Nitrogen Fertilization Impacts on Soil Organic Carbon and Structural Properties under Switchgrass

Dissertation

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ABSTRACT

Switchgrass is an important herbaceous bioenergy crop selected by the Department of Energy (DOE) due to its high potential for biomass production and costeffective growth characteristics. Benefits of growing switchgrass as a bioenergy crop include high productivity of plant biomass and a large potential for sequestering soil organic carbon (SOC) through root production. Increasing SOC pools provides numerous ecosystem services while also can mitigate anthropogenic carbon dioxide (CO_2) emissions. Hitherto, there has been considerable emphasis on nitrogen (N) management research and plant breeding to increase aboveground biomass. However, creating a sustainable biomass production system by maintaining or improving SOC concentration/pools is as important as enhancing biomass yield from switchgrass plantations. Most positive responses of N fertilization to SOC pools in agricultural fields are generally associated with an increase in the amount of residues returned because of improved biomass production through fertilization. However, because the aboveground biomass is harvested and removed for use as bioenergy feedstock, it is important to assess whether N fertilization would positively impact SOC sequestration under switchgrass. Moreover, the attendant changes in soil structural properties with N fertilization or under different cultivars need to be evaluated for sustainable biomass production. Therefore, the overall objectives of this study were to assess the potential of improving biomass yields

with N fertilization and cultivars of switchgrass, changes in SOC concentration and pool size, and effects on soil structural properties. These objectives were realized by conducting field experiments in Ohio and Tennessee, and a laboratory incubation study in Ohio.

First, the effects of N fertilization on switchgrass biomass production and SOC sequestration were determined in Ohio. This study was conducted at three research stations (Northwest, Jackson, and Western) of the Ohio Agricultural Research and Development Center (OARDC). In 2008 and 2009, four rates of N (0, 50, 100, and 200 kg N ha⁻¹) were applied to switchgrass plots established in 2004. Measurements included aboveground and belowground biomass, SOC concentration, soil bulk density, and C and N concentrations in plant tissues. To consider the effects of land use changes while establishing plots, SOC concentrations and pools were measured from the reference soils which had been maintained as the same land use before switchgrass plots were established at each site. No differences in SOC concentrations or pools were observed among plots receiving different rates of N except at Jackson. Similarly, no differences were observed in comparison with the reference soils at either of any three sites. However, slightly positive correlation between SOC pools and N rates were observed at Northwest and Jackson sites. While aboveground biomass and shoot/root ratios increased with increasing N rates, there were no significant differences in belowground biomass among N treatments. The aboveground biomass was the highest at the Western site. The amount of N exported by harvesting biomass increased with increasing rates of N application due to higher biomass production and higher concentrations of N in plant tissues. The point at which the rate of N application was equivalent to its amount removed in harvested biomass was calculated at 32 kg N ha^{-1} (95% CI: 11-53 kg N ha⁻¹).

Second, laboratory incubation studies were conducted on organic matter (OM) decomposition in response to the addition of the root-C substrate and inorganic N. A factorial experimental design was used with three levels of root-C [no addition of C (C0)] = 0, low level of C (CL) = 5 mg root/g soil, and high level of C (CH) = 10 mg root/g soil] and three levels of inorganic N [no addition of N (N0) = 0, low level of N (NL) = 0.021mg N/g soil, and high level of N (NH) = 0.083 mg N/g soil]. The efflux of CO_2 , inorganic N, soil pH, enzyme activities, SOC concentration, and SOC derived from root-C were measured during a 200-day lab incubation. In general, there was no interaction between C and N rates on the parameter measured. The addition of inorganic N resulted in a decrease in decomposition of OM. Several mechanisms for the negative effect of N on decomposition were tested. One possible explanation was an N mining theory, which entailed microbial mineralization of OM to obtain N from soil organic matter (SOM) under the N-limited conditions. However, a higher concentration of available N in soil even in the N0+CH treatment (the most N limiting condition) rejected the N mining hypothesis. Another hypothesis is that the addition of N causes a decrease in oxidative enzyme activities, which decompose even the recalcitrant SOM fraction. However, no particular trends were observed in oxidative enzyme activities either. The third hypothesis is that addition of N enhances the formation of recalcitrant compounds. Indeed, a non-linear regression model showed a decrease in mineralizable C pools with the addition of N. Among C treatments, increased CO₂ effluxes and cumulative CO₂ production were observed with the addition of root-C substrates. Although there was no

difference in total SOC concentration among C treatments, the isotopic discrimination showed a decrease in the native SOC concentration in the CH treatment. This trend indicated that addition of root-C substrates induced a priming effect on native SOC.

Third, the effects of N fertilization on soil structural changes under switchgrass were assessed in a field experiment. Soil samples were obtained from a field study in Milan, Tennessee in April and October, after four consecutive years of N fertilization (0, 67, and 202 kg N ha⁻¹). Root weight density (RWD), root length density (RLD), SOC concentration, aggregate stability, and soil moisture characteristics curves (SMCC) were measured. The RWD in April and the RLD in October were the lowest under the 202 kg N ha⁻¹ treatment. In contrast, the SOC concentration was the highest under the 202 kg N ha⁻¹ treatment. No consistent trends were observed in SMCC among N treatments. The ratio of mean weight diameter (MWD), after and before wet-sieving was lower in the 202 kg N ha⁻¹ than in the 0 and 67 kg N ha⁻¹ treatments. This trend in MWD ratio showed that excessive N fertilization could negatively impact soil structure by reducing root biomass and/or length despite the increase in SOC concentration.

Fourth, soil structural changes under four switchgrass cultivars (Alamo, GA992, GA993, SL-93-2) were evaluated in Milan, Tennessee during the fourth year after switchgrass establishment. The RWD, C and N concentrations in roots, aggregate stability by wet-sieving, C concentrations in aggregates (4.75-8 mm size fraction) before and after wet-sieving, and total porosity and pore size distribution in aggregates (4.75-8 mm size fraction) were measured. No significant treatment differences were observed either in plants or in aggregate properties among cultivars during the growing season. Yet, there were seasonal differences in properties of aggregates. These seasonal differences

may be attributed to differences in soil moisture content at the time of sampling. These seasonal differences indicate that the effects of antecedent moisture content (abiotic factors) could be more important than the effects of cultivars.

Overall, the data from Ohio and Tennessee experiments showed that the aboveground biomass was more strongly influenced by N fertilization than the belowground biomass. Even when the aboveground biomass was harvested and removed, N fertilization led to an increase in SOC pools, both in Ohio and Tennessee. Data from the laboratory incubation study showed that N additions could retard the decomposition of OM, which may contribute to higher SOC pools in N fertilized plots. Although no clear relationship between root growth and N fertilization were found in the short-term (2 years) experiments conducted in Ohio, reduced root biomass and lower root length were observed with high fertilization (202 kg N ha⁻¹) in the 5-year experiments in Tennessee. The results from the Tennessee experiments indicated the important role of roots in stabilizing soil structure. Despite higher SOC concentrations in plots receiving high rates of N fertilization, higher soil structural stability was associated with greater root biomass and longer root length in plots receiving none or low rates of N fertilizer. These data indicate that root growth is a crucial driver of surface soil structure. Moreover, soil structural properties are strongly affected by the antecedent moisture content and other environmental factors at the time of sampling.

DEDICATION

사랑하는 부모님께

이 논문을 바칩니다.

This dissertation is dedicated to

my parents

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CHAPTER 1

INTRODUCTION

1.1 Soil organic carbon (SOC)

1.1.1 The role of SOC in the global C cycle

Since the beginning of the Industrial Revolution in 1750, concentrations of carbon dioxide (CO₂) in the atmosphere have increased from 280 ppm to 390 ppm in 2010 mainly due to anthropogenic activities (IPCC, 2007; NOAA/ESRL). Changes in greenhouse gases (GHGs) and aerosols in the atmosphere impact the energy balance in the earth system and have led to climate changes (IPCC, 2007). The carbon (C) in the earth is stored in five major pools: oceanic (38,400 Pg), geologic (4,130 Pg), pedologic (2,500 Pg), atmospheric (760 Pg), and biotic (560 Pg) pools (Lal, 2008). The pedologic (soil) C pool to 1 m depth is comprised of organic (1550 Pg) and inorganic (950 Pg) C pools (Batjes, 1996). Being the largest C pools in terrestrial ecosystems, knowledge of soil carbon dynamics is important in comprehending a rapidly changing global C cycle (GCC) and to mitigating emission of GHGs. Of the 120 Pg yr⁻¹ of atmospheric C taken up by plants through photosynthesis (Lal, 2008), half returns to the atmosphere through plant respiration, and the other half through soil respiration. Fossil fuel combustion is the

primary contributor to the increase in CO_2 in the atmosphere (8.7 Pg C yr⁻¹ from the geologic pool in 2008), and deforestation and soil erosion contribute to CO_2 –C emissions at a rate of 1.6 and 0.8-1.2 Pg C yr⁻¹, respectively (Lal, 2008; Le Quéré et al., 2009).

Despite being a relatively small proportion of soil mass, soil organic matter (SOM) is an important determinant of soil quality. Qualitative and quantitative changes in SOM alter physical (moisture retention, aggregation, soil temperature, etc.), chemical (cation exchange capacity [CEC], pH, nutrient availability, etc.), and biological (food sources for microorganisms, mineralization, biodiversity etc.) properties of soils (Tate, 1992; Wander, 2004). Soil organic carbon (SOC) is a proxy surrogate for SOM. The sequestration of SOC, a type of biosequestration, is worthwhile because it can offset some anthropogenic CO₂ emissions in the GCC as well as provide numerous ecosystem services, such as supplying nutrients, retaining water, buffering soil pH, etc. (Lal, 1997; Wander and Nissen, 2004).

The magnitude of SOC sequestration depends on inherent soil properties, factors limiting biomass production, and management practices. Ingram and Fernandes (2001) described three levels of SOC sequestration: potential, attainable, and actual. The potential for SOC sequestration includes the inherent soil properties such as mineralogy, texture, soil depth, etc. However, the amount of C in the system is limited by input of biomass-C which is determined by climate, net primary production (NPP), and biomass partitioning into components. These factors affect the attainable SOC pool. Any factors that exacerbate the loss of C from the SOC pool (e.g., erosion, tillage, residue removal, or drainage) determine the actual rate of SOC sequestration. This conceptual approach to understanding SOC sequestration needs to be elaborated and evaluated through site-

specific and mechanistic studies. Although many studies have been conducted to understand the GCC and SOC sequestration, there are some areas which need more research to achieve the goal of biosequestration such as partitioning of plant biomass, plant-soil interactions, microbial processing of plant litter, and stabilization mechanisms of SOC (US DOE, 2008).

1.1.2 Inputs of biomass-C to soil

1.1.2.1 Importance of root-derived C on SOC concentration

The SOC originates from remains of plant materials with the assumption that there is no C input from other organic materials such as manure and remains of animals. Photosynthetically fixed C is transferred to soils as plant litter, sloughed plant cells, exudates from roots, and so on, and the majority of C input among them is derived from roots in non-agricultural ecosystems (van Groenigen et al., 2006). Comparatively greater contribution of roots than shoots to the SOC pool is of interest to soil scientists. The substantial differences in chemical composition between above- and below-ground plant tissues can affect residue decomposability (Johnson et al., 2007). Balesdent and Balabane (1996) reported that the contribution of roots to SOC was 1.5 times greater than that of shoots because of the complex C compounds in the roots. Crow et al. (2009) also showed that aliphatic compounds derived from roots were more resistant to degradation than those from shoots at the deciduous forest in Pennsylvania. Similary, Rasse et al. (2005) synthesized data from several published studies and concluded that the mean residence time (MRT) of root-derived C was 2.4 times longer than that of shoot-derived C. However, Rasse and colleagues (2005) argued that rather than mere differences in root

chemical composition, there may be other mechanisms which favor relatively greater stability in root-derived carbon compounds. These mechanisms are root distribution at greater depths, aggregation (reduction of surface area for degradation reactions to take place) by roots and mycorrhizal hyphae, and chemical interactions with metal ions which accentuate physical and chemical protective mechanisms for root-derived C (Rasse et al., 2005).

In addition to the sloughed root tissues, rhizodeposition (root exudates) is another important source of root-derived C (Johnson et al., 2006). Rhizodeposits are comprised mostly of easily decomposable compounds with a short MRT (Kuzyakov, 2002; Gregory, 2006). Even though rhizodeposits are rapidly consumed by microorganisms, they are synthesized into microbial cells and thus converted to more recalcitrant forms of C through microbial processes (Tisdall, 1996; Gregory, 2006). Facilitated microbial activities and exudates contribute to aggregation which plays a role in protecting SOC against decomposition. Additionally, rhizodeposition may protect inherent SOC against decomposition by providing substrates that favor the preferential microbial consumption of rhizodeposits over recalcitrant SOC as food sources (Torbert et al., 2000; Kuzyakov, 2002). In contrast, other hypotheses suggest that stimulated microbial activity by rhizodeposits can increase the decomposition of inherent SOC (Kuzyakov, 2002; Cheng et al., 2003). These contrasting views about rhizodeposition indicate the need for additional research to understand the roles of root-derived C in SOC dynamics (Johnson et al., 2006).

1.1.2.2 Effects of new C input on native SOC (priming effect)

Addition of fresh organic or mineral materials can result in a short-term change in SOM decomposition rates (Dalenberg and Jager, 1989; Kuzyakov et al., 2000). This priming effect can be either positive (stimulation of SOM decomposition) or negative (retardation of SOM decomposition) (Kuzyakov et al., 2000). Löhnis (1926) reported that green manure additions stimulated turnover of native organic matter (OM) by increasing bacterial activity and its consequent release of native humus-N. Subsequent studies on the priming effect have shown that plants uptake more non-labeled N with the application of ¹⁵N-fertilizer than under no N fertilization. This phenomenon, called "added nitrogen interaction (ANI)" (Jenkinson et al., 1985), was suggested as evidence that mineral N addition facilitated mineralization of SOM-N. However, many studies have disagreed with this hypothesis of stimulated mineralization of SOM-N by fertilizer N by considering it as "salt effects" which lead to microbial death due to NH₄⁺, changes in soil pH, or substitution of N pools (Jansson and Persso, 1982; Jenkinson et al., 1985).

With the aid of isotopic labeling and detection of the CO_2 production from SOM decomposition, pathways of the priming effect have been elucidated. After addition of OM compared to the control soil (no addition of OM), an increased efflux of CO_2 with the same isotopic signal as the native SOM does not mean that the rate of SOM decomposition has been changed. Changes in CO_2 efflux can be caused by changes in active microbes' turnover or microbial metabolisms, as is the case with N. In this case, the added substrates do not alter the pattern of SOM decomposition, that is, an "apparent priming effect" (Kuzyakov et al., 2000). On the other hand, the change in microbial activity can indeed alter SOM decomposition. Higher enzyme production or co-

metabolism induced by added materials can enhance SOM decomposition. The actual change in the SOM decomposition by added materials is a "real priming effect" (Kuzyakov et al., 2000).

Because priming effects are the results of microorganisms' actions, microbial diversity or enzyme activities can help understand the underlying mechanisms. The microbial group of r-strategists characterized by rapid growth in a short time after C addition can lead the apparent priming effect because they can only decompose the easily decomposable substrates (Blagodatskaya and Kuzyakov, 2008). On the other hand, the real priming effect can be observed under the stimulated activities of K-strategists which can break down recalcitrant compounds present in SOM. The activities of extracelluar enzymes can be an indicator for the real priming effect because those enzymes function outside of microbial cells (Blagodatskaya and Kuzyakov, 2008). The stimulation of enzymes degrading cellulose or lignin can be considered as a cause of the real priming effect (Fontaine and Barot, 2005).

