) for pure pine (P-B); pine + switchgrass bed, edge and interbed (PSG-B, PSG-E, PSG-I); and pure switchgrass (SG-I) treatments. P-values in upper right corner indicate the test effects for treatment and time for the Proc Mixed analysis. Values represent the average of three subsamples and four blocks and error bars represent ± one standard error from the mean. A star on the graph indicates significant treatment differences.



	Mar 30	May 10	Jun 16	Jul 10	Sept 15	Oct 26	Dec 09	Jan 19
PSG-B	b[1]	b	b	b	b	b	b	b
P-B	b	b	b	b	b	b	b	b
PSG-E	a	a	a	a	a	a	a	a
PSG-I	a	a	a	a	a	a	a	a
SG-I	a	a	a	a	a	a	a	a

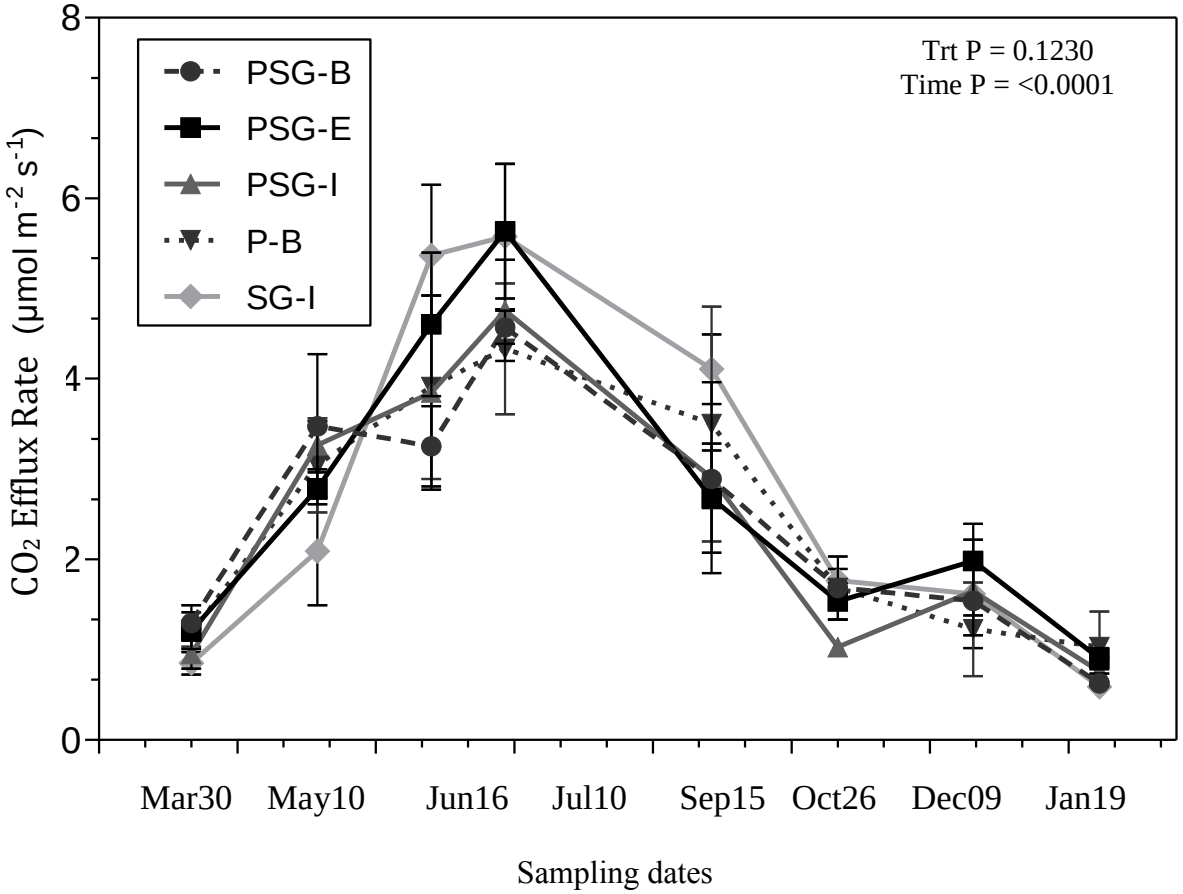
[1] Means with different letters show significant differences for treatments by each date using Tukey's HSD at the 0.05 level.

Figure 5.1: Statistical analysis of mean soil moisture (%) for pure pine (P-B); pine + switchgrass bed, edge and interbed (PSG-B, PSG-E, PSG-I); and pure switchgrass (SG-I) treatments. P-values in upper right corner indicate the test effects for treatment and time for the Proc Mixed analysis. Values represent the average of three subsamples and four blocks and error bars represent ± one standard error from the mean. A star on the graph indicates significant treatment differences.

4.6 Soil CO_2 Efflux

Repeated measures analysis of mean soil CO_2 efflux shows a significant time ($P<0.001$) effect, but treatment ($P = 0.1230$) effects were not significant. Soil CO_2 efflux increased during the growing season and peaked in July 2012 before declining to its lowest point for all treatments in January 2013 (Figure 5.0).

Multiple linear regression analysis was used to examine the relationships between CO_2 efflux and the other environmental parameters measured during over the course of this study. The model developed for predicting CO_2 efflux for all treatments showed that soil temperature, DOC, acetate and oxalate were significant parameters and explained 57% of the variance observed (Table 4.2). Partial R^2, which measures the contribution of one explanatory variable when all others are already included in the model showed that soil temperature with the greatest contribution to the model with 21% followed by oxalate with 14%, DOC with 13% and acetate with 10%.



	Mar 30	May 10	Jun 16	Jul 10	Sept 15	Oct 26	Dec 09	Jan 19
PSG-B	a[1]	a	a	a	a	a	a	a
PSG-E	a	a	a	a	a	a	a	a
PSG-I	a	a	a	a	a	a	a	a
PB	a	a	a	a	a	a	a	a
SG-I	a	a	a	a	a	a	a	a

[1] Means with different letters show significant differences for treatments by each date using Tukey's HSD at the 0.05 level.

Figure 5.2: Statistical analysis of mean soil CO_2 efflux (µg C-CO_2/g soil/h) for pure pine (P-B); pine + switchgrass bed, edge and interbed (PSG-B, PSG-E, PSG-I); and pure switchgrass (SG-I) treatments. P-values in upper right corner indicate the test effects for treatment and time for the Proc Mixed analysis. Values represent the average of three subsamples and four blocks and error bars represent ± one standard error from the mean. A star on the graph indicates significant treatment differences.

Table 4.2: Multiple linear regression was used to define relationships between CO_2 efflux and other environmental parameters.

Significant parameters influencing Soil CO_2 Efflux in all Treatments				
Parameter	Soil CO_2 Efflux (μmol m^{-2} s^{-1})			
	Std. error	Estimate	Partial R^2	P-value
Soil Temperature (°C)	0.029	0.139	0.205	<0.0001
DOC (mg C/kg soil)	0.006	0.020	0.126	<0.0001
Oxalate (μM/g soil)	0.008	0.017	0.136	0.0008
Acetate (μM/g soil)	0.011	-0.007	0.098	0.0569
Model	R^2=0.565	MS(E) = 1.202	Intercept = -0.918	<0.0001
CO_2 = -0.918 + 0.139 (temp) + 0.020(DOC) + 0.017(oxalate) - 0.007(acetate)				

4.7 Correlations between Soil Parameters and CO_2 Efflux

Spearman's correlation coefficients for all associations between each measured soil chemical and biotic property are listed in Appendix C, with the associated p-values. Of the 20 soil properties and 189 correlations tested, 96 are significantly correlated at ($\alpha < 0.05$) and 8 are additionally significant at an ($\alpha < 0.10$). Table 4.3 shows the correlations of all of the parameters only with microbial activity, microbial biomass, soil temperature, moisture, DOC and CO_2 efflux. The correlations between the major parameters and all of the soil properties measured show the strongest relationships in the analysis. These include positive relationships between soil CO_2 efflux and temperature ($r = 0.7332$) and between CO_2 efflux and oxalic acid ($r = 0.6716$) and between DOC and CO_2 efflux ($r = 0.5715$) listed in Table 4.6. The strongest negative relationships are between soil temperature and acetic acid ($r = -0.7165$) between CO_2 efflux and acetic acid ($r = -0.5904$) and soil temperature and citric acid ($r = -0.5899$) also listed in Table 4.6.

Table 4.3: Spearman's Correlations of selected parameters in this study. Values within parenthesis are the correlation coefficients and outside are p-values. Values in bold represent significant ($p < 0.1$) correlations.