The amount and types of priming effects are regulated by the composition, amount, and availability of substrates (Blagodatskaya and Kuzyakov, 2008). In general, easily degradable C sources could induce a greater priming effect than low available substrates (Conde et al., 2005; Kuzyakov and Bol, 2006). Moreover, the magnitude of priming effects vary depending on the ratio of the amount of added C to microbial biomass C: (1) a linear increase of the priming effect if the ratio is < 0.15, and (2) an exponential decrease of that when the ratio is > 0.5 (Blagodatskaya and Kuzyakov, 2008). Availability of N can also alter the priming effects on SOC by activating different groups of microbes such as r- and K-strategists (Fontaine et al., 2004). Additionally, the existence of plants can cause a priming effect which is a rhizosphere priming effect by root exudates, drying and rewetting of soil, destruction of aggregate, stimulation of microbial activities, etc. (Kuzyakov, 2002; Cheng and Kuzyakov, 2005). The magnitude of priming effects can also vary depending on soil chemical (nutrients, C:N ratio, or pH) and physical properties (aggregation which determine the pore space as the habitat), and mineralogical composition (Rasmussen et al., 2007; Blagodatskaya and Kuzyakov, 2008; Hartley et al., 2010).

Priming effects are not always considered important in influencing the SOC dynamics, since it is thought to be either a short-term effect or CO_2 originated from sources other than SOC decomposition. However, the types and/or duration of priming effects can vary, and magnitudes of the priming effect on SOM decomposition are not well understood yet (Blagodatskaya and Kuzyakov, 2008). Furthermore, rhizosphere priming effects can occur wherever plants exist, and the transfer of dissolved organic carbon after litter input showed a priming effect in a deeper soil profile which was away from the source of C (Steinbeiss et al., 2008). Therefore, in-depth research is needed on the mechanisms of priming effects.

1.1.3 Coupled cycles of carbon and nitrogen

Carbon and N are major elements in ecosystems, and their cycling processes are strongly coupled (Asner et al., 1997). Available forms of N (NH_4^+ and NO_3^-) to plants or microbes come from mineralization of SOM (ca. C:N = 12:1), N fertilization, or N deposition (US DOE, 2008). Nitrogen is generally the most limiting element in plant growth (Gruber and Galloway, 2008). Thus, N applications to plants increase rates of photosynthetic CO₂ fixation through photosynthesis. McGill and Cole (1981) proposed a conceptual model showing that N-transformations during SOM mineralization are related to C-transformations as a result of their association as elemental constitutes of C:N compounds synthesized by plants and microbes. In this manner, C and N are closely linked together from photosynthesis to decomposition. Increases in CO₂ emission and atmospheric-N deposition are major environmental concerns recently (Vitousek et al., 1997; Heimann and Reichstein, 2008; Reay et al., 2008; Rockström et al., 2009). Response of SOC to the increases in CO₂ and N are not fully understood (Reay et al., 2008; Janssens and Luyssaert, 2009). Interactions with other elements such as N, P, K increase the difficulty to predict the impacts of increasing CO₂ on plant productivity and SOC pools (van Groenigen et al., 2006).

1.1.3.1 Effects of N addition on SOC

In general, N fertilization leads to an increase in SOC concentration (Johnson & Curtis, 2001; Alvarez, 2005; Christopher and Lal, 2007; Nave et al., 2009; Jagadamma et al., 2009). Under appropriate N fertilizer application, plant residues returned to soils result in SOC and total N pools increased (Gregorich et al., 1996). Alvarez (2005) studied the effects of N fertilizer on SOC concentration with a range of data sets from different climates, soil types, and management. That NPP was linearly related to SOC concentration (Alvareze and Lavado, 1998), any increase in crop residue returns following N fertilization leads to a positive response to SOC. The positive effect of N fertilization on SOC pools is generally more pronounced in humid and temperate than in dry and tropical climates (Alvarez, 2005). Such a trend may be attributed to greater

responses of crops to N in humid climates and to the higher conversion efficiency from crop residues to SOC in temperate climates (lower temperature) (Alvarez, 2005). Allmaras et al. (2004) used natural C-isotopes abundance in corn (*Zea mays*) fields to detect corn-derived SOC and evaluate the contribution of non-harvestable parts (crown, root, and rhizodeposits) to SOC as a function of fertilizer application rates. Fertilization with N led to increases in stover-derived C and rhizodeposition. However, an increase in SOC pools due to increasing N-fertilization was found only when corn stover was returned to the soil.

In contrast to the positive response of SOC to N fertilization, Khan et al. (2007) reported from experiments in the Morrow plots, the oldest agricultural experimental site in the United States, that SOC concentrations declined with an increase in the amount of inorganic N fertilizer applied. Despite a higher residue input in NPK fertilized plots, the concentration of SOC decreased with time, and this trend was more pronounced in plots receiving the highest rate of fertilizers. Khan and colleagues (2007) concluded that the increase in heterotrophic decomposition of SOC with N additions resulted in declining SOC concentration. Concentrations of total soil N in the Morrow plots followed similar trend to those of SOC (Mulvaney et al., 2009).

Studies involving fractionation of SOC have shown varying effects of N-addition on SOC concentration depending on the SOC fractions. For example, Gregorich et al. (1996) and Nyborg et al. (1999) attributed increasing SOC pools with increasing Napplication to increases in new SOC from increased residue inputs. Hagedorn et al. (2003) also reported that increased N deposition increased the content of total SOM in an acidic loam soil after four years. The N-deposition led to increased root biomass which was related to an increase in SOC in the coarse sand fraction (labile SOM). In addition, Hagedorn et al. (2003) observed higher contents of SOM associated with the silt and clay fractions (old SOM) under high N deposition rates compared to SOM contents of the same size fractions under the lower N deposition treatment. Bradford et al. (2008) reported non-linear responses of sequestered SOC to the N application rates. Within a range of 0 to 100 kg N ha⁻¹ yr⁻¹ treatments, the sequestered root-derived C was the highest at the intermediate level of 30 kg N ha⁻¹ yr⁻¹. C stored in the particulate organic matter (POM) fraction increased with N-fertilization and mineral-associated C was lower in soil receiving the higher N rates (60 and 100 kg N ha⁻¹ yr⁻¹) compared to that in the no N application treatment (Bradford et al., 2008).

No differences in the total SOC pool by N application but differences among SOC fractions have been observed. Neff et al. (2002) observed that N fertilization decreased SOC associated with the light-fractions but stabilized plant materials in heavier, mineral-associated fractions despite no differences in the total SOC pool. Hoffmann et al. (2009) also reported no influence of N fertilization on old and new SOC pools, or ligninderived from old C substrates, however, N fertilization enhanced the decomposition of lignin-derived from new C substrates. These studies allude to the complex transformations of SOC fractions in response to N addition.

1.1.3.2 Effects of N addition on plants

The application of N increases biomass production. In C3 photosynthesis, the enzyme for carbon fixation, ribulose-biphosphate carboxylase-oxygenase (Rubisco), and other photosynthetic enzymes account for about 50% of leaf N (Chapin III et al., 2002).

Accordingly, N addition can enhance photosynthesis by fulfilling the large N requirement of plants. Plant production and biomass accumulation are enhanced by N, especially where N limits plant production such as in boreal and temperate regions (Högberg, 2007). In N-limited forests, N deposition increases leaf N concentration and enhances photosynthetic rates (Hyvönen et al., 2007). Grasslands could also be N-limited or N and P co-limited systems (Crain et al., 2008). Thus, the addition of N to grasslands could result in higher productivity and greater C input to the soil (Conant et al., 2001; Baer and Blair, 2008).

In contrast to the readily apparent positive responses in aboveground biomass to N addition, the responses of belowground plant tissues are not so obvious. The addition of N can change biomass partitioning in plants. The optimal partitioning theory states that plants allocate more fixed C to those organs which enhance the uptake of the most limiting elements (Thornley, 1972). Thus, alleviation of nutrient deficiency through the external N addition can induce plants to invest a higher amount of C to aboveground parts compared to belowground parts. Several studies have supported the optimal partitioning theory by showing altered biomass allocation in plants due to N-fertilization; the production of leaves, wood, and coarse roots is enhanced, but the production of fine roots is decreased (Nilsson and Wiklund, 1995; Oren et al., 2001; Iivonen et al., 2006; Högberg, 2007). Decrease in soil respiration with N addition is often reported, and it is attributed to reduced fine roots production (Magill et al., 2004; Olsson et al., 2005). The reduced translocation of assimilated C to the belowground is also observed in crops and pastures (Kuzyakov et al., 2000). Thus, plants can reduce the "ineffective" C losses such as root respiration and exudation when available forms of N are sufficient.

On the other hand, studies on root production rather than maximum root biomass have presented another view indicating a strong relationship between root productivity and N availability. The low root biomass under high available N may be attributed to a faster turnover rather than to a lower production of roots (Raich and Nadelhoffer, 1989; Burton et al., 2000; Nadelhoffer, 2000). The basic assumption of this hypothesis is that both root growth and mortality can occur together and continuously, thus the maximum root biomass measurement does not adequately represent root production. Raich and Nadelhoffer (1989) observed that a constant proportion of total NPP was allocated to roots in a mature forest ecosystem. Thus, lower root biomass in fertile sites may be attributed to a faster turnover of roots despite a higher amount of NPP allocation.

Inorganic N application can alter plant community composition, and the amount and/or quality of litter produced, such changes can indirectly affect the rate of decomposition (Wardle et al., 2004; Suding et al., 2005; Manning et al., 2008). The high amount of available N can lead plants to produce litter containing a low C:N ratio. In the Harvard Forest, biomass of fine roots was not changed, but N content in roots increased with a chronic N deposition (Magill et al., 2004). Substrates with a lower C:N ratio can be preferably consumed by microorganisms, and thus lead to a faster decomposition of litter after N addition (Waldrop et al., 2004; Hobbie, 2005; Knorr et al., 2005). Increased N availability in a peat bog facilitated litter peat decomposition by altering accumulated litter quality as well as by alleviating N limitation for microbes (Bragazza et al. 2006). Thus, changes in litter quality by addition of N can influence decomposition and feedback to the overall C and N cycles. 1.1.3.3 Effects of N addition on microbes

Inorganic N can directly affect species composition or activities of microorganisms. The addition of N fulfills the nutrient demands of microorganisms, and can thus alter microbial activities (Hessen et al., 2004). In most ecosystems, N is a limiting element for growth, and microbes require energy to obtain N. The supply of inorganic N relieves the demands for N and alleviates competition for N with plants. The addition of N increased soil microbial biomass and activity in a 10-year study (Lovell et al., 1995). Thus, an increase in N availability can enhance microbial decomposition and turnover of C (Knorr et al., 2005; Bragazza et al. 2006). Conversely, a chronic N deposition at Harvard forest caused a reduction in soil respiration (Bowden et al., 2004) as well as microbial biomass and potential microbial activity (Compton et al., 2004). Decline in forest productivity by a chronic N deposition might lower root activity and rhizodeposition, and thus it is likely to reduce microbial activity. Meta-analysis showed that the addition of N reduced 15~20% of microbial biomass.(Treseder, 2008; Liu and Greaver, 2010).

The addition of N can induce changes in the microbial community (Compton et al., 2004; Bradley et al., 2006; Chu et al., 2007; Treseder, 2008). Fontaine and Barot (2005) suggested several possible cases indicating the effects of nutrient availability on microbial competition. While r-strategists are abundant with available nutrients, K-strategists can be dominant under nutrient limiting conditions. The shift from a fungal- to bacterial-dominated community is frequently documented as a result of addition of N (Bradely et al, 2005; Treseder, 2008). Frey et al. (2004) reported that active fungal biomass was less in fertilized compared to unfertilized plots. Using molecular techniques,

Allison et al. (2007) documented a decrease in fungal diversity and richness in boreal ecosystems with N application.

Enzyme production or composition is also affected by the addition of N (Bragazza et al. 2006). Peroxidase and phenol oxidase are related to C cycling because they can decompose recalcitrant SOM as well as lignin-containing materials (Sinsabaugh, 2010). The addition of N can change enzyme activities from oxidative enzymes to hydrolase enzymes (Ajwa et al., 1999; Saiya-Cork et al., 2002; Sinsabaugh et al., 2002). A significant decrease in lignin-degrading enzymes produced by white-rot fungi (basidiomycetes) was found with chronic N addition (Carrio et al. 2000). Therefore, the addition of N could decrease decomposition of SOM through changes in oxidative enzymes or from changes in microbial community composition.

1.1.4 Relationships between SOC and soil structure

Soil structure refers to an arrangement of mineral particles and voids. The soil aggregate is a basic unit of soil structure. Several physical and chemical properties (environmental variables, and organic and inorganic binding agents) and biological factors (microorganisms, roots, and soil fauna) are involved in aggregate formation and stabilization (Six et al., 2004). Hierarchical organization of fundamental particles into aggregates is observed where SOM is the main binding agent. According to the aggregate hierarchy model, primary mineral particles are bound together by various cementing agents including persistent organic materials, crystalline oxides, and highly disordered aluminosilicates which form microaggregates (53-250 µm). Then, temporary binding

agents such as microbial exudates, fungal hyphae, and plant roots contribute to form macroaggregates (> 250 μ m) (Tisdall and Oades, 1982). It is hypothesized that microaggregates are generally formed within macroaggregates (Oades, 1984; Golchin 1994; Gale et al., 2000; Six et al., 2004). Macroaggregates are formed around new inputs of POM. The POM-C is decomposed by microbes, which produce microbial exudates as binding agents which enhance the stability of macroaggregates. The exudates from microorganisms acting as glue to bind clay particles into microaggregates within the macroaggregates. As the labile POM is decomposed and macroaggregates.

While SOC plays crucial roles in aggregation, as is shown in the previous paragraphs, aggregates can also provide protective mechanisms for SOC against decomposition and thus result in the accumulation of SOC by increasing its MRT. The spatial inaccessibility of occluded SOC within aggregates is one of the several SOC protection mechanisms in conjunction with chemically recalcitrant SOC compositions and SOC interactions with minerals or metal ions (Sollins et al., 1996; Christensen, 2001; Six et al., 2002; Jastrow et al, 2007). The rate of SOC decomposition is affected by its spatial distribution within soil aggregates. Lignin was in a less decomposed state where located within the interior of aggregates compared to a more decomposed state where located at or near the aggregates surface (Amelung et al., 2002). Soil porosity affects the distribution, movement, and activities of soil microorganisms (van Veen and Kuikman, 1990). Pore neck diameters < 1 μ m offered limited access of decomposers to SOC, extending its MRT in the soil (van Veen and Kuikman, 1990). Within pores, the availability of oxygen and water can also restrict microbial activities. Strong et al. (2004)

described the relationship between pore-sizes and decomposition rates. The initial decomposition rate was faster in the pores with 15-60 μ m diameter than in the larger size pores (60-300 μ m). Although abundant oxygen was supplied to larger pores (60-300 μ m), less favorable conditions for microbes (i.e., slower diffusion of nutrients, existence of water as thinner water films rather than water filled pores) limited the decomposition rate (Strong et al., 2004). Therefore, soil structure is particularly important in SOC dynamics.

Macroaggregates are somewhat unstable and have shorter MRT compared to stable (not easily destroyed by external disturbances) microaggregates (Puget et al., 2000; Six et al, 2004; De Gryze et al, 2006). Even though macroaggregates provide a short-term protection for SOM, the turnover rate of macroaggregates is important to stabilize SOC (Puget et al., 2000; Six et al., 2004). If the turnover rate of macroaggregates is too rapid due to disturbances or other factors, the formation or stabilization of microaggregates can be limited. Several studies have shown that formation and stabilization of microaggregates as well as microaggregate-protected SOC levels decreased under conditions of frequent disturbances (i.e. tillage operation) (Six et al., 2000; John et al., 2005; Pullman et al., 2005). Soil organic carbon and soil aggregation are strongly interrelated.