	Microbial biomass	**Microbial Activity**	**Temp**	**Moisture**	**DOC**	**CO_2**
Microbial biomass	-					
Microbial Activity	**0.39(<0.0001)**	-				
Temp	**0.27(0.0007)**	**0.34(<0.0001)**	-			
Moisture	**0.18(0.025)**	**0.30(0.0001)**	**0.45(<0.0001)**	-		
DOC	0.10(0.226)	**0.15(0.059)**	**0.52(<0.0001)**	**0.27(0.006)**	-	
CO_2	**0.25(0.0013)**	**0.36(<0.0001)**	**0.73(<0.0001)**	**0.29(0.0002)**	**0.57(<0.0001)**	-
TDN	**0.15(0.06)**	**0.14(0.074)**	**0.28(0.0003)**	-0.002(0.979)	**0.46(<0.0001)**	**0.26(0.0009)**
DON	**0.18(0.034)**	**0.26(0.0015)**	**0.27(0.0009)**	**0.33(<0.0001)**	**0.31(0.0001)**	**0.31(0.0002)**
NO_3	**0.19(0.014)**	0.10(0.217)	-0.12(0.136)	**-0.22(0.0048)**	0.05(0.495)	0.08(0.321)
NH_4	**0.19(0.016)**	-0.139(0.111)	-0.03(0.67)	**-0.245(0.0019)**	0.05(0.518)	0.016(0.8376)
%C	-0.12(0.2798)	**0.26(0.021)**	**0.19(0.091)**	**0.33(0.0026)**	0.16(0.143)	0.15(0.174)
%N	-0.15(0.197)	**0.24(0.033)**	**0.45(<0.0001)**	**0.49(<0.0001)**	0.11(0.325)	**0.19(0.088)**
Phosphate	0.02(0.761)	-0.013(0.87)	**0.275(0.0004)**	**0.016(0.043)**	**0.469(<0.0001)**	**0.33(<0.0001)**
Acetate	**-0.21(0.009)**	**-0.21(0.008)**	**-0.717(<0.0001)**	**-0.22(0.0005)**	**-0.325(<0.0001)**	**-0.59(<0.0001)**
Lactate	-0.06(0.583)	-0.15(0.178)	-0.157(0.168)	-0.07(0.521)	0.12(0.301)	-0.019(0.865)
Oxalate	**0.39(<0.0001)**	**0.40(<0.0001)**	**0.79(<0.0001)**	**0.50(<0.0001)**	**0.44(<0.0001)**	**0.67(<0.0001)**
Succinate	**-0.03(0.765)**	**-0.17(0.069)**	**-0.54(<0.0001)**	**-0.36(<0.0001)**	**-0.24(0.008)**	**-0.37(<0.0001)**
Citrate	-0.09(0.355)	**-0.17(0.09)**	**-0.59(<0.0001)**	**-0.20(0.04)**	**-0.36(0.0002)**	**-0.37(0.0001)**
Formate	**-0.48(<0.0001)**	**-0.25(0.002)**	**-0.17(0.040)**	-0.08(0.312)	-0.009(0.91)	**-0.22(0.0074)**
Malate	0.18(0.113)	-0.06(0.592)	0.16(0.172)	0.038(0.744)	**0.22(0.052)**	**0.25(0.04)**

Chapter 5
Discussion

5.1 Soil Properties Influencing CO_2 Efflux

Several soil properties and processes were associated with soil CO_2 efflux in support of our hypothesis. Soil temperature, DOC, oxalate and acetate were significant parameters in explaining some of the variance in soil CO_2 efflux (R^2= 0.565). The contribution of soil temperature to soil respiration is well documented in the literature and our observations agreed with these assessments concluding that soil temperature explained the greatest amount of variance in soil CO_2 efflux (Partial R^2 = 0.205) in our model (Raich and Schlesinger 1992; Davidson et al. 1998; Lin 1999; Maier and Kress 2000; Pangle and Seiler 2002). Q_{10} is the factor by which CO_2 efflux is multiplied when temperature increases by 10°C (van't Hoff 1898; Ryan 1991). Our calculated Q_{10} values ranged from 1.67 to 3.18 and averaged 2. Ryan (1991) and Amthor (1984) found Q_{10} values also ranging from 1.6 to 3 with averages around 2 similar to our study.