1.2 Switchgrass as a bioenergy crop

1.2.1 The potential of biofuel production

Biomass has been and continues to be an important energy source in developing countries, and more recently has been considered as a renewable energy source in developed countries (Sagar and Kartha, 2007). The US's Energy Independence and Security Act of 2007 mandated increases in the use of renewable biofuels from 18 billion L in 2007 to 136 billion L by 2022 (Energy Independence and Security Act, 2007). Thus, attempts are being made to develop efficient technology for lignocellulosic ethanol production. Biofuels can supplement energy supply from fossil fuel resources, reduce net anthropogenic CO₂ emission, and be a new source of income for farmers (Lemus and Lal, 2005; Luque et al., 2008). Because of the technological constraints in converting biomass into liquid fuels, biofuels are still more expensive than fossil fuels (Sanderson et al., 2006). However, it is expected that these constraints can be overcome in the near future through technological advances such as improved efficiency of biomass conversion into the fuels and use of biotechnology of developing beneficial plant traits as bioenergy crops such as high yield, C allocation, and biochemical composition (McLaughlin and Kszos, 2005; Sanderson et al., 2006; Yuan et al., 2008).

Theoretically, biofuels can be a C-neutral energy source since the CO_2 fixed through photosynthesis is recycled. Nevertheless, whether biofuels in practice are a Cneutral energy source remains a highly debatable issue (Cowie et al., 2006; Hill et al, 2006; Fargione et al., 2008; Searchinger et al., 2008). Scharlemann and Laurance (2008) assessed the total environmental impact of a wide range of biofuels. Among 26 biofules species, nearly half (including corn ethanol, sugarcane ethanol, and palm oil diesel) had more adverse impacts on environments than fossil fuels. On the other hand, lignocellulose ethanol production from non-food plants (e.g., switchgrass, trees, or algae) can benefit environments and reduce the costs for biofuel production. Tilman et al. (2006) suggested that using low input high diversity (LIHD) prairie to produce bioenergy feedstock on degraded lands could yield high energy and reduce the use of fertilizers and pesticides, and accordingly result in the reduction of GHG emissions. Along with these benefits, growing perennial grasses or woody plants can improve quality of soil and water by enhancing SOC and filtering pollutants through deep root systems (Tolbert et al., 2002; Tufekciolu et al., 2003; Sartori et al., 2006; Wright and Turhollow, 2010).

1.2.2 General characteristics of switchgrass

Switchgrass has been selected as one of the promising herbaceous crops among 34 species by the U.S. Department of Energy because of its potential for high biomass production and its cost-effective growth characteristics (McLaughlin and Walsh, 1998; McLaughlin, 1992; Bransby et al., 1998). Being a native grass, it is geographically distributed in a wide range of environments and can be grown on a marginal land or under poor growth conditions (Sanderson et al., 2006; Wright and Turhollow, 2010). Moreover, as a C4 species, switchgrass has high N and water use efficiency and can gain some N through fixation by associated soil bacteria (Bassam, 1998; Parrish and Fike, 2005). There are two ecotypes of switchgrass: lowland and upland cultivars. Lowland cultivars generally produce more biomass, have thicker stems and are taller than upland cultivars (Parrish and Fike, 2005; Wullschleger et al., 2010). While lowland cultivars are

adapted to flood plains, upland cultivars are more drought tolerant. Results of experiments conducted by the Bioenergy Feedstock Development Program (BFDP) showed that the average aboveground biomass production of switchgrass ranged from 10 to 21 Mg ha⁻¹ across the 13 states in the United States (McLaughlin and Kszos, 2005).

Ma et al. (2000) studied growth of switchgrass roots up to 330 cm. Despite the deep rooting characteristics, 70-80% of roots are distributed in the surface 30 cm (Ma et al., 2000; Sanderson, 2008; Garten Jr. et al., 2010). The deep and extensive roots of switchgrass can transfer C into soil, which can contribute to improving soil quality (McLaughin and Walsh, 1998; Ma et al., 2000). Liebig et al. (2005) showed that SOC distribution in a soil profile under switchgrass was greater below 30 cm than that under cultivated crops. Frank et al. (2004) reported a high rate of SOC increase of 1.01 kg m⁻² yr⁻¹ under switchgrass in frigid Mollisols. In addition to the increase in SOC concentration, the establishment of switchgrass enhanced C dynamics such as C mineralization, microbial biomass C, and C turnover (Ma et al., 2000).

1.2.3 Establishment and management of switchgrass

Major obstacles for switchgrass establishment are seed dormancy and weed competition (Parrish and Fike, 2005). Freshly harvested seed has a low rate of germination (< 5%) (Knapp, 2000). Germination rate can be increased when viable seed is treated by stratification to break dormancy (Haynes et al., 1997). Using pre-emergence chemicals with no-till planting, no fertilization in the establishment year, or late seeding can reduce the encroachment and competition from cool season grasses (Parrish and Fike, 2009).
Additionally, the management of N is particularly important to a successful establishment of switchgrass (Parrish and Fike, 2005; Haque et al., 2009; Heggenstaller et al., 2009; Mooney et al., 2009). High rates of N fertilization have been recommended for switchgrass as a forage crop to improve the feedstock quality for animal feeding or to minimize biomass loss by frequent cutting (Smith, 1981; Hall et al., 1982; Reid et al., 1988). However, lower rates of N fertilization can be applied to switchgrass for the bioenergy feedstock production if the harvest is delayed until after the major nutrients, especially N, are translocated belowground (Garten Jr. et al., 2010). Switchgrass has high N use efficiency and can recycle N in the subsequent year's growth (Parrish and Fike, 2005). However, when multi-biomass harvesting in a year is needed, higher rates of N should be considered since more N will be exported from the system (Parrish and Fike, 2005). Accordingly, the rate of N fertilization must be determined by considering the site-specific factors such as soil fertility, precipitation, and harvesting frequency.

1.2.4 Changes in soil quality followed by growing biofuel crops

Several societal and environmental objections have been raised concerning the cultivation of bioenergy crops: competing lands for food production, degradation of soil quality, fertilizer use, and loss of wildlife habitats (Righelato and Spracklen, 2007; Sagar and Kartha, 2007; Field et al., 2008; Johnson et al., 2010; Lal et al., 2010). In some cases, the energy output might be lower than the energy input depending on fuel consumption for cultivation, transportation, conversion processes, etc. Therefore, the question whether biofuels can be produced sustainably needs to be addressed prior to discussing its potential (Doornbosch and Steenblik, 2007). Among several concerns regarding biofuel

production, changes in soil quality with the cultivation of bioenergy crops is discussed in the following paragraphs.

Cultivation of bioenergy crops usually entails the removal of plant biomass from the land. Based on the accumulated knowledge about no-till farming, it is possible to surmise the probable consequences of continuous removal of aboveground biomass feedstock on soil quality (Blanco-Canqui et al., 2007; Blanco-Canqui, 2010; Johnson et al., 2010). First of all, the removal of biomass changes energy and water balance in the soil ecosystem. The residue cover reduces fluctuations of soil temperature due to the soil surface exposure from direct radiation and heat (McCalla, 1943; Blanco-Canqui et al., 2006). The surface roughness of residue changes the aerodynamic boundary layer which moderates the exchanges of water and heat (Hagen, 1996). Decreased evaporation and increased water infiltration resulting from residue present on the soil surface generally result in high soil moisture (Dao, 1993; Kumar et al., 1999; Hobbs et al., 2008). Thus, soil from which residue has been removed maybe prone to accelerated soil erosion because it is not protected against climatic erosivity.

Furthermore, residues, being a C source, can influence the level and dynamics of SOC and aggregation (Johnson et al., 2010; Cruse et al., 2010). The contribution of the C input from aboveground biomass may have a lesser impact on SOC than that from belowground biomass (Rasse et al., 2005). Nevertheless, SOC levels in the surface layer can be strongly influenced by the input of aboveground residue-C. Because microbial activities are the most active and aggregation is stable in the surface soil, any decrease in C input from the aboveground biomass would adversely impact its functions and structure (Blanco-Canqui and Lal, 2008). Abiotic changes which accentuate

decomposition of SOC along with a lesser input of C can aggravate depletion of SOC pool and decline of soil structure (aggregation), and jeopardize the sustainable production of bioenergy crops.

Biomass harvesting also involves a removal of nutrients from the system (Cruse et al., 2010). In this context, Tilman et al. (2006) suggested that using a diverse plant community as biofuel feedstock may not need any external input of nutrients for maintaining a high biomass production. Mikhailova et al. (2000) reported that the SOC and total nitrogen concentrations under a hay field that was cut annually for at least 50 years did not differ from that of a nearby native grassland. As a rule of thumb, however, extracted nutrients must be replenished through external inputs to minimize the mining of soil nutrient stocks from soils (Russelle et al., 2007; Johnson et al., 2010). The export of nutrients can be minimized by harvesting biomass after crop-senescence. Hence, biofuels may not be the ultimate option for solving the energy scarcity, but sustainable production of biofuel could be an intermediate option until improved non-C fuel sources can be developed and brought online. Therefore, proponents of bioenergy crops need to seek sustainable methods of cultivation by considering all expected changes in soil quality associated with cultivation of bioenergy crops.

1.3 Research objective

As a renewable energy source, biofuel advocates aim at mitigating the increase in anthropogenic CO_2 emission by recycling C from photosynthates and by increasing SOC pools. Fertilization with N is one of the most common management practices in growing bioenergy crops. The responses of SOC to N input should be understood because of the coupled cycling of C and N. However, the mechanisms underlying the changes in SOC dynamics as driven by exogenous N are not fully understood. Since the availability of N can control both biomass production and decomposition simultaneously, it is difficult to predict the overall effect of N on SOC pools. Since a major source of soil C input is root biomass in bioenergy crops, it is critical to understand the roles of roots in SOC sequestration and the probable shift in biomass allocation associated with N addition. Moreover, the potential of switchgrass production as a biofuel feedstock has not been well recognized in the Midwestern region, especially in Ohio.

Changes in SOC by N addition can influence soil structural properties. Therefore, changes in soil quality with N input needs to be evaluated under switchgrass cultivation for sustainable production of biofuel feedstock. Therefore, the overall goal of this study is to assess the effects of inorganic N addition on switchgrass biomass production, SOC dynamics, and soil structural properties. Specific objectives of this research are: (1) to assess the changes in SOC and in the aboveground and belowground biomass production of switchgrass under different rates of N application in Ohio, (2) to study decomposition of organic matter by addition of inorganic N and C substrates, and (3) to evaluate soil structural properties under switchgrass followed by N addition.

1.4 Dissertation Outline

This dissertation consists of six chapters. Chapter 1 is an introductory section, outlining the rationale and objectives of the research. The general introduction includes related research reviews such as the importance of the SOC, C inputs especially the root-derived C and priming effects, coupled cycles of C and N in plant biomass production

and decomposition of SOM, and aggregate formation and stabilization. Moreover, general traits of switchgrass as a bioenergy crop and environmental concerns regarding aboveground biomass removal are discussed.

Chapter 2 evaluates the effects of N fertilization on switchgrass biomass production and SOC concentration and pools at three sites in Ohio for two consecutive years. The objective was to assess whether changes in biomass allocation were induced by N application and whether SOC was changed in a positive or negative direction. The SOC pool was also compared with that from the adjacent soils under previous land uses, and the land conversion effects are discussed. The potential of switchgrass production in Ohio is also evaluated.

In Chapter 3, the effects of inorganic N and C substrates on OM decomposition were assessed under laboratory conditions. Several possible mechanisms for the negative effects of N on OM decomposition were tested whether N limitation leads microbes to decompose OM to obtain N or whether N addition decreased specific enzyme activities. An exponential model was used to test the possibility of recalcitrant materials formation with N addition. Priming effects by C substrates (root-C) on inherent SOC were also evaluated with the natural abundance isotope (¹³C) technique.

The research on the soil structural changes induced by N fertilization is presented in Chapter 4. This research was conducted on soils under switchgrass in Tennessee after four years of consecutive N fertilization. Several soil physical parameters were examined including the aggregate stability. The causes of the differences in soil aggregate stability were related to the changes in root attributes after N fertilization. Chapter 5 describes soil aggregate properties assessed under different switchgrass cultivars during a growing season in Tennessee. The goal was to assess differences in root characteristics among cultivars and the attendant changes in soil structure and SOC concentrations after four years of switchgrass growth. The relationships among physical properties of aggregates are also investigated.

Chapter 6 presents the overall conclusions and further research directions on SOC dynamics and soil quality measurement under biofuel crops in general, and switchgrass in particular.

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CHAPTER 2

IMPACTS OF NITROGEN FERTILIZATION ON BIOMASS PRODUCTION OF SWITCHGRASS AND CHANGES IN SOIL ORGANIC CARBON IN OHIO

2.1 Abstract

Switchgrass (*Panicum virgatum*) is a promising bioenergy crop that requires nitrogen (N) fertilization to improve biomass yield, depending on soil type and site characteristics. N fertilization can also increase the soil organic carbon (SOC) stock, an important determinant of soil quality. Therefore, this study was conducted to assess the effects of N fertilization on switchgrass biomass production and the SOC stock under different locations in Ohio. Switchgrass was established at three research stations (Northwest, Jackson, and Western site) of the Ohio Agricultural Research and Development Center (OARDC) in spring 2004. N fertilizers was applied at four rates (0, 50, 100, and 200 kg N ha⁻¹) in 2008 and 2009. The SOC concentrations under switchgrass were analyzed before (spring 2008) and after N application (fall 2009) and from reference soils under the same previous land uses for 0-10, 10-20, and 20-30 cm depths. Also, aboveground and belowground biomass and the C and N concentrations in plant tissues were measured at the end of growing season. The SOC concentration at different depth did not vary among N treatments except for 10-20 cm depth at Jackson (15.3 and 10.1 g SOC kg⁻¹ for 200 and 50 kg N ha⁻¹ treatments, respectively). The SOC stock sizes

under switchgrass to 30 cm depth ranged from 102~123, 55~70, and 59~67 Mg C ha⁻¹ at the Northwest, Jackson, and Western sites, respectively. There were no differences in SOC stocks among treatments at Northwest and Western sites, but the SOC stock in the plot receiving fertilizer at a rate of 200 kg N ha⁻¹ was significantly higher than that of 50 kg N ha⁻¹ at Jackson site. While the SOC stocks increased linearly with increase in N rates for Northwest and Jackson sites, this positive correlation was not observed for the Western site. Aboveground biomass (10.4, 12.9, 13.8, and 16.6 Mg ha⁻¹) and shoot/root ratios (3.0, 3.1, 3.5, and 4.5) increased with N rate (0, 50, 100, and 200 kg N ha⁻¹, respectively), but the belowground biomass $(4.6 - 5.2 \text{ Mg ha}^{-1})$ did not vary among N rates. The aboveground biomass production in 2009 (15.5 Mg ha⁻¹) increased by 4.2 Mg ha⁻¹ from 2008. Among three sites, aboveground biomass was the highest at the Western site. The amount of N export by the harvest of aboveground biomass increased with increase in N rates, but did not differ among sites. The amount of N export which was balanced with the rate of N application was estimated as 32 kg N ha⁻¹ with a 95% confidence interval (11-53 kg N ha⁻¹). The results indicated a potential of growing switchgrass as a bioenergy crop in Ohio and positive responses of both aboveground biomass and the SOC stock to N fertilization.

Keywords: switchgrass, biomass production, soil organic carbon (SOC), nitrogen fertilization

2.2 Introduction

The issues of energy security and the increase in carbon dioxide (CO_2) in the atmosphere underscore the need for the production of bioenergy crops (McLaughlin et al., 2002). However, the concerns of ethanol production using crop residues have also been increased because of the competition for lands, accelerated soil erosion and degradation of soil quality by continuous and perpetual residue removal (Blanco-Canqui and Lal, 2007; Varvel et al., 2008; Pimentel, 2010). Switchgrass (Panicum virgatum L.) is an important herbaceous bioenergy crop because of its high biomass production and costeffective growth characteristics (Dunn et al., 1993; Wright, 1996). Along with these positive traits for biofuel feedstock, switchgrass can be grown on degraded and/or agriculturally marginal soils since it is adapted to these soil conditions (Wright and Turhollow, 2010). Furthermore, switchgrass can improve soil quality by increasing soil organic carbon (SOC) and by reducing soil erosion (McLaughin and Walsh, 1998; Tolbert et al., 2002; Frank et al., 2004; Liebig et al., 2005; Cowie et al., 2006). Increase in SOC stocks can also help mitigate anthropogenic emissions of CO₂ as well as provide other ecosystem services (Lal, 1997; Wander and Nissen, 2004).

N fertilization at a judicious level is one of the most important management practices for increasing biomass yields of switchgrass. The effects of N addition on SOC stocks could be positive, negative, or neutral (Johnson & Curtis, 2001; Christopher and Lal, 2007; Reay et al., 2008). In agricultural ecosystems, N fertilizer may increase the SOC stocks but only when it is applied in conjunction with crop residue return (Gregorich et al., 1996; Alvarez, 2005). Allmaras et al. (2004) observed that N-fertilization resulted in an increase in stover C and rhizodeposition and thus maintained a

higher SOC concentration. Application of N fertilizer to the level of the yield sufficiency increased residue returns which lead to higher SOC.

Some studies, however, have reported contrasting results about the response of SOC to the application of inorganic N fertilizer. The data from the Morrow plots, the oldest agricultural experimental site in the United States, showed that the concentration of SOC decreased with time despite the higher residue input in NPK fertilizer plots, and this trend was more pronounced in plots receiving high rates of fertilizer (Khan et al., 2007). Total soil N in the Morrow plots followed the same trend (Mulvaney et al., 2009). In soddy-podzolic soils, high inorganic N converted the stable state of Ca-forms of SOC to labile humic substances and increased mineralization, and thus decreased the steady state C (Shevtsova et al., 2003). These studies showed that SOC stocks can decrease with the application of N fertilizers. Furthermore, Neff et al. (2002) showed no net change in bulk soil C after N fertilization but different responses were observed depending on SOC fractions: decreased C in light fractions and stabilized plant materials to the heavier fractions.

The addition of N enhances plant production and biomass accumulation in Nlimited systems (Högberg, 2007). In addition, N fertilization can alter biomass allocation in plants; relatively more C is translocated to leaves and woody tissues with N addition (Nilsson and Wiklund, 1995; Högberg, 2007). Alleviation of nutrient deficiency through fertilization can increase allocation of C to the aboveground biomass to facilitate competition for light (Tilman, 1987). However, the degree and the direction of these changes may vary depending on species and the types of plant functional groups (Wedin and Tilman 1996; Sage and Kubien, 2003). The input of C to soil under switchgrass cultivation is mainly through belowground biomass since the aboveground biomass is harvested and removed from the plot for bioenergy feedstock. Thus, the change in biomass allocation induced by N application can affect the input of C and subsequently SOC stocks. Studies on biomass partitioning of switchgrass with N fertilization are limited (Ma et al., 2001; Heggenstaller et al., 2009), and additional research is needed on the responses of roots to the addition of N.

Switchgrass as a bioenergy crop generally requires a low level of N fertilization. Yet, fine tuning is necessary depending on the site and soil conditions (Parrish and Fike, 2005) to maximize the aboveground biomass production, to improve N use efficiency, and to enhance SOC sequestration. Thus, the objective of this research was to assess the effects of N fertilization on the aboveground and belowground biomass production of switchgrass on three soils in Ohio. It is hypothesized that the aboveground biomass, shoot/root ratio, SOC stock, and amount of N export by the aboveground biomass harvesting increase with increasing rates of N application.

2.3 Materials and Methods

2.3.1 Field sites and N fertilization

This study was conducted in switchgrass plots in the Ohio Agricultural Research and Development Center (OARDC). The research sites were Western (39° 51′ 21" N, 83° 40′ 40" W), Northwest (41° 13′ 49" N, 83° 45′ 34" W), and Jackson (39° 1′ 39" N, 82° 36′ 17" W) branches (Table 2.1). During the 6 years of switchgrass growth (2004-2009), the average annual temperature and precipitation in Northwest, Jackson, and Western sites were 10.4°C and 849 mm, 11.7°C and 1061 mm, and 10.2°C and 950 mm, respectively (<u>http://www.oardc.ohio-state.edu/ newweather/default.asp</u>).

The predominant soil series in Jackson are Rarden-Wharton silt loams (Fine, mixed, active, mesic Aquultic Hapludalfs, Fine-loamy, mixed, active, mesic Aquic Hapludults) and the Shelocta-Rarden association, steep (Fine-loamy, mixed, active, mesic Typic Hapludult, Fine, mixed, active, mesic Aquultic Hapludalfs) (Soil Survey Staff, 2008). At Jackson, cool season grasses, such as tall fescue (Schedonorus phoenix) and orchard grass (*Dactylis glomerata*), were planted in the early 1980's for hay production. Warm season grasses, such as switchgrass (Panicum virgatum), eastern gammagrass (Tripsacum dactyloides), Indiangrass (Sorghastrum nutans), and Caucasian bluestem (Bothriochloa caucasica), were planted during the mid 1980's to early 1990's, and were grazed once a year. The soil at the Western site is a Kokomo silty clay loam (Fine, mixed, superactive, mesic Typic Argiaquolls) and Strawn-Crosby complex (Fine-loamy, mixed, active, mesic Typic Hapludalfs, Fine, mixed, active, mesic Aeric Epiaqualfs) (Soil Survey Staff, 2008). At the Western site, the previous land use was a no-till (NT) corn (Zea mays)-soybean (Glycine max) rotation since the 1990s. Fertilizer was applied at 202 kg N ha⁻¹ during corn cultivation, but no fertilizer was applied for the soybean crop. Corn had been planted the year before switchgrass was established. The soil at the Northwest site is Hoytville silty clay loam and Hoytville clay loam (Fine, illitic, mesic Mollic Epiaqualfs) (Soil Survey Staff, 2008). The soil was under grass before 1985, apple trees from 1985 to 1997, and sod after 1997. No drainage had been installed at any of these three sites. The bioenergy plots in all three sites were established in spring 2004. Soil was plowed for better seed bed preparation before planting switchgrass. Each site had four

plots of switchgrass, and each plot was 6×6 m. The seeding rate was 11.2 kg ha⁻¹ pure live seed (PLS). Nitrogen fertilizer (56 kg N ha⁻¹) was applied at the time of plot establishment, but afterwards no N fertilizer was applied until the N experiments were initiated in 2008. Since the time of establishment, aboveground biomass of switchgrass was cut at 15 cm above the soil surface at the end of the growing season every year, and the harvested biomass was removed from the plots. In one plot in Jackson, the establishment of switchgrass was failed due to weed competition, and therefore that plot was not included in this study.

Each species plot was divided into four sub-plots (3 m \times 3 m), and four rates of N fertilizer (0 (control), 50, 100, and 200 kg N ha⁻¹) were broadcasted in randomly selected subplots in 2008 and 2009. The amount of N fertilizer was split into two and applied once in late May to early June, and again in July 2008. These rates were repeated in May and July 2009. Ammonium nitrate was used as N fertilizer in 2008, and urea was applied in 2009.

2.3.2 Soil sampling and analyses

Soil samples were obtained during spring 2008 before N fertilization to establish baseline data, and subsequently in fall 2008 and 2009 to assess the treatment effects. In spring 2008, the reference soils, which were maintained under the same land uses as before establishing the switchgrass, were obtained from close proximity to the plots at each site. Soil under tall fescue was sampled at Jackson, and that under sod was selected at the Northwest site as a reference soil. At the Western branch, NT corn plots were chosen as a reference soil. A plot under NT corn was established at the time of starting the switchgrass plot, but N fertilizer (202 kg N ha⁻¹) was applied every year, and after harvesting, corn stover was not removed from the NT plot.

Soil cores were obtained for bulk density (ρ_b) and root biomass measurements, and bulk soil samples were collected for the other soil analyses for 0-10, 10-20, and 20-30 cm depth between two rows (inter-row). In each subplot, one 4.65 cm diameter core (5 cm height) was used for 0-5 and 5-10 cm depth, and these two core samples were pooled for the 0-10 cm depth sample. A core with 5.4 cm diameter and 6 cm height was obtained for 10-20 and 20-30 cm depths. Bulk soil samples were air-dried, gently ground, and passed through a 2-mm sieve.

Gravimetric moisture content was determined from field-moist soils after drying at 105°C. Soil volume was calculated based on the core size. Soil ρ_b was calculated by oven-dried weight divided by soil volume (Topp and Ferré, 2002). The volume and weight of the soil were corrected for gravel contents in the samples before ρ_b calculation. Soil texture was determined by the pipette method without treatment with H₂O₂ (Kilmer and Alexander, 1949). Soil (10 g) was dispersed with sodium hexametaphosphate and sodium carbonate solution by shaking for 16 hrs. The clay fraction was measured by pipetting, and the sand fraction was measured after washing silt and clay fractions out through a 0.05 mm-sieve. Soil pH was determined in a 1:2 soil/water (w/v) ratio (Thomas, 1996). For SOC and total N (TN) analyses, soil samples (< 2 mm) were ground in a roller-mill grinder (Sampletek 200 Vial Rotator, USA) for 2 days, and then passed through a 0.25-mm sieve. The SOC and TN concentrations were measured by dry combustion (900°C) using a CN analyzer (Vario Max Elementar Americas, Inc., Germany) (Nelson and Sommers, 1996).

2.3.3 Plant sampling and analyses

Aboveground and belowground biomass were sampled in October 2008 and in November 2009. Standing aboveground biomass (a 50×50 cm quadrat) was sampled in the center of the subplot to minimize a marginal effect by leaving ca. 15 cm stubbles from the ground. Fresh weight was measured, and a subsample was taken for moisture content correction and C and N analyses. Green and senesced parts were not distinguished. The aboveground biomass was dried in the oven at 60°C until a constant dry weight (ca. 48 hrs). The aboveground subsample was chopped, finely ground with a Wiley Mini Mill (Thomas Scientific, USA), and passed through a 0.64 mm-sieve. For the root biomass estimation, soil core samples taken for ρ_b measurement were used. Since soil cores were taken from the inter-row, a few core samples included rhizomes. Rhizomes were excluded for root biomass and length measurement in this study. All roots were separated manually as much as possible from soils, then washed, dried at 60°C for 48 hrs, and weighed. No distinction was made between live and dead roots. In 2009, total root length was measured before drying roots through scanning and image analysis with a WinRhizo software program (Regent Instruments, Inc., Québec, Canada). Root length density (RLD) was calculated by dividing total root length by the soil volume. After root biomass measurements, roots in the same soil profile were combined for C and N analyses. After being dried at 60°C for 48 hours, roots were ground as the same way for the aboveground biomass. The CN analyzer (Vario Max Elementar Americas, Inc., Germany) was also used to determine C and N concentrations in plant tissue samples.

2.3.4 Calculation and statistical analyses

N export through the removal of aboveground biomass at the end of growing season was calculated based on the area (Eq.[1]).

N export (kg N ha⁻¹) = N concentration in above ground tissues (kg N Mg⁻¹) × harvested biomass (Mg ha⁻¹) Eq. [1]

Area-based SOC and TN stocks to 30-cm depth (equivalent volume approach, EV) (Eq. [2]) and those stocks in equal soil mass with different depths (equivalent mass approach, EM) (Eq. [3]) were calculated. In the EM approach, one soil profile which had the lowest soil mass among all samples was used as a reference (Paul et al., 2001).

In the EV approach (Gregorich et al., 1995),

SOC (or TN) (g C (or N) ha⁻¹) = $\sum_{i=1}^{3} \{$ SOC (or TN) concentration_i (g C (or N) Mg soil⁻¹) × ρ_{bi} (Mg soil m⁻³) × 0.1 m (depth) $\} × 10^{4}$ m² ha⁻¹ Eq. [2] where *i* = soil depth (1 = 0-10 cm; 2 = 10-20 cm; 3 = 20-30 cm).

In the EM approach (Paul et al., 2001),

SOC (or TN) (g C (or N) ha⁻¹) = $\sum_{i=1}^{2} \{ \text{ SOC(or TN) stock}_i \} + \{ \text{SOC (or TN)} \text{ concentration}_{i=3} (g C (or N) Mg \text{ soil}^{-1}) \times \rho_{bi=3} (Mg \text{ soil m}^{-3}) \times x \text{ m (depth)} \} \times 10^4 \text{ m}^2 \text{ ha}^{-1}$

Eq. [3]

where i = soil depth (1 = 0.10 cm; 2 = 10.20 cm; 3 = 20.30 cm depth; x was calculated to render total soil mass equal for all treatments).

The normality of data and constant variance of errors were checked prior to conducting statistical analyses. When the data distribution was not normal, or the error assumption was violated, the data were log-transformed.

Aboveground and belowground biomass and the shoot:root (S/R) ratio (aboveground/ belowground biomass) were analyzed by a repeated measures (year) randomized complete block (RCB) design ANOVA (factor = N rates and sites). A covariance structure chosen by AIC criteria was used in the model. Because the interaction between N rate and year was not significant, the model used for this experiment was (Eq. [4])

$$Y_{ijkl} = \mu + \beta_i + \tau_j + \gamma_k + \delta_l + (\tau\gamma)_{jk} + (\beta\gamma)_{ik} + \varepsilon_{ijk}$$
 Eq. [4]

where Y_{ijkl} was the observed values for the *i*th block assigned to the *j*th N rates at site *k* in year *l*; μ was the overall mean; β_i was the *i*th block effect; τ_j was the *j*th N main effect; γ_k was the *k*th site main effect; δ_l was the *l*th year main effect; $(\tau \gamma)_{jk}$ was the interaction effect between N rates and sites; $(\beta \gamma)_{ik}$ represented the random effects; and ε_{ijkl} represented error terms.

The repeated measures RCB design ANOVA was performed using PROC MIXED in SAS release 9.2 (SAS Institute, 2002). When significant differences were observed (p < 0.05) in the ANOVA, post-hoc mean comparisons were conducted by a Tukey's HSD method (p < 0.05).

The RLD in each depth increment and the C and N concentrations in plant tissues were analyzed with a two-way ANOVA model (N rates, sites, and N rates*sites interaction). Because of using different reference soils for each site, ρ_b , SOC and TN concentrations, and C/N ratios in soil, SOC and TN stocks, and soil pH were analyzed with one-way ANOVA model (variable: N rates) by each depth in each site (JMP 7.0). When significant differences were observed (p<0.05), mean separation was followed by a Tukey's HSD method (p<0.05). Linear regression analysis was conducted to evaluate the change in SOC stocks and the N export depending on N rates (R Development Core Team, 2009).

2.4 Result

2.4.1. Soil bulk density and pH

Overall, there were no significant differences in ρ_b among N treatments in the switchgrass plots. At 0-10 cm depth, ρ_b under the reference soil under sod in the Northwest site was significantly lower than in soil under switchgrass in 2008 (Table 2.2). In the Western site, ρ_b in the reference under NT corn and the switchgrass soil in 2008 was significantly higher than that of non-fertilized plots in 2009 for 10-20 cm depth. In other depths and sites, there were no significant differences in ρ_b among treatments. At all three research sites, soil pH was not statistically different among N treatments (Table 2.3). However, the mean pH tended to decrease with the increase in N rates for the Northwest and Jackson sites. No specific trend in soil pH was observed for the Western site.

2.4.2. Soil C and N concentrations and stocks

Across all sites and depths, no significant differences in SOC concentration were found among the reference and before and after N application, except for the Jackson site (10-20 cm depth) (Table 2.4). At Jackson, the SOC concentration under the 50 kg N ha⁻¹ treatment was significantly lower than that for the 200 kg N ha⁻¹ treatment for 10-20 cm depth. Although the effects of N fertilization were not statistically significant, the mean of SOC concentration tended to increase with increasing N application rates for both Jackson and Northwest sites. While soil under switchgrass had a higher SOC concentration than in the reference soil for the deeper layers at Jackson, the reference soil had a higher SOC concentration than soil under switchgrass at the Western site. However, there were no statistical differences in SOC concentrations between a reference soil and switchgrass plots at any of the three sites.