The presence of DOC (Partial R^2 = 0.126), oxalate (Partial R^2 = 0.136) and acetate (Partial R^2 = 0.098) in the model show the importance of highly labile C in influencing respiration rates. DOC in soil solution, and the contribution of LMWOAs to the DOC pool, provide an important energy source for microbial communities. Based on the model we believe that LMWOAs, as a labile component of DOC, are influencing total CO_2 efflux because they are being consumed by microbial community, increasing heterotrophic respiration and as a result overall total CO_2 efflux. Other studies have also concluded that the amount and distribution of labile C controls microbial community dynamics as well as the stabilization of soil C (Kalbitz 2000; Michalzik 2001).

Correlations showed several significant associations between soil CO_2 efflux and other parameters studied. Several other LMWOAs, including citrate and formate, show strong negative relationships with soil CO_2 efflux and microbial biomass and activity have strong positive relationships.

As the microbial biomass and activity increase and labile C is consumed, respiration increases. Significant positive correlations between DON, PO_4^{3-}, malic and oxalic acids show the importance of other factors that could potentially influence soil CO_2 efflux and the soil C balance.

5.2 Treatment Effects on C Dynamics

Differences in soil properties and processes of intercropped treatments and pine only treatments were sporadic and most did not show clear trends. These trends lead us to believe that the presence of pine in intercropped treatments does not alter the microbial activity, DOC, DON and LMWOAs of intercropped switchgrass and vice versa. According to Tilman (1985), successful coexistence occurs where resource requirements differ among the species allowing for greater utilization of both non-limiting and limiting resources (Tilman 1985). Since the site was only established in 2009, the effects of intercropping switchgrass on soil properties and processes may not be discernible yet. There may also still be a temporary flush of nutrients from the post-harvest assart effect, site disturbance from biomass removal and bedding (Kimmins 1997; Portnoy 1999). These findings are consistent with other short-term studies that have shown no significant treatment differences in soil respiration and microbial biomass in young alley cropping systems (Chander 1998; Kaur 2000; Lee 2003). Conversely, C budgets of alley cropping systems show significant increases in soil respiration, microbial biomass and SOC in older intercropping systems ranging from 7 to 47 years (Table 1.1 and 1.2). None of these systems were pine-switchgrass, therefore further work needs to be done to assess the effects on these soil parameters in this intercropping system as it ages.

The edge microsite location (PSG-E) showed higher rates of C cycling than other areas of the system. Pine-switchgrass intercropped treatments had significantly higher microbial activity at the PSG-E. We attribute these increases in edge treatments to variations in the quality and quantity of C inputs from two processes: (1) enhanced root C supply from root exudation, and (2) the additive effect of

having both pine and switchgrass roots present (Sparling 1996; Jones 1998; Nardi 2000; van Hees 2005). Higher concentrations of DOC in edge treatments relative to bedded treatments in June and July of 2012 also support this conclusion. Werth (2008) stated that 16% to 39% of microbially derived C comes from roots, with the majority resulting from root exudates (Wreth 2008). The concentrations of acetate, formate and lactate declined as we moved into the growing season, suggesting rapid microbial immobilization. Other studies also concluded that LMWOAs, as a component of DOC, are sustained at low levels in soil solution because they are being directly consumed by microorganisms (Marscher and Kalbitz 2003; Smolander et al. 2001; van Hees 2005). Krzysaowska (1996) also looked at the temporal effects of LMWOAs in soil solution in Florida Spodosols. Concentrations of acetate and formate remained low from July to August while oxalate concentrations were higher in June and July and declined in August. Trends developed from our work showed seasonal variability in LMWOA concentrations similar to Krzysaowska (1996). The edge or the interaction point between roots the two species at this location could be having an additive effect since both pine and switchgrass roots are present making DOC and microbial activity higher than they would be in PSG-B or PSG-I treatments alone.

LMWOAs may contribute other vital uses other than providing an important energy source for microbial communities. Oxalate, malate, succinate, and citrate are behaving differently than acetate formate and lactate in this system. Oxalate and malate both increased as we moved into the growing season while citrate and succinate only increased during the fall months. They are more complex, not as easily degraded and potentially play a very different role in the soil system. Some LMWOAs such as malate, citrate, and oxalate have been tied to the increased solubility of inorganic P in soil solution. Elevated levels of these acids desorbed P from surface complexes by the dissolution of amorphous coatings or by substituting for it in ligand exchange reactions (Fox and Comerford1990a; Fox and Comerford 1990b; Fox 1995; Jones and Darrah 1994). The increase in concentrations of oxalate,

malate, citrate and succinate in the growing season suggest that they are being exuded to help plants acquire nutrients (Cleveland 2007). Since our LMWOA data consisted of point measurements analyzed with 36 hours of collection microbes could also be consuming these acids as an energy source before they can be processed.