The concentration of TN in soil followed a trend similar to that of SOC, and there were no significant differences among treatments except for the 10-20 cm depth at Jackson. The TN concentration in the 50 kg N ha⁻¹ treatment was significantly lower than at the highest N rate. The TN concentration in the reference soil was slightly higher than that in soil under switchgrass at the Western site.

The C/N ratio at 0-10 and 10-20 cm depths at Northwest was marginally significant (p = 0.08), and soil samples at the end of growing season in 2009 tended to have a higher C/N ratio regardless of N application than the reference soil or soils under switchgrass in spring 2008. At Jackson, soils with no N fertilization at 20-30 cm depth had a significantly higher C/N ratio than the reference soil under tall fescue. At the Western site, there were no statistical differences in TN concentrations and C/N ratios as

were the cases at other sites, but the mean value of TN concentrations in switchgrass plots seemed to be lower than those in the reference soil.

The SOC stock up to 30-cm depth with N application was in the range of 102~123, 55~70, and 59~67 Mg C/ha at Northwest, Jackson, and Western site, respectively (Table 2.5). The SOC and TN stocks to 30-cm depth (EV) did not differ among treatments at Northwest and Western sites. At Jackson, however, the SOC and TN stocks (EV) were significantly higher at the 200 kg N ha⁻¹ than those for the 50 kg N ha⁻¹ treatment. Although the p-value was somewhat higher (p = 0.07) in the EM approach, the lower SOC and TN stock sizes for the 50 kg N ha⁻¹ treatment at Jackson was consistent with the results of the EV approach. This represented that SOC stocks in the EV approach were not strongly affected by ρ_b at Jackson.

The positive correlations between N rates and the magnitude of the SOC stock were observed at Northwest (r = 0.74; p < 0.01) and Jackson (r = 0.61; p < 0.05) sites, but not at the Western (r = 0.16; p = 0.61) site. When the data were fitted to a linear regression model, it showed an increase in the SOC stock with increase in the rate of N fertilization at Northwest and Jackson sites (Fig. 2.1). The increase rate in the SOC stock per unit increase in N application seemed more responsive at Northwest rather than at Jackson. However, the SOC stock was less for the 50 kg N ha⁻¹ than no N fertilization treatment at Jackson, so the linear fitting could not exactly describe the non-linear response of the SOC stock to N fertilization at Jackson.

2.4.3. Switchgrass biomass and root length

Although the aboveground biomass tended to increase with N application rates at each of three sites, there were no statistical differences due to the high variability of the data, except at the Western site for the highest rate of N application (200 kg N ha⁻¹). Thus, all biomass data from three sites were pooled and analyzed with a repeated measures RCB design ANOVA (Table 2.6). The interaction between sites and N rates was not significant. The 200 kg N ha⁻¹ fertilization produced the highest aboveground biomass yield, the yields did not differ between 50 and 100 kg N ha⁻¹ treatments, and no N fertilization treatment produced the least yield. The aboveground biomass was the highest at the Western site and did not differ between Northwest and Jackson sites. Aboveground biomass increased significantly in 2009 compared to 2008. In contrast to the aboveground biomass, neither of any factors, i.e. site, N rate, and year, did affect belowground biomass. Despite no statistical differences, the mean value of belowground biomass at Jackson was higher than those from the other two sites, and it was lower under the 200 kg N ha⁻¹ compared to N rates. The S/R ratio at the Western site was significantly higher than that at Jackson. Among N rates, the 200 kg N ha⁻¹ treatment was characterized by the highest S/R ratio and was significantly higher than that of the control. Because of high aboveground biomass production in 2009, the S/R ratio also significantly increased in 2009.

More than 90% of total root was less than 2-mm in diameter, and the effects of N fertilization on root mass was mainly on fine roots (< 2 mm in diameter). Thus, no further classification for roots based on the diameter was made. The RLD did not vary among N treatments for any depth intervals, but the mean value of the RLD at the 50 kg N ha⁻¹

treatment was higher than that from the other N treatments for 0-10 cm depth (Table 2.7). Although root biomass did not show any difference among sites, the RLD at Jackson was significantly higher than that at Northwest and Western sites for 0-10 cm depth, and that at the Western site for 10-20 cm depth. No significant differences were observed among either N treatments or sites in RLD for 20-30 cm depth.

2.4.4. Plant C and N concentrations

The C and N concentrations in plant tissues generally increased with increasing N rates (Table 2.8). The concentration of C was 45-46% in the aboveground biomass compared with 42-45% in the belowground biomass. The C concentration in aboveground biomass for higher N rates was significantly higher at Jackson among all three sites. However, the difference in C concentration was rather small. In contrast to C, the concentration of N in plant tissues generally increased with increasing a N application rate. Among N rates, the N concentration in switchgrass tissue for 200 kg N ha⁻¹ was significantly higher than that for other rates of N fertilizer. The N concentration in aboveground part was significantly higher at Jackson than those in other sites, yet that in belowground part was the highest at the Northwest site. The C and N concentration both in the above- and below-ground biomass was the lowest, and the C/N ratio was the highest at the Western site. Due to extremely narrow changes in the concentration of C and relatively greater changes in that of N with N fertilization, the C/N ratios decreased with increase in rates of N fertilization.

Export of N by aboveground biomass harvesting significantly increased with an increase in N application rates (Fig. 2.2), which ranged from 25 to 70 kg N ha⁻¹

depending on the fertilization rates. The magnitude of N export was less than the rates of N application except in the control. Export of N at the highest rate of N application was almost double compared to that of the control. The linear regression equations between N fertilization rates and the amount of N export did not vary among sites (the hypothesis of coincidence test in comparing regression lines, p > 0.05), and thus a single linear regression was used across all three sites. Under no N fertilization, 26 kg N ha⁻¹ was removed on average from the soil in the aboveground biomass harvest at the end of growing season. High aboveground biomass production in control plots at the Western site led to higher amount of N export by biomass removal.

2.5 Discussion

2.5.1. Soil bulk density and pH

Jagadamma et al. (2007) reported a decrease in ρ_b with increase in N fertilization under continuous corn and corn-soybean rotation in some Mollisols in Illinois. The decrease in ρ_b was attributed to the increased residue return associated with N fertilization. In contrast, the present study did not show any significant increase in SOC concentration or root biomass due to N fertilization (Tables 4 and 6). Therefore, it is not surprising that there were no differences in ρ_b among N treatments after only 2 years.

Soil ρ_b at the Western site was higher than for the other two sites. The previous land use at the Western site was a corn-soybean rotation for 20 years, which could have increased ρ_b due to heavy traffic for farming operations. Growing a perennial plant like switchgrass can reduce ρ_b by reducing vehicular traffic. Deep and extensive roots can also increase porosity and improve soil structure through SOC accrual (Tolbert et al.,
2002). However, the short duration (2 years) in this study of growing switchgrass was probably not long enough to observe any change of ρ_b .

The use of inorganic N fertilizer can decrease soil pH due to N transformation and the attendant acidification (Helyar and Porter, 1989). Although the differences were not statistically significant, there was a decreasing trend in soil pH with increasing rate of N fertilizer. Since N fertilizer has been applied just for two years, a long-term monitoring is needed to verify the effects of N fertilization on soil pH.

2.5.2. SOC and TN concentrations and stocks

In general, the SOC and TN concentrations and the corresponding stock sizes did not differ among reference soil, before and after N fertilization (Tables 2.4 and 2.5). A paired t-test was performed to check whether there was any change in SOC concentration before and after N fertilization, however, no change was observed either. A high variability in SOC stocks also made it difficult to detect any small change in SOC concentration over a short period of time. Garten and Wullschleger (1999) demonstrated that detecting a change of 5 Mg SOC ha⁻¹ required 16 samples with a 0.90 statistical power. Therefore, it may be possible not to detect a change in SOC over short duration of 18 months and a small number of replicates in this present study. Since a small change in SOC concentration could cause large differences in SOC stock sizes, a calculation of SOC sequestration rates by comparing mean values of stock sizes between 2008 and 2009 would not be meaningful in such a short term study.

Although there were no statistical differences, the mean value of SOC concentration was generally lower under no N fertilization and higher under the higher

rates of N application compared to the reference soils (Table 2.4). Soil was tilled in 2004 when the switchgrass plots were established. Tillage-induced perturbation could have caused a loss of SOC in all three sites. Anderson-Teixeira et al. (2009) pointed out that a land conversion effect must be considered in computing an increasing rate of SOC under biofuel crops plantation. Through a model analysis, Conant et al. (2007) showed that even one tillage event could reduce SOC concentration by 1 to 11%. Not only tillage but also C inputs are generally low during the first or second year as switchgrass gets established. In this study, the SOC concentration and the SOC stock size did not differ among the reference soil and that under switchgrass. This lack of difference might be a result of a small number of replicates (n = 3). However, it could also be possible that either switchgrass growing for a maximum of five to six years compensated the land conversion effect, or the loss of SOC during the plot establishment was not large enough to be of a consequence in this study.

Although the SOC concentrations among N treatments for each depth increment were not significantly different, the regression analysis showed that there was a positive relationship between N rates and SOC stock sizes at Northwest and Jackson sites but not at the Western site (Fig. 2.1). The significant differences in SOC stocks might be observed after integrating small changes which were not significant individually for each depth. Different responses of SOC stocks in three sites to N application could be explained by differences in soil texture. Fine mineral particles with a high surface area can contribute to increase SOC stocks by providing strong association between mineral particles and SOC and by preventing decomposition of present mineral-associated SOC (Christensen, 2001). The Northwest site is comprised of higher clay contents than at other sites (Table 2.1), and this might lead to a positive response in SOC to N application with the highest SOC stock size. On the contrary, the Western site has a higher sand content which might function in an opposite way. Additionally, no responses in SOC to N application in the Western site might be attributed to less root C input. The RLD in the Western site was significantly lower than that in Jackson and, the mean value in the RWD in the Western site was lower than Jackson. High ρ_b in the Western site could have a dilution effect for the amount of root C input to soil. Thus, this could make more difficult to change SOC concentration (mg C g⁻¹ soil). Moreover, a critical level of SOC, that is, soil quality can be declined below the level, is suggested as 2% in temperate regions (Kemper and Koch, 1966; Greenland et al., 1975). The SOC concentrations might have no effects on N application because of the lowest initial SOC concentrations. In addition, the SOC concentrations showed higher spatial variability in the Western site than the other two sites. All of these factors might have been attributed to the no response of SOC in the Western site.

In contrast to the positive responses to N in SOC in this present study, Ma et al. (2000b) did not observe significant changes in SOC by N fertilization, and a short period of research was interpreted as a likely reason. Khan et al. (2007) also argued that N fertilization can decrease SOC concentration by increasing heterotrophic decomposition. However, Powlson et al. (2010) pointed out that the decline in soil N with fertilizer applications from the Morrow plot might be related to a trend in declining soil C and N concentrations after the conversion from undisturbed natural lands to arable lands. Moreover, soil spatial variability and changes in sampling methods during a long term

period could make it difficult to explain the trend of declines in soil C and N (Powlson et al., 2010).

Despite some reports on the negative correlation between N application rate and SOC, the positive relationship between the addition of N and SOC is a more general trend due to increased crop residue returns by increasing fertility (Johnson and Curtis, 2001; VandenBygaat et al., 2003; Christopher and Lal, 2007). Fertilization in grassland increased the production of both aboveground and belowground biomass which could contribute to the increase in SOC (Russell and Williams, 1982; Conant et al., 2001). In the present study, the aboveground biomass positively responded to N fertilization, but root biomass did not (Table 2.6). Theoretically, the aboveground biomass is harvested for bioenergy feedstock, so the increase in yields of aboveground biomass might not contribute to the increase in SOC stocks under switchgrass. However, Garten Jr. et al. (2010) showed that switchgrass produced remarkably high amounts of surface litter which was approximately half of the aboveground biomass. Thus, the increased surface litter production which is not measured as the aboveground biomass may have played a role in increasing SOC stocks following N addition. However, the impact of litter production on the SOC stock was not examined in the present study.

At the Western site, the mean value of the SOC stocks in the reference soil were 15.5 Mg C ha⁻¹ higher than those in switchgrass plots (Table 2.5). These trends might be attributed to the different field operations between switchgrass and reference soils. Corn under NT has been grown in the reference soil with application of 202 kg N ha⁻¹ every year, and the residues were not removed from the plots. Such NT operations associated with N fertilization might contribute towards a higher SOC concentration in the reference

soil at the Western site. At Jackson, however, the SOC stocks in 2009 fall were lower than those prior to the application of N and compared with the reference soil under 0 and 50 kg N ha⁻¹ application. The SOC stock size is determined by the balance between the input and output of C. Harvesting aboveground biomass at the end of the growing season can affect SOC stocks both by directly reducing the C input from the aboveground residue and by indirectly facilitating decomposition through increasing soil temperature and evaporation in early spring owing to less litter cover (Johnson et al., 2010). Thus, a loss of SOC could happen with growing switchgrass if the C input could not counterbalance the loss of C due to the inappropriate managements.

The SOC stock sizes after 5-6 years of switchgrass at the Western site were similar or lower to those reported under the NT management, and were higher than those under conventional tilled plots for the same research site (Ussiri and Lal, 2009; Mishra et al., 2010). At Northwest, SOC stock under switchgrass was higher than under conventional till and NT plots reported by Mishra et al. (2010). These results indicate a high potential for increasing the SOC stocks by growing switchgrass on agricultural soils.

2.5.3. Switchgrass biomass and root length

Use of N fertilization increased the aboveground biomass but did not affect the root biomass, and thus the S/R ratio increased with increase in N rates (Table 2.6). The positive responses of both aboveground biomass and the S/R ratio to N fertilization are a general phenomenon under the N limiting condition. A 2-year N fertilization experiment conducted on switchgrass in Iowa showed similar results indicating that the rate of 220 kg N ha⁻¹ application produced the highest aboveground (Heggenstaller et al., 2009). On

the other hand, root biomass of switchgrass showed a quadratic response to N addition and was maximized at 140 kg N ha⁻¹ (Heggenstaller et al., 2009). In the present study, the highest belowground biomass and RLD were associated with 50 kg N ha⁻¹ application, and root biomass and length tended to decrease at the highest N rates. Yet, there were no statistical differences in root biomass among N treatments. Similarly, Ma et al. (2000a) also showed that the root weight density (RWD) of switchgrass did not respond to N fertilization (0, 112, and 224 kg N ha⁻¹).

The change in the S/R ratio by N fertilization might be explained by the optimal partitioning theory which states the strategy of plants: allocation of photosynthates into a plant's organs to maximize obtaining the most limiting resources (Thornley, 1972). Kuzyakov and Domanski (2000) showed the reduction of ineffective C losses by decreasing translocation of C to the belowground biomass when available forms of N were sufficient. This present study observed the change in aboveground biomass and S/R ratio, and no differences in root biomass following N fertilization. However, root biomass measurement at one time a year may not be enough to represent root production throughout the entire year. Higher turnover rates of root under higher N fertilization might offset the biomass gains from higher root production. Moreover, the translocation of C from the aboveground parts at the end of the growing season might obscure the probable differences in root biomass during the growing seasons.

Aboveground biomass was significantly higher in 2009 than 2008 (Table 2.6), and this trend might be attributed to a carryover effect and more appropriate timing of N fertilization in 2009. However, root biomass within the surface 30 cm did not vary between the two years.

Aboveground biomass and the S/R ratio at the Western site were the highest among three sites despite lower SOC and TN concentrations in soil and lower root biomass. These trends are difficult to explain with the limited information. The annual precipitation at the Western site was higher than that at Northwest, and it could contribute to higher aboveground biomass at the Western site (Table 2.1). However, the lower annual precipitation and temperature in the Western site than the Jackson site negate any climatic effect on differences in biomass between two sites. Even root production was not always positively correlated to the aboveground biomass production. If the key elements for plant growth are adequate in soils, aboveground parts can grow even without developing extensive root systems (Van Noordwijk and Brouwer, 1997). Due to the previous agricultural land use history and management practices at the Western site, there might be residual nutrients available that could enhance switchgrass growth. This hypothesis can be corroborated by analyzing nutrients other than the TN concentration in soils. Even though the root biomass was not statistically different, the RLD at the Jackson site was significantly higher than that at the Western site. The soil series at the Jackson site have a deep effective rooting depth and low plant available water capacity (Soil Survey Staff, 1985). The previous pasture land use and lower $\rho_{\rm b}$ along with these inherent soil properties might have supported a deep root system development at Jackson.

Root biomass within a 30-cm depth ranged from 3.2 to 5.3, 6.1 to 7.1, and 4.2 to 4.9 Mg ha⁻¹ at Northwest, Jackson, and Western site, respectively, after 6 years of switchgrass establishment (Table 2.6). Although root biomass was not measured for deeper soil layers, estimated root biomass could represent the belowground biomass in the inter-row zone. The roots of switchgrass could grow up to 3.3 m, but 73-81% of root

biomass is concentrated in the surface 30 cm depth (Ma et al., 2000a). Furthermore, 94% of coarse roots (Sanderson, 2008) and 71% of total roots (dead + live) (Garten Jr. et al., 2010) were distributed within the surface 30 cm depth in a 90-cm soil profile. Root biomass in this study is lower, and the S/R ratio is higher than those reported in the literature. Heggenstaller et al. (2009) showed that root biomass of switchgrass (Cave-in-Rock) at 1.0 m depth ranged from 15 to 30 Mg ha⁻¹ under different rates of N fertilization. Ma et al. (2000a) reported that root biomass at 30 cm depth ranged from 3.4 to 21.5 Mg ha⁻¹. Such a wide range of root biomass estimates in switchgrass could be explained by differences in measurement methodology. In the present study, soil for root biomass measurement was sampled in-between two rows (inter-row), and thus fewer roots would present at the sampling spots which were away from the plant crowns. In addition, rhizome biomass was not included in the present study. Heggenstaller et al. (2009) used average values from two soil cores: one on plant crowns and the other between the crowns. Ma et al. (2000a) clearly showed that there was a big difference in root biomass especially in surface soils between intra-row and inter-row sampling; root biomass in the intra-row zone was 6-10 times higher than that in the inter-row zone. Thus, if root biomass included root data from close to the plants crowns or was measured by root excavation in wider sampling area, then higher root biomass and lower S/R ratio than estimated values in this present study would be obtained.

2.5.4 N export by harvesting aboveground biomass

The N concentration in aboveground biomass was less than that in roots (Table 2.8). The C and N in plants were analyzed in the senesced aboveground tissue. Thus, N would be translocated to the belowground part at the end of the growing season. Garten Jr. et al. (2010) showed that the N concentration in the roots was higher in April than in July and October, but that in rhizomes increased from July to October through N translocation. Recycling of N for the subsequent year's growth is a good trait of perennial bioenergy crops because of the low N requirement for the growth, and high quality biomass with low N concentration for cellulosic ethanol production.

The concentration of N in aboveground biomass at harvest time influences the amount of N exported with the biomass removal. Harvesting biomass after nutrients are translocated to the belowground parts at the end of growing season can reduce the amount of N export by biomass removal. The amount of N export increased with N rates, but it did not differ among sites (Fig. 2.2). The point that an N rate is concurrent to the amount of N export was calculated as 32 kg N ha⁻¹ with a 95% CI (11-53 kg N ha⁻¹). This rate can be considered as a minimum rate of N application which does not mine N from soils or replenish soil N extracted by plants growth, however, this rate did not consider any other loss of N through leaching, denitrification, etc. In Tennessee, the N export by aboveground biomass removal was 63 kg N ha⁻¹ which was higher than observed in the present study (Garten Jr. et al., 2010). The lower amount of N export in the present study compared to the result from Tennessee (Garten Jr. et al., 2010) was due to the lower aboveground biomass production rather than due to the differences in the N concentration in biomass.

2.5.5 Potential of growing switchgrass in Ohio

There is not much available information about switchgrass biomass production and SOC dynamics for Ohio soils. The Bioenergy Feedstock Development Program (BFDP) did not emphasize the potential of switchgrass production in Ohio (McLaughlin and Kszos, 2005), and Geophyta did not recommend switchgrass as the most promising energy crop through the screening studies conducted at three northeast sites in Ohio (Wright, 1990). However, the results from Geophyta showed that switchgrass (Cave-in-Rock) was well established in all three sites which covered a range of wetness regimes, and the aboveground biomass increased over time (Wright, 1990; Wright and Turhollow, 2010). Moreover, although the upland cultivar (Cave-in-Rock) was tested, the present study showed that switchgrass biomass in Ohio can be produced at a level similar to other states in the Mid-West. Aboveground biomass at Northwest, Jackson, and Western sites ranged from 10 to 14, 7 to 12, and 13 to 22 Mg ha⁻¹, respectively. These data support the conclusion that switchgrass has the potential to be grown as a bioenergy crop in Ohio. A principal constraint to switchgrass cultivation in Ohio could be weed infestation, which is a common problem in switchgrass establishment (Parrish and Fike, 2005). Indeed, establishment of switchgrass in one plot at Jackson failed because of the severe weed competition.

2.6 Conclusions

N fertilization has both positive and negative aspects in bioenergy crop cultivation. The aboveground biomass and the SOC stocks in the surface 30 cm showed positive responses to N rates. However, increasing rates of N application increased the amount of N export due to biomass removal. Thus, the judicious rate of N application should be determined by a life cycle analysis (Alder et al., 2007) and a breakeven price analysis (Mooney et al., 2009) with a consideration of a minimum rate of N fertilization which can be calculated like this study.

Although a difference in the SOC stocks among N treatments was found at the Jackson site, no differences were generally detected from the other sites or from the comparisons between the reference soils and the soils under switchgrass. Thus, a long-term monitoring on SOC concentration is required to conclusively determine the effects of N on SOC storage because this study was based on a 2-year experiment. In addition, the SOC stocks under switchgrass after five years of growth were similar to those under previous land use. The land conversion effects could be counterbalanced in the environment of no further disturbances under a perennial grass.

Root biomass was not affected by N treatments despite the increases in the S/R ratio. The change in translocation of fixed C in roots due to N fertilization is particularly important to SOC sequestration because root is considered as the main C input source. Despite no differences in root biomass, positive relationships between SOC and N rates suggest other sources of C input (surface litter or root exudates) may have contributed. Additional research on these mechanisms should be conducted to understand SOC dynamics further.

Switchgrass biomass was measured where drainage systems had not been installed at any of the three sites in Ohio. Comparable amount of harvested biomass feedstock in this study showed the possibility of growing switchgrass as a bioenergy crop in Ohio. However, this plot scale research needs to be extended to a large scale to cover spatial variability through the landscape.

2.7 References

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	Annual Annual Temp- Precip- Previous			Soil texture						
Site	erature (°C)*	itation (mm)*	land use	Latitude	Taxonomic class	Depth (cm)	Sand (%)	Silt (%)	Clay (%)	texture
Northwest	10.4	849	Sod	41° 13′ 49" N	Fine, illitic, mesic Mollic Epiaqualfs	0-10	20.3	42.1	37.5	Clay loam
				83° 45′ 34" W		10-20	18.9	40.9	40.2	Silty clay
						20-30	18.3	39.9	41.9	Clay
Jackson	11.7	1061	Cool	39° 1′ 39" N	Fine, mixed, active, mesic Aquultic Hapludalfs	0-10	14.2	68.2	17.7	Silt loam
			season	82° 36′ 17" W	Fine-loamy, mixed, active, mesic Aquic Hapludults	10-20	12.9	67.6	19.3	Silt loam
			grasses		Fine-loamy, mixed, active, mesic Typic Hapludult		10.7	66.1	23.1	Silt loam
Western	10.2	950	Corn-	39° 51′ 21" N	Fine, mixed, superactive, mesic Typic Argiaquolls	0-10	20.5	56.1	23.4	Silt loam
			Soybean	83° 40′ 40" W	Fine-loamy, mixed, active, mesic Typic Hapludalfs	10-20	19.4	55.3	25.2	Silt loam
			rotation		Fine, mixed, active, mesic Aeric Epiaqualfs	20-30	19.0	55.9	25.1	Silt loam

Table 2.1. Sites characteristics and previous land use in three research sites.

*Temperature and precipitation are the average values for six years of switchgrass cultivation (2004~2009)

Site	Depth (cm)	Reference	2008	2009 N fertilization rate (kg N ha ⁻¹)			
			-	0	50	100	200
Northwest	0-10	$1.22 (0.03)^{\rm b}$	1.43 (0.04) ^a	1.40 (0.02) ^{ab}	1.34 (0.03) ^{ab}	1.25 (0.04) ^{ab}	1.32 (0.06) ^{ab}
	10-20	1.50 (0.03)	1.54 (0.02)	1.56 (0.02)	1.49 (0.03)	1.48 (0.03)	$1.48 (0.04)^{\rm NS}$
	20-30	1.54 (0.02)	1.61 (0.03)	1.50 (0.09)	1.48 (0.05)	1.45 (0.04)	1.42 (0.08) ^{NS}
Jackson	0-10	1.39 (0.03)	1.40 (0.04)	1.42 (0.01)	1.35 (0.03)	1.37 (0.03)	1.31 (0.04) ^{NS}
	10-20	1.50 (0.05)	1.52 (0.02)	1.50 (0.08)	1.50 (0.03)	1.48 (0.04)	1.45 (0.03) ^{NS}
	20-30	1.56 (0.01)	1.58 (0.01)	1.64 (0.05)	1.54 (0.03)	1.54 (0.03)	1.60 (0.03) ^{NS}
Western	0-10	1.58 (0.03)	1.55 (0.03)	1.55 (0.04)	1.51 (0.05)	1.52 (0.03)	1.53 (0.03) ^{NS}
	10-20	1.63 (0.02) ^a	1.63 (0.01) ^a	$1.53 (0.02)^{b}$	1.55 (0.02) ^{ab}	1.57 (0.03) ^{ab}	1.60 (0.01) ^{ab}
	20-30	1.61 (0.05)	1.66 (0.01)	1.59 (0.01)	1.65 (0.03)	1.64 (0.03)	1.63 (0.01) ^{NS}

Table 2.2. Bulk density (Mg m⁻³) for reference soils in each site, soils under switchgrass in spring 2008 before N fertilization, and soils under switchgrass in fall 2009 after N fertilization

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Different letters within a row for each depth indicate significant differences (p < 0.05). Values in parentheses are standard errors (n = 4 for Northwest and Western and n = 3 for Jackson). NS: not significantly different.

Site	Donth (am)	N fertilization rates (kg N ha ⁻¹)								
Site	Depth (cm)	0		50		10	100		200	
Northwest	0-10	6.88	(0.19)	7.02	(0.31)	6.91	(0.16)	6.71	(0.38)	NS
	10-20	7.14	(0.18)	7.15	(0.30)	7.01	(0.17)	6.96	(0.35)	NS
	20-30	7.29	(0.25)	7.24	(0.28)	7.12	(0.10)	7.10	(0.35)	NS
Jackson	0-10	6.24	(0.07)	6.23	(0.07)	6.12	(0.18)	6.03	(0.18)	NS
	10-20	6.38	(0.09)	6.52	(0.05)	6.34	(0.22)	6.32	(0.20)	NS
	20-30	6.50	(0.07)	6.58	(0.05)	6.49	(0.24)	6.51	(0.24)	NS
Western	0-10	5.44	(0.06)	5.42	(0.02)	5.44	(0.12)	5.41	(0.11)	NS
	10-20	5.32	(0.14)	5.49	(0.04)	5.58	(0.12)	5.53	(0.07)	NS
	20-30	5.43	(0.18)	5.72	(0.09)	5.81	(0.09)	5.78	(0.08)	NS

Table 2.3. Soil pH after N fertilization in fall 2009.

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Values in parentheses are standard errors (n = 3). NS: not significantly different at the p = 0.05 level.

	C :4a	Depth	Defense	2008	N fertilization rates (kg N ha ⁻¹) in 2009					
	Site	(cm)	Reference	2008	0	50	100	200	_	
SOC	Northwest	0-10	34.67(4.09)	35.43(6.77)	31.74(3.31)	35.03(2.96)	37.16(3.73)	39.60(3.79)	NS	
$(g kg^{-1})$		10-20	21.38(0.38)	25.44(5.50)	21.88(0.97)	22.63(0.79)	25.22(1.85)	27.40(1.80)	NS	
		20-30	20.55(0.39)	19.99(1.42)	16.56(5.57)	20.11(1.36)	21.13(0.73)	22.15(1.10)	NS	
	Jackson	0-10	22.17(1.15)	19.61(1.72)	20.21(1.61)	19.18(0.45)	19.92(1.60)	23.15(4.39)	NS	
		10-20	$14.46(0.93)^{ab}$	13.46(0.37) ^{ab}	13.06(1.95) ^{ab}	$10.05(0.66)^{b}$	13.57(0.25) ^{ab}	15.33(0.26) ^a		
		20-30	8.00(1.44)	9.91(1.21)	7.89(0.68)	8.96(1.28)	9.69(1.36)	11.42(0.91)	NS	
	Western	0-10	18.71(1.26)	16.33(1.88)	17.48(2.29)	16.82(2.17)	18.96(2.53)	17.63(1.89)	NS	
		10-20	15.44(0.72)	11.97(2.33)	11.76(1.74)	11.07(2.05)	12.74(0.88)	13.42(2.34)	NS	
		20-30	14.55(1.19)	10.60(1.35)	10.28(1.42)	10.36(2.30)	10.53(1.09)	10.35(2.03)	NS	
TN	Northwest	0-10	3.72(0.39)	3.73(0.64)	3.25(0.44)	3.49(0.39)	3.61(0.49)	3.86(0.36)	NS	
$(g kg^{-1})$		10-20	2.49(0.04)	2.72(0.53)	2.16(0.12)	2.30(0.04)	2.52(0.16)	2.69(0.13)	NS	
		20-30	2.39(0.06)	2.23(0.13)	1.74(0.61)	2.05(0.08)	2.16(0.11)	2.22(0.15)	NS	
	Jackson	0-10	2.31(0.11)	2.02(0.19)	2.00(0.18)	1.98(0.07)	2.06(0.22)	2.36(0.45)	NS	
		10-20	$1.53(0.12)^{ab}$	$1.49(0.08)^{ab}$	$1.34(0.21)^{ab}$	$1.02(0.08)^{b}$	$1.42(0.02)^{ab}$	$1.58(0.03)^{a}$		
		20-30	0.94(0.19)	1.10(0.13)	0.78(0.05)	0.93(0.12)	1.00(0.14)	1.12(0.12)	NS	
	Western	0-10	1.82(0.10)	1.54(0.14)	1.60(0.14)	1.74(0.26)	1.88(0.26)	1.73(0.22)	NS	
		10-20	1.49(0.05)	1.15(0.19)	1.26(0.18)	1.11(0.22)	1.33(0.14)	1.30(0.24)	NS	
		20-30	1.40(0.09)	1.07(0.10)	1.00(0.16)	1.00(0.24)	1.04(0.15)	0.94(0.15)	NS	
C/N	Northwest	0-10	9.30(0.16)	9.43(0.18)	9.87(0.38)	10.09(0.27)	10.39(0.39)	10.27(0.21)	NS	
		10-20	8.59(0.01)	9.29(0.38)	10.19(0.74)	9.83(0.22)	10.01(0.26)	10.18(0.34)	NS	
		20-30	8.59(0.06)	8.96(0.18)	9.73(0.99)	9.79(0.33)	9.82(0.18)	9.99(0.25)	NS	
	Jackson	0-10	9.61(0.17)	9.72(0.12)	10.15(0.13)	9.68(0.21)	9.70(0.30)	9.80(0.12)	NS	
		10-20	9.48(0.22)	9.04(0.29)	9.72(0.13)	9.86(0.17)	9.57(0.22)	9.73(0.20)	NS	
		20-30	$8.60(0.30)^{c}$	$9.00(0.07)^{bc}$	$10.06(0.19)^{ab}$	$9.59(0.22)^{abc}$	$9.70(0.14)^{abc}$	$10.21(0.34)^{a}$		
	Western	0-10	10.24(0.16)	10.55(0.42)	10.89(0.77)	9.79(0.47)	10.13(0.12)	10.25(0.57)	NS	
		10-20	10.34(0.24)	10.34(0.27)	9.40(0.58)	10.06(0.13)	9.62(0.39)	10.38(0.55)	NS	
		20-30	10.36(0.17)	9.87(0.72)	10.34(0.22)	10.42(0.16)	10.25(0.80)	10.93(0.40)	NS	