5.3 Conclusions

Due to the temporal variations associated with our data we can conclude that intercropping species supports the need for multiple seasonal measurements to get an accurate look at the heterogeneity of soil and site features. Our study also indicates that intercropping switchgrass in already existing loblolly pine plantations does not have a major short-term effect on soil properties and processes. The site was only established in 2009 and both loblolly pine trees and switchgrass are not fully established. We believe that the main drivers in the system are still the disturbance from site establishment in 2009 and the post-harvest assart effect. Carbon budgets in young alley cropping systems in the literature agree with our results showing no significant increases in soil respiration and microbial biomass. Increases in soil respiration, microbial biomass, and SOC only occurred in older systems. Increases in microbial activity and DOC in the edge treatments of summer months show potential differences in the litter quality where the two species are interacting. This work also shows important relationships and potential drivers of total soil CO_2 efflux. Soil temperature, DOC, oxalate and acetate are significant parameters in the regression analysis but there are also several highly significant relationships between CO_2 efflux and DON, PO_4^{3-}, citric and succinic acids. These relationships underline the complexity of these systems and the need for better understanding in the variability in processes that govern C cycling. More work needs still needs to be done to access the affects of intercropping on soil parameters and long-term site quality in this pine-switchgrass system as it ages.

5.4 References

Amthor, J.S., 1984. The role of maintenance respiration in plant growth. Plant, Cell and Environment 6: 595-599.

Chander K., Goyal S., Nandal D.P., Kapoor. K., 1998. Soil organic matter, microbial biomass and enzyme activities in a tropical agroforestry system. Biology and Fertility of Soils 27: 168–172.

Chen, G., Yang, Y., Guo, J., Xie, J., Yanf, Z., 2011. Relationships between carbon allocation and partitioning of soil respiration across world mature forests. Plant Ecology. 212: 195–206.

Cleveland, C.C., Liptzin, D., 2007. C:N:P stoichiometry in soil: is there a 'Redfield ratio' for the microbial biomass? Biogeochemistry 85: 235–252.

Davidson, E.A., Belk, E., Boone, R.D., 1998. Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. Global Change Biology 4: 217-227.

Fox, T.R. and Comerford, N.B. 1990a. Low-molecular-weight organic- acids in selected forest soils of the Southeastern USA. Soil Science Society America Journal 54: 1139–1144.

Fox, T.R. and Comerford, N.B. 1990b. Phosphorus and Aluminum Release from a Spodic Horizon Mediated by Organic Acids. Soil Science Society America Journal 54.

Fox, T.R. 1995. The influence of Low-Molecular-Weight organic acids on properties and processes in forest soils, in: Carbon Forms and Functions in Forest Soils. Soil Science Society of America Journal 43–62.

Jones, D.L., 1998. Organic acids in the rhizosphere-a critical review. Plant and Soil 205: 25–44.

Jones, D.L., Darrah, P.R., 1994. Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. Plant and Soil 166: 247–257.

Kalbitz K., Solinger S., Park J.H., Michalzik B., Matzner E., 2000. Controls on the dynamics of dissolved organic matter in soils: A review. Soil Science 165: 277–304.

Kaur, B., Gupta, S.R., Singh, G., 2000. Soil carbon, microbial activity and nitrogen availability in agroforestry systems on moderately alkaline soils in northern India. Applied Soil Ecology 15: 283–294.

Kimmins, J.P., 1997. Forest ecology. A foundation for sustainable management. 2nd ed. Prentice- Hall Inc., Upper Saddle River, New Jersey.

Krzysaowska A.J., Baylock M.J., Vance G.F., David M.B., 1996. lon-Chromatographic Analysis of Low Molecular Weight Organic Acids in Spodosol Forest Floor Solutions. Soil Science Society America Journal 60.

Lee K.H., Jose S., 2003. Soil respiration and microbial biomass in a pecan–cotton alley cropping system in southern USA. Agroforestry Systems 58: 45–54.

Lin, G., Ehleringer J.R., 1999. Elevated CO_2 and temperature impacts in different components of soil CO_2 efflux in Douglas-Fir terracosms. Global Change Biology 5: 157-168.

Maier, C. A., Kress, L. W., 2000. Soil CO_2 evolution and root respiration in 11 year-old loblolly pine (*Pinus taeda*) plantations as affected by moisture and nutrient availability. Canadian Journal of Forest Research 30 3: 347-359.

Marschner, B., Kalbitz, K., 2003. Controls of bioavailibity and biodegradability of dissolved organic matter in soils. Geoderma 113: 211–235.

Mungai, N.W., Motavalli, P.P., Kremer, R.J., Nelson K.A. 2005. Spatial variation of soil enzyme activities and microbial functional diversity in temperate alley cropping systems. Biology and Fertility of Soils 42: 129–136.

Nardi, S., Concheri G., Pizzeghello D., Sturaro A., Rella R., Parvoli G., 2000. Soil organic matter mobilization by root exudates. Chemosphere 5: 653–658.

Portnoy, J.W., 1999. Salt marsh diking and restoration: biogeochemical implications of altered wetland hydrology. Environmental Management 1: 111-120.

Raich, J.W., Schlesinger W.H., 1992. The global carbon-dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus Series B-Chemical and Physical Meteorology 44: 81–99.

Ryan, M.G., 1991. Effects of climate change on plant respiration. Ecological Applications 1(2): 157-167.

Smolander, A., Kitunen, V., 2002. Soil microbial activities and characteristics of dissolved organic C and N in relation to tree species. Soil Biology and Biochemistry 34: 651-660.

Sparling, G.P., 1992.Ratio of Microbial Biomass Carbonto Soil Organic Carbon as a Sensitive Indicator of Changes in Soil Organic Matter. Australian Journal of Soil Research 30: 195-207.

van Hees P.A.W., Jones, D.L., Finlay, R., Godbold, D.L., 2005. The carbon we do not see? The impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. Soil Biology and Biochemistry 37:1-13.

Van't Hoff, J.H., 1898. Lectures on Theoretical and Physical Chemistry. Part 1. Chemical Dynamics. London: Edward Arnold.

Wardle, D.A., Lavelle P., 1997. Linkages between soil biota, plant litter quality and decomposition. In: Driven by nature: plant quality and decomposition. CAB International, Cambridge, UK, 107–124.

Werth, M., Kuzyakov. Y., 2008. Root-derived carbon in soil respiration and microbial biomass determined by C-14 and C-13. Soil Biology and Biochemistry 40 3: 625-637.

Appendix A
Greenhouse Experiment

6.1 Methods

To determine the quantities and types of the specific LMWOAs produced by each species, 14 loblolly pine seedlings and 14 switchgrass plugs were established in pots in a controlled greenhouse study. The seedlings used were provided by Weyerhaeuser Company and were seedlings were injected with fertilizer before being planted in pots. Seedlings were established in pots using acid washed sand with 53 micron opening nylon mesh at the bottom of each pot to keep the sand in place. A known amount of sand (roughly equal within 5 kg) was placed in each pot and both seedlings and plugs were left to establish for roughly 6 months (October 2013). After 6 months the plants were extracted from pots and lightly shaken to remove the mineral soil component, defined as bulk soil. Then the plant root systems were vigorously shaken, and the remaining soil was defined as rhizosphere soil (Fox and Comerford, 1990). Bulk and rhizosphere soils were weighed and a ratio of 5 parts deionized water to every 1-part soil was added to Nalgene bottles and shaken at 100 rpm for 30 minutes. Samples were allowed to settle before the solution was filtered using a 0.45μm membrane filter and stored at -4°C. The rhizosphere and bulk soil were analyzed for LMWOAs on an ion chromatograph (Dionex Corp., Sunnyvale, CA). The same LMWOA species analyzed in the field component were also analyzed. This pot study was used to establish a baseline for LMWOA production of both loblolly pine and switchgrass. All data and information can be found in Appendix A of this document.

6.2 Results

There are no statistical difference between bulk and rhizospheric soil when analyzed in JMP using Tukey-Kramer HSD. Lactic and succinic acid concentrations were found in a field setting but not in greenhouse settings. Citrate concentrations were found in switchgrass pots but not in pine. There was a lot of variability between the 14 pots. In a field setting citric acid was found in all treatments but switchgrass and other C4 species including Big Bluestem and Broomsedge were in some of the bedded locations in all of the treatments (Table 4.4).

Table 6.1: Greenhouse study data showing LMWOAs from an average of 14 pine and 14 switchgrass pots in bulk and rhizospheric soil. Significant treatments indicated by letter. Analysis done in JMP using 1-way ANOVA and Tukey HSD.