Table 2.4. Soil organic carbon (SOC), total nitrogen (TN), and C/N ratios for reference soils in each site, soils under switchgrass in spring 2008 before N fertilization, and soils under switchgrass in fall 2009 after N fertilization Values in parentheses are standard errors (n = 3). NS: not significantly different at the p = 0.05 level

								N for	20	09 roto (ka N	b e ⁻¹)		
Site	Parameter	Method	Reference	20	. 800			IN Tel	unzation	rate (kg N	na)		
						0		50		100		200	
Northwest	SOC	EV	105.7 (3.8)	121.1	(17.1)	102.3	(6.1)	110.6	(3.4)	114.0	(4.9)	123.4	$(3.9)^{ns}$
	(Mg C ha ⁻¹)	EM	99.6 (3.8)	109.0	(17.9)	94.9	(5.1)	103.6	(2.9)	109.6	(6.0)	118.2	(3.0) ^{ns}
	TN	EV	11.9 (0.3)	13.0	(1.6)	10.4	(1.1)	11.2	(0.6)	11.3	(0.7)	12.1	$(0.1)^{ns}$
	$(Mg N ha^{-1})$	EM	11.2 (0.3)	11.7	(1.7)	9.6	(1.0)	10.4	(0.5)	10.9	(0.8)	11.6	(0.3) ^{ns}
	Depth (cm)*		28.1	26.2		26.7		27.6		28.6		28.3	
Jackson	SOC	EV	64.9 (2.1) ^{ab}	63.4	(1.6) ^{ab}	60.9	(3.3) ^{ab}	54.8	$(3.1)^{b}$	62.1	(1.0) ^{ab}	70.3	$(4.7)^{a}$
	(Mg C ha ⁻¹)	EM	61.0 (1.5)	58.0	(1.4)	56.2	(3.7)	51.0	(2.5)	58.1	(1.2)	65.9	(5.0) ^{ns}
	TN	EV	7.0 (0.4)	6.8	(0.2)	6.1	(0.4)	5.7	(0.3)	6.4	(0.2)	7.1	(0.4) ^{ns}
	(Mg N ha ⁻¹)	EM	$6.5 (0.3)^{ab}$	6.2	(0.1) ^{ab}	5.6	$(0.4)^{ab}$	5.3	(0.2) ^b	6.0	(0.2) ^{ab}	6.7	(0.4) ^a
	Depth $(cm)^*$		26.8	26.6		26.3		27.2		27.2		27.5	
Western	SOC	EV	78.1 (4.5)	62.2	(8.5)	61.3	(6.8)	59.4	(9.4)	66.3	(8.0)	65.3	(8.9) ^{ns}
	(Mg C ha ⁻¹)	EM	65.6 (3.8)	53.1	(7.5)	53.9	(5.7)	51.8	(8.1)	58.1	(6.4)	57.0	(7.6) ^{ns}
	TN	EV	7.6 (0.4)	6.0	(0.6)	6.0	(0.7)	6.0	(1.0)	6.7	(1.0)	6.3	(0.8) ^{ns}
	$(Mg N ha^{-1})$	EM	6.4 (0.3)	5.1	(0.6)	5.3	(0.6)	5.2	(0.8)	5.9	(0.8)	5.5	(0.7) ^{ns}
	Depth $(cm)^*$		24.7	24.8		25.5		25.5		25.3		25.1	

Table 2.5. SOC and TN stock by an equal volume approach (EV, depth = 30 cm) and by an equal mass approach (EM)

*Depth was determined for the equal mass approach. Different letters within a row indicate significant differences (p < 0.05). Values in parentheses are standard errors (n = 3). NS: not significantly different.

		Aboveground biomass (Mg ha ⁻¹)	Belowground biomass (Mg ha ⁻¹)	S/R ratio
Site	Northwest	12.58 (0.85) ^b	4.27 (0.25)	3.35 (0.37) ^{ab}
	Jackson	10.31 (0.74) ^b	6.53 (0.47) ^{ns}	1.73 (0.18) ^b
	Western	16.63 (1.01) ^a	4.54 (0.42)	5.05 (0.67) ^a
N rate	0	10.42 (0.90) ^c	5.11 (0.56)	3.03 (0.74) ^b
$(kg N ha^{-1})$	50	12.93 (0.87) ^b	5.21 (0.44) _{ns}	3.08 (0.52) ^{ab}
· • /	100	13.77 (1.02) ^b	5.03 (0.44)	3.50 (0.60) ^{ab}
	200	16.62 (1.46) ^a	4.60 (0.50)	4.50 (0.62) ^a
Year	2008	11.33 (0.74) ^b	4.87 (0.38) _{ns}	3.19 (0.47) ^b
	2009	15.54 (0.80) ^a	5.11 (0.30)	3.86 (0.41) ^a
ANOVA	df		— p - value ———	
Site	2	0.002	0.129	0.038
Ν	3	< 0.0001	0.697	0.017
Year	1	< 0.0001	0.528	0.002
Site * N	6	0.175	0.424	0.565
Data trans- formation		None	None	Log

Table 2.6. Above and belowground biomass yield

Different letters within sites, N rates, and years indicate significant differences, respectively (p < 0.05). Values in parentheses are standard errors. NS: not significantly different.

		Depth (cm)						
	_	0-10		10-20		20-30		
N rate	0	3.62 (0.25)		1.78 (0.21)		1.08 (0.13)		
(kg N ha ⁻¹)	50	4.49 (0.62)	NS	1.94 (0.24)	NS	1.37 (0.18)	NS	
	100	3.54 (0.49)		2.02 (0.20)		1.05 (0.11)		
	200	3.62 (0.61)		1.70 (0.27)		0.98 (0.22)		
Site	Northwest	3.45 (0.28)	b	1.83 (0.13)	ab	1.09 (0.12)		
	Jackson	5.34 (0.61)	а	2.31 (0.30)	а	1.39 (0.22)	NS	
	Western	3.05 (0.25)	b	1.55 (0.14)	b	0.95 (0.09)		
ANOVA	df -	,		— p-value				
Ν	3	0.463		0.736		0.190		
Site	2	0.001		0.040		0.159		
N*sit	6	0.480		0.862		0.653		
Data trans- formation		Log		None		Log		

Table 2.7. Root length density (RLD, cm cm⁻³ soil) among N rates and among sites

Different letters among sites and N rates indicate significant differences (p < 0.05). Values in parentheses are standard errors. NS: not significantly different.

				Aboveground			Belowground				
	-	C (%)		N (%)	C/N	C (%)	N (%)	C/N			
Site											
Nort	thwest	45.6 (0.1) ^{ab}	0.27 (0.02) ^b	183.4 (13.8) ^a	43.3 (0.4)	$0.88 (0.07)^{a}$	52.7 (4.0) ^b			
Jack	son	45.8 (0.1) ^a	0.34 (0.03) ^a	148.6 (13.1) ^b	43.0 (0.2) ^{ns}	$0.76 (0.05)^{ab}$	59.4 (4.3) ^{ab}			
Wes	tern	45.5 (0.1) ^b	0.24 (0.01) ^b	197.5 (10.9) ^a	43.3 (0.2)	0.69 (0.04) ^b	64.9 (3.1) ^a			
N rate (kg]	N ha ⁻¹)										
	0	45.3 (0.1) ^b	0.24 (0.02) ^b	199.0 (13.5) ^a	42.9 (0.2) ^{ab}	0.66 (0.06) ^b	67.9 (4.7) ^a			
	50	45.6 (0.2	2) ^{ab}	0.24 (0.02) ^b	204.8 (14.2) ^a	44.0 (0.5) ^a	0.73 (0.05) ^b	62.2 (3.8) ^a			
	100	45.7 (0.1) ^a	0.28 (0.02) ^b	167.9 (11.7) ^{ab}	43.2 (0.2) ^{ab}	0.75 (0.05) ^b	59.2 (3.6) ^{ab}			
	200	45.8 (0.1) ^a	0.37 (0.03) ^a	134.3 (13.4) ^b	42.8 (0.2) ^b	0.96 (0.08) ^a	46.8 (3.6) ^b			
ANOV A	df -				p-va	lue					
Site	2	0.03		0.001	0.005	0.54	0.02	0.04			
Ν	3	0.004		<.001	0.001	0.04	0.002	0.003			
Site * N	6	0.56		0.08	0.34	0.22	0.18	0.38			

Table 2.8. C and N concentration in plant tissues (aboveground and belowground) at the end of growing seasons

Different letters among sites and N rates indicate significant differences (p < 0.05). Values in parentheses are standard errors. NS: not significantly different.



Fig. 2.1. The SOC stock with N fertilization at each site.



Fig. 2.2. The amount of the N export by aboveground biomass removal at the end of growing season.

CHAPTER 3

ORGANIC MATTER DECOMPOSITION IN RESPONSE TO INORGANIC NITROGEN AND SWITCHGRASS ROOT-CARBON

3.1 Abstract

Soil organic carbon (SOC) sequestration can mitigate the increase in carbon dioxide (CO₂) in the atmosphere. However, whether the addition of nitrogen (N) contributes to SOC sequestration is questionable, and the effect of added N on SOC decomposition is not clearly understood. Moreover, the impact of newly added C substrates on the native SOC is not yet understood. Thus, this study was conducted to investigate the effect of inorganic N and root-C addition on decomposition of organic matter (OM). Soil was incubated for 200 days with nine treatments [three levels of N (no addition of N (N0) = 0, low level of N (NL) = 0.021, high level of N (NH) = 0.083 mg N/g soil) x three levels of C (no addition of C (CO) = 0, low level of C (CL) = 5, high level of C (CH) = 10 mg root/g soil)]. The efflux of CO₂, inorganic N concentration, soil pH, the potential activities of oxidative enzymes and β -glucosidas, SOC, and SOC derived from root-C were measured during six months of incubation. Overall, the interaction between C and N was not significant for the measured parameters. The efflux of CO₂ in the NH treatment was significantly lower than that in the N0 treatment, mostly

before day 45. The cumulative CO₂ at day 200 in N0, NL, and NH treatments was 3.10, 2.94, and 2.65 mg C/g soil respectively. The remaining SOC at day 200 in N0, NL, and NH treatments was 19.25, 19.06, and 19.44 mg C/g soil respectively. These measurements in C dynamics among N treatments showed that the NH treatment had a negative effect on OM decomposition. An N mining theory, which states that microbes decompose soil organic matter (SOM) to obtain N, could not account for the negative effect of N because inorganic N concentration increased over time without showing any N immobilization. Further, oxidative enzyme activities could not explain the negative effect of N either. When CO₂ data were fitted to the exponential model, the potentially mineralizable C pool (Co) decreased with the addition of N (3.6, 3.3, and 3.1 mg C/g soil in the N0, NL, and NH treatments, respectively). This trend indirectly suggested the possibility that added N contributed to the formation of recalcitrant OM. Soil pH was also correlated with the change in CO_2 efflux rates under the same levels of root-C. The CO_2 efflux rate was generally in order of CH > CL > C0 over the incubated samples. The native SOC in the CH treatment (18.29 mg C/g soil) was significantly lower than that in the C0 treatment (19.16 mg C/g soil). However, total SOC concentration did not differ among C treatments. The SOC-derived from root-C may have compensated for the loss of native SOC. The data suggested that the addition of N could increase SOC sequestration by reducing SOC decomposition. Also, the addition of root-C may have accentuated the decomposition of native SOC (priming effect).

Keywords: organic matter decomposition, nitrogen (N) addition, root-C, incubation, N mineralization, enzyme activity, priming effect

3.2 Introduction

Growing switchgrass (Panicum virgatum L.) as a bioenergy crop may reduce net carbon dioxide (CO₂) emission in two ways. Ethanol production using aboveground biomass is a way of recycling carbon (C) and reducing fossil C signature in the atmosphere (McLaughlin et al., 2002). In addition, belowground C input via roots can sequester soil organic carbon (SOC) (Frank et al., 2004; McLaughin and Kszos, 2005). Nitrogen (N) fertilization is crucial to improving switchgrass biomass yield as feedstock for ethanol production and to influence the level of SOC (Parrish and Fike, 2005). However, many uncertainties on SOC sequestration following N addition exist. Addition of N can influence SOC pools through an increase in biomass production and changing SOC decomposition. The net effects of N on SOC pools can either be positive, negative, or neutral depending on the amount of C input and output controlled by the N effect (Johnson and Curtis, 2001; Christopher and Lal, 2007; Reay et al., 2008). In general, N fertilization increases aboveground biomass production, and this has been frequently suggested to increase SOC pools following N application (Alvarez, 2005; Gregorich et al., 1996). However, the effect of N addition on decomposition is inconsistent and often contradictory (Fog, 1988; Mack et al., 2004; Hobbie, 2008).

Several changes in abiotic and biotic factors following N addition can subsequently affect the decomposition process. Since two major elements in ecosystems, C and N, are strongly coupled (Asner et al., 1997), the change in N levels could induce chages in SOC as well. In a conceptual model, McGill and Cole (1981) proposed that N mineralization is related to the decomposition of C because of the coupled characteristics of C and N bound in soil organic matter (SOM). Alleviating N-deficits by fertilization can facilitate the microbial activity needed to decompose C substrates (Hessen et al., 2004; Mack et al., 2004). However, readily available N can also suppress the decomposition of recalcitrant SOM to mineralize N by inducing microbes to use easily-available C substrates according to the N mining theory (Fontaine et al., 2004; Crain et al., 2007). Furthermore, all changes derived from N application, such as a shift in microbial community structure, soil pH or formation of recalcitrant materials, may influence the decomposition processes (Fog et al., 1988; Ågren et al., 2001; Hyvönen et al., 2007; Xia and Wan, 2008; Treseder, 2008).

The fractionation of SOC into different pools could help in understanding the responses of SOC to N addition. Studies of SOC fractionation have shown that the effects of N addition on SOC decomposition vary depending on SOC pools. For example, N fertilization over 10 years did not change bulk soil C, but decreased the light fraction C ("labile" SOC) (Neff et al., 2002). In a 90-day incubation, addition of mineral N increased the amount of humin ("stable" SOC fraction) (Moran et al., 2005). In contrast, high rates of inorganic N application accentuated the conversion of stabilized forms of SOM into labile humic substances in soddy-podzolic soils (Shevtsova et al., 2003), which decreased the SOC pool by increasing its decomposition. Hofmann et al. (2009) observed that N fertilization did not influence the old SOC pool, new SOC pool, and lignin derived from old C substrates but facilitated the decomposition of the lignin derived from new C substrates. Thus, the effect of N on decomposition can vary depending on the specific pools of SOC.

In the presence of plant cover, new C is continuously added into soil by rhizodeposits, root turnover, or litter. However, the increase in C input is not directly reflected in an increase in the SOC pool (Gill et al., 2002). The effects of the addition of new C substrates on decomposition of native SOC (i.e., priming effect) have been debated (Kuzyakov et al., 2000). The priming effect denotes a short-term change in SOC decomposition rates following the addition of fresh organic or mineral materials (Dalenberg and Jager, 1989). The addition of fresh C substrates can alter microbial community structure, activate the synthesis of enzymes, or be served as an energy source for the production of enzymes, such that SOM decomposition is enhanced (Blagodatskaya and Kuzyakov, 2008). However, the rapid increase in CO₂ production after the addition of C substrates might come from an acceleration of microbial metabolism or a C pool substitution due to the microbial turnover rather than additional CO₂ production from SOC, which is the "apparent priming effect" (Kuzyakov et al., 2000). To confirm the "real priming effect," a change in SOC should be measured, and isotopic discrimination can be used to distinguish the source of decomposing C. Since switchgrass is a C4 plant, mixing switchgrass for C3-C in soil using isotopic discrimination by natural abundance of isotopic C could be useful in fractionating the decomposition of organic matter (OM) from each source.