Parameter	Acetate	Lactate	Formate	Succinate	Oxalate	Malate	Citrate
Pine Bulk (µM/g soil)	0.188a	n.a.	0.037a	n.a.	0.145a	0.042a	n.a.
Pine Rhizosphere (µM/g soil)	0.213a	n.a.	0.041a	n.a.	0.210a	0.029a	n.a.
SG Bulk (µM/g soil)	0.233a	n.a.	0.286a	n.a.	0.500a	0.110a	0.089a
SG Rhizosphere (µM/g soil)	0.551a	n.a.	0.513a	n.a.	0.956a	0.0327a	0.0155a

Appendix B
Lenior 1 Site Timeline

Table 6.2: Lenior 1 establishment and site history including switchgrass fertilization.

Month	Day	Year	Lenior 1 Site History
April	8	2008	Site Selection
May	15-30	2008	Plot Layout
September	11-12	2008	Layout treatment boundaries
September	15-20	2008	Pre-Site Prep
September	22-1	2008	Biomass removal with excavator
October	16-31	2008	V-shearing
October	30-31	2008	Raking of switchgrass only plots
November	1-14	2008	Raking of switchgrass only plots
November	17-18	2008	Aerial herbicide application: Rate 48 oz. chopper/acre
November	24-27	2008	Bedding and Pre-plant fertilizer
December	4-5	2008	Planting of Containerized seedlings on beds
December	28-29	2008	Planting of 3rd gen. bare root seedlings on extra row treatments
January	28-31	2009	Installed soil moisture probes
February	1-12	2009	Installed soil moisture probes
March	25	2009	Installed weather station
April	20-24	2009	Install rain gauges
March-June		2009	Install electric fences around monitoring equipment
April	9	2009	Tip Moth Application
May	19-20	2009	Herbicide switchgrass and pine x switchgrass plots
May	26-30	2009	V-shearing streaks for switchgrass alleys
June	1-5	2009	Planting of switchgrass
April	1	2010	Switchgrass Maintenance Mowing
June	10-15	2010	Herbicide switchgrass and pine x switchgrass plots
June	29	2010	Fertilized Switchgrass and Intercropped plots
December	9-14	2010	Switchgrass cutting, raking, and bailing
January	21-29	2011	Switchgrass cutting, raking, and bailing
December	18-21	2011	Fertilization at 50lbs N rate
April	13-Dec	2012	Fertilization at 50lbs N rate
April	10	2013	Switchgrass Maintenance Mowing

Appendix C
Complete Correlation Matrix

Table 6.3: Spearman's Correlations of all parameters in study. Values within parenthesis are correlation coefficients and outside are p-values.

	Microbial biomass	Microbial Activity	Temp	Moisture	DOC	TDN	DON	NO_3^-	NH_4^+	CO_2	%C	%N	Phosphate	Acetate	Lactate	Oxalate	Succinate	Citrate	Formate	Malate
Microbial biomass	-	0.39(**<0.0001**)	0.27(**0.0007**)	0.18(**0.025**)	0.10(**0.226**)	0.15(**0.06**)	0.18(**0.034**)	0.19(**0.014**)	0.19(**0.016**)	0.25(**0.0013**)	-0.12(**0.28**)	-0.15(**0.197**)	0.02(**0.761**)	-0.21(**0.009**)	-0.06(**0.583**)	0.388(**<0.0001**)	-0.03(**0.765**)	-0.09(**0.355**)	-0.48(**<0.0001**)	0.18(**0.113**)
Microbial Activity		-	0.44(**<0.0001**)	0.30(**0.0001**)	0.15(**0.059**)	0.14(**0.074**)	0.26(**0.0015**)	0.10(**0.217**)	-0.139(**0.111**)	0.36(**<0.0001**)	0.26(**0.021**)	0.24(**0.033**)	-0.013(**0.87**)	-0.21(**0.008**)	-0.15(**0.178**)	0.40(**<0.0001**)	-0.17(**0.069**)	-0.17(**0.09**)	-0.251(**0.002**)	-0.06(**0.592**)
Temp			-	0.45(**<0.0001**)	0.52(**<0.0001**)	0.28(**0.0003**)	0.27(**0.0009**)	-0.12(**0.136**)	-0.03(**0.67**)	0.73(**<0.0001**)	0.19(**0.091**)	0.45(**<0.0001**)	0.275(**0.0004**)	-0.72(**<0.0001**)	-0.157(**0.168**)	0.79(**<0.0001**)	-0.54(**<0.0001**)	-0.59(**<0.0001**)	-0.17(**0.040**)	0.16(**0.172**)
Moisture				-	0.27(**0.006**)	-0.002(**0.979**)	0.33(**<0.0001**)	-0.22(**0.005**)	-0.245(**0.0019**)	0.29(**0.0002**)	0.33(**0.0026**)	0.49(**<0.0001**)	0.016(**0.043**)	-0.22(**0.0005**)	-0.07(**0.521**)	0.50(**<0.0001**)	-0.36(**<0.0001**)	-0.20(**0.04**)	-0.08(**0.312**)	0.038(**0.744**)
DOC					-	0.46(**<0.0001**)	0.31(**0.0001**)	0.05(**0.495**)	0.05(**0.518**)	0.57(**<0.0001**)	0.16(**0.143**)	0.11(**0.325**)	0.47(**<0.0001**)	-0.33(**<0.0001**)	0.12(**0.301**)	0.44(**<0.0001**)	-0.24(**0.008**)	-0.36(**0.0002**)	-0.009(**0.91**)	0.22(**0.052**)
TDN						-	0.17(**0.038**)	0.19(**0.015**)	0.24(**0.0018**)	0.26(**0.0009**)	0.02(**0.8396**)	-0.104(**0.325**)	0.40(**<0.0001**)	-0.16(**0.045**)	-0.141(**0.216**)	0.28(**0.0009**)	-0.04(**0.665**)	-0.16(**0.104**)	-0.099(**0.223**)	-0.18(**0.111**)
DON							-	-0.07(**0.388**)	-0.08(**0.31**)	0.31(**0.0002**)	0.025(**0.835**)	0.25(**0.0068**)	0.08(**0.345**)	-0.09(**0.284**)	-0.055(**0.648**)	0.34(**<0.0001**)	-0.11(**0.247**)	-0.268(**0.0095**)	-0.21(**0.016**)	0.21(**0.088**)
NO_3^-								-	0.35(**<0.0001**)	0.08(**0.321**)	-0.238(**0.033**)	-0.32(**0.0039**)	-0.016(**0.839**)	-0.11(**0.168**)	0.20(**0.072**)	-0.06(**0.475**)	0.23(**0.01**)	0.11(**0.264**)	-0.39(**<0.0001**)	-0.037(**0.754**)
NH_4^+									-	0.016(**0.8376**)	-0.024(**0.029**)	-0.23(**0.0042**)	-0.05(**0.563**)	-0.12(**0.151**)	-0.007(**0.955**)	-0.08(**0.335**)	0.24(**0.0075**)	0.04(**0.684**)	-0.32(**<0.0001**)	-0.05(**0.662**)
CO_2										-	0.15(**0.174**)	0.19(**0.088**)	0.33(**<0.0001**)	-0.59(**<0.0001**)	-0.019(**0.865**)	0.67(**<0.0001**)	-0.37(**<0.0001**)	-0.37(**0.0001**)	-0.22(**0.0074**)	0.25(**0.04**)
%C											-	0.30(**0.0068**)	-0.08(**0.509**)	0.047(**0.684**)	-0.094(**0.865**)	0.07(**0.577**)	-0.20(**0.125**)	-0.15(**0.329**)	0.29(**0.0095**)	0.26(**0.25**)
%N												-	-0.056(**0.621**)	-0.178(**0.116**)	-0.195(**0.536**)	0.15(**0.271**)	-0.08(**0.508**)	-0.24(**0.114**)	0.23(**0.042**)	-0.55(**0.01**)
Phosphate													-	-0.20(**0.013**)	0.141(**0.195**)	0.160(**0.062**)	-0.13(**0.144**)	-0.11(**0.293**)	-0.006(**0.942**)	0.11(**0.361**)
Acetate														-	-0.10(**0.216**)	-0.62(**<0.0001**)	0.268(**0.003**)	0.44(**<0.0001**)	0.37(**<0.0001**)	-0.24(**0.04**)
Lactate															-	-0.16(**0.1966**)	0.22(**0.085**)	0.091(**0.543**)	-0.13(**0.248**)	0.21(**0.256**)
Oxalate																-	-0.50(**<0.0001**)	-0.45(**<0.0001**)	-0.26(**0.003**)	0.19(**0.098**)
Succinate																	-	0.28(**0.015**)	-0.03(**0.752**)	-0.17(**0.218**)
Citrate																		-	0.06(**0.587**)	-0.31(**0.01**)
Formate																			-	-0.12(**0.348**)
Malate																			-	-