Several studies have been conducted on OM decomposition combined with N addition, but few have discussed the probable mechanisms for the specific observed results. Therefore, the overall goal of this study was to assess the effect of inorganic N and switchgrass root-C addition on decomposition of OM and to identify possible mechanisms. A lab incubation study was conducted to assess the effects of N availability

and the addition of root-C on SOC decomposition. The following hypotheses were tested: (1) increased availability of N from inorganic N additions reduces the decomposition of SOM (2) addition of switchgrass roots accentuates SOM decomposition, and (3) a higher level of root biomass addition (CH treatment) shows a smaller priming effect than that from a small amount of root biomass addition (CL treatment).

3.3 Materials and Methods

3.3.1 Field sampling and experimental design

Bulk soil from for 0-10 cm depth was obtained with a shovel from four randomly selected points in tall fescue (*Schedonorus phoenix*) plots in Jackson, OH (39°10′ N, 82°36′ W) in early September 2008, and composited. The soil type was Rarden-Wharton silt loam, 15-25% slope (mesic Aquultic Hapludalfs and fine-loamy, mixed, active, mesic Aquic Hapludults) (Soil Survey Staff, 2008). The soil had 13.7% sand, 70.5% silt, and 15.8% clay based on the pipette method without destruction of SOM (Kilmer and Alexander, 1949). Soil pH was 7.15 (1:2, w/v), inorganic N was 10 µg N/g soil, and total C and N were 2.2 and 0.24%, respectively. Two mm-sieved field moist soil equivalent to 50 g (oven dry weight) per sample was used for this study. Visible roots and residues were manually removed from the sieved soil prior to the incubation study.

Switchgrass roots were sampled from 5-year old switchgrass plots next to the tall fescue plots at the time of soil sampling. Switchgrass roots were manually separated and washed with deionized water. Switchgrass roots were dried at 45°C to a constant weight, then chopped into 0.5-1.0 cm lengths and added as a C source at 0 (C0), low C at 5 (CL), and high C at 10 (CH) mg root DW/g soil. Average root weight density of switchgrass

on-site (Jackson) for 10 cm soil depth was 4.3 mg root/g soil with a range of $2 \sim 9$ mg root/g soil. The root C and N concentrations were 44.0% and 0.61%, respectively. The source of inorganic N, KNO₃ was applied at 0 (N0), low rate of 0.021 (NL), and high rate of 0.083 (NH) mg N/g soil to provide an equivalent of 0, 25, and 100 kg N/ha by assuming a bulk density 1.2 g/cm³ for the top 10 cm soil depth. After mixing switchgrass roots and KNO₃ solution with soil, DI water was added to adjust water potential at -33 kPa for each sample. In the soil which did not receive KNO₃ solution (N0 treatments), the DI water was added to adjust the moisture content to the level same to those for the other treatments.

A factorial experimental design was used in this study. A total of nine treatments, comprising three levels of N and three levels of C with three replicates constituted one set of destructive sampling. A total of five sets of destructive samples were prepared over the course of incubation. Among them, one set was used for CO_2 evolution measurement and destructively sampled following the last CO_2 sampling after 200 days. Four intermediate sets were destructively sampled at 0, 10, 45, and 100 days of incubation.

3.3.2 Incubation and CO₂ monitoring

Soil samples were incubated in the dark under aerobic conditions for 200 days at room temperature (25°C). Soil moisture potential was maintained at -33 kPa on average within a range of -6 kPa to -66 kPa by spraying water once a week. Head space gas samples were collected initially at day 0, followed by day 1, 2, 3, 5, 7, 11, 15, 20, 25, 30, 37, 45, 51, 64, 78, 90, 100, 120, 140, 160, 185, and 200. At each sampling date, samples were transferred to a 500 mL Mason jar, sealed and head space air was sampled at 0, 2, 4, and 8 hrs intervals and analyzed for CO_2 concentration (a closed dynamic chamber method). The CO_2 sampling intervals increased over the course of the incubation. The CO_2 concentration was analyzed using a gas chromatograph (Shimadzu GC-14A, Kyoto, Japan) equipped with a thermal conductivity detector (TCD). Gaseous separation was achieved through a 3 m and 0.3 cm internal diameter Hayesep D column (Grace Davidson, Deerfield II). Helium was used as a carrier gas at a flow rate of 25 cm³ min⁻¹. Oven and detector temperatures were 70 and 150°C, respectively. Concentration of CO_2 was estimated by analyzing CO_2 standard samples (Grace Davidson, Deerfield, IL) simultaneously.

3.3.3 Soil sampling and analyses

Each set was destructively sampled at 0, 10, 45, 100 and 200 days of incubation. Soil moisture content was measured gravimetrically by drying a subsample at 105°C for each sampling. Enzyme activities and inorganic N content were measured for the moist soil. Other parameters were determined from air-dried soils for each sampling.

Inorganic N (NH₄⁺ and NO₃⁻) was extracted from 6~7 g of moist soil with 30 ml of 2 M KCl. The extracts were filtered with Whatman #42 filter paper and were analyzed for NO₃⁻ and NH₄⁺ contents colorimetrically using an autoanalyzer (Astoria Oregon, USA). The color formation (indophenol blue) was achieved by reaction of alkaline phenol and hypochlorite for NH₄⁺ (US EPA, 1984). The NO₃⁻ was reduced to NO₂⁻ by cadmium column and reacted with buffered (cupric imidazole pH 7.5) sulfanilamide and N-ethylenediamine dihydrochloride to form pink color (US EPA, 1984).

The activity of β -1,4-glucosidase was measured from samples collected at 10, 45, 100, and 200 days of incubation according to the Tabatabai (1994) method, using pnitrophenyl (pNP)- β -glucopyranoside (PNG) as a substrate. Briefly, moist soil equivalent to 0.5 g of dry weight was incubated for 1 hour at 37°C with 4 mL of acetate buffer (50 mM, pH 6.0) and 1 mL of the PNG solution (25 mM). Immediately after the incubation, 4 mL of 0.1 N Tris(hydroxymethyl)aminomethane (THAM) buffer (pH 12) and 1 mL of 0.5 M CaCl₂ were added to cease the reaction. This mixture was centrifuged at 3000 G for 5 min, and the optical density of the surpernant was measured at 410 nm in a photometer (Spectronic 401, USA).

The activities of phenol oxidase and peroxidase were assayed at 10, 45, and 200 days of incubation as described by Sinsabaugh et al. (1999). For the phenol oxidase activity, 1 g of soil was homogenized in a 20 mL acetate buffer (50 mM, pH 5), and 2 mL of sample homogenate and 2 mL of L-dihydroxyphenylalanine (L-DOPA, 10 mM) were mixed and incubated for 1 hr at the ambient temperature (25°C). For the peroxidase activity, 0.2 mL of 0.3% H₂O₂ was added to the 2 mL of sample homogenate and 2 mL of L-DOPA, and the other steps were same as the phenol oxidase assay. The reaction was terminated by centrifuging at 3000 G for 5 min, and the optical density of the surpernant was measured at 460 nm. In calculation, 1.6 μ mol⁻¹ was used as a micromolar (μ M) extinction coefficient for L-DOPA (Sinsabaugh et al., 1999). A sum of phenol oxidase and peroxidase activity was considered as the activity of oxidative enzyme.

Soil pH was determined for air dried soil in a 1:2 soil/water (w/v) mixture (Thomas, 1996). The SOC and total N (TN) concentrations were determined by dry combustion (900°C) from acid washed soils and switchgrass roots using a CN analyzer
(Vario Max Elementar Americas, Inc., Germany). The δ^{13} C was determined from acidwashed soils before and after the incubation and from switchgrass root samples using an elemental analyzer (Euro EA, EuroVector Instruments & Software, Milan, Italy) interfaced with an isotope ratio mass spectrometer (IsoPrime, GV Instruments, Manchester, UK). Graphite [National Institute of Standards & Technology (NIST 8541)] referenced to Pee Dee Belemnite (PDB) was used as a standard reference material. The isotopic signature was expressed in the delta notation ($\delta^{13}C$) relative to international standard PDB (Craig, 1957).

$$\delta^{13}C(\%) = \left[\frac{{}^{13}C/_{12}Csample}{{}^{13}C/_{12}Creference} - 1\right] 1000 Eq. [1]$$

where ${}^{13}C/_{12}C_{sample}$ was the stable isotope ratio of the sample and ${}^{13}C/_{12}C_{reference}$ was the stable isotope ratio of the PDB standard. The $\delta^{13}C$ measurements were reproducible at $\pm 0.3\%_0$.

3.3.4 Calculation and statistical analyses

A two-compartment model (Eq.[2]) (Boutton, 1996; Bernoux et al., 1998) was used for calculating the size of C pools from C3-derived (native SOC) and C4-dervied SOC (switchgrass root derived SOC).

$$f = \frac{\delta_s - \delta_{C_3}}{\delta_{C_4} - \delta_{C_3}}$$
 Eq.[2]

where *f*: proportion of SOC derived from switchgrass root; δ_{C3} : $\delta^{13}C$ of the initial soil used in this experiment (-26.40); δ_{C4} : soil $\delta^{13}C$ of the steady state soil under switchgrass; δ_s : soil $\delta^{13}C$ at the end of the incubation

The δ^{13} C signal from switchgrass root (-15.00) was substituted for the value of δ_{C4} . The pool size of C derived from switchgrass root (C4) was calculated by multiplying the proportion *f* (Eq. [2]) by SOC (Eq. [4])

$$C_{C3} = (1-f) \times C_{Tt} \qquad Eq.[3]$$

 C_{C3} : the SOC pool size of the initial soil (C3); C_{C4} : the SOC pool size of C derived from switchgrass roots (C4); C_{Tt} : the total size of the SOC pool.

The difference between initial SOC and C_{C3} was considered as a loss of SOC during the incubation. The CO₂-C efflux data were converted to cumulative CO₂-C production by interpolation. An exponential model (Stanford and Smith, 1972; Alvarez and Alvarez, 2000) was used to describe carbon mineralization kinetics (R Development Core Team, 2009) (Eq. [5]).

 C_{min} = mineralized carbon; C_o = potentially mineralizable C pool; k = mineralization constant; day = incubation period

A two-way ANOVA model (JMP 7.0) was used to test the effects of the addition of root-C and N on CO₂-C efflux, cumulative CO₂-C, C_o and k from the exponential model, inorganic N concentration, soil pH, enzyme activities, SOC, SOC-derived from C3 and C4 by each sampling date. In most cases, the interaction between root-C and N was not significant, thus the main effects of root-C and N were independently described in this study. When there were significant differences, a post-hoc analysis was performed with a Tukey's HSD to compare mean differences at p < 0.05.

3.4 Results

3.4.1 CO₂ efflux rates and cumulative CO₂ production

 CO_2 efflux rates were the highest on Day 2 (Fig. 3.1 a,c,e). The second peak occurred after 15 days for all treatments, and then CO_2 efflux rates decreased gradually thereafter. Although there was an interaction effect between C and N on Day 25, 45, and 64, the CO_2 efflux rates in the CH treatment were always the highest among C treatments and lower with N addition. The root-C addition had significant effects on CO_2 efflux rates which continued through the entire incubation period of 200 days. Except Day 51 and 78, the CO_2 efflux rates were consistently in order of CH > CL > C0 for the entire incubation period. For day 51 and 78, the CO_2 efflux order was CH = CL > C0.

The effect of N addition was generally significant at the beginning of the incubation (i.e. day 1, 5 ~ 45, and 140) but did not last until the end of incubation. During the first 30 days, the NL treatment showed the similar or higher CO₂ efflux rates compared to the control. Except for Day 11, the CO₂ efflux rate was significantly lower under the highest N addition treatment than that under no N addition treatment during 5 to 45 days of incubation period.

The effects of both root-C and N addition were evident more for cumulative CO_2 production than for CO_2 efflux rates (Fig.3.1). Overall, the cumulative CO_2 production consistently followed the order of CH > CL > C0 among C treatments. In addition, the NH treatment had significantly lower cumulative CO_2 production than for the N0 and NL treatments throughout the sutdy (Fig. 3.1 b, d, f). Despite no statistical differences, the cumulative CO_2 production for NL was slightly higher than N0 during the initial stage of incubation (day 2~7). The interaction between C and N was significant (p<0.05) between Day 64 and 140. However, the trend of the cumulative CO_2 production with root-C and N addition did not change; the higher cumulative CO_2 was associated with higher addition of root-C among C treatments and with lower addition of N among N treatments (Fig. 3.1 b, d, f).

The decomposition constant (*k*) and the potentially mineralizable C pool (C_0) were estimated by an exponential model (Bonde et al., 1988; Alvarez and Alvarez, 2000). Decomposition constants did not vary among both root-C and N treatments (Table 3.1). However, the magnitude of C_0 was the largest under the CH and the smallest under the C0 treatment among root-C treatments, while the N0 treatment had significantly larger pool of C_0 than those for NL and NH Treatments (Table 3.1).

3.4.2 Inorganic N concentration in soil

Inorganic N concentration increased with the incubation time for all treatments (Fig. 3.2 a,b). More than 90% of inorganic N was in the form of NO₃-N because the added N was KNO₃, and the predominant form of inorganic N through transformation of organic N would be NO_3^- under the aerobic incubation conditions. There was no

interaction effect between root-C and N on the inorganic N concentration in soil (Table 3.2), which was significantly higher under NH than under N0 and NL among N treatments on 0, 10, 45, and 200 days. Although there was no statistical difference in inorganic N concentration after 100 days, the mean value was the highest under the NH treatment as was the case on other sampling dates. While the addition of root-C did not affect the inorganic N concentration in soil on day 0 and 10, it was significantly lower under the CH treatment than no C addition treatment on Day 45, 100, and 200.

3.4.3 Soil pH

In general, soil pH decreased with time (Fig. 3.2 c,d). In the N0 and NL treatment, however, pH increased slightly on day 10 then decreased with time. Among N treatments, the pH under NH was the lowest on 0, 10, 45, and 100 days, but there were no statistical differences among N treatments on the last day of the incubation. Among root-C treatments, pH was the lowest in the CH treatment at the beginning of the incubation. From 45 days, the decline in soil pH was more significant under no or less root-C addition than for the CH treatment among C treatments.

3.4.4 Enzyme assay

The differences in β -glucosidase activity among C treatments were significant on day 10 and 100 (Fig. 3.3 a, b). β -glucosidase activity was significantly higher in treatments receiving root-C on day 10. On day 45, the differences among C treatments were not statistically significant, but the increasing trend with the addition of root-C was consistently observed. On day 100, the activity of β -glucosidase in the CH treatment was greater than the C0 treatment. The addition of root-C did not affect β -glucosidase activity on day 200. The effect of N was only significant on day 100; β -glucosidase was significantly higher in the NH treatment than in the N0 treatment.

The activity of phenol oxidase and peroxidase differed significantly among C treatments on day 10 (Fig. 3.3 c, d). The response of phenol oxidase activity was not linearly correlated to the level of root-C, but that of the oxidative enzyme activity was significantly higher in the CH than for C0 treatment on day 10. On day 45, there were no statistical differences in phenol oxidase and peroxidase activity among C and N treatments, but there was a significant interaction between C and N treatments. On day 200, phenol oxidase activity increased with addition of N in the CH treatment, but that of the peroxidase was the highest in the NL among N treatments and in the C0 among C treatments. Overall, based on three destructive sampling, responses of the phenol oxidase and peroxidase activity to the level of C and N addition did not show any specific trend.

3.4.5 Soil organic carbon and $\delta^{13}C$

The initial soil had 2.05% of SOC and 0.21% of TN (C:N = 9.8:1). The SOC concentration at the end of the incubation was the highest under the NH treatment which was significantly higher than that in the NL treatment, i.e., the loss of SOC was the greatest under the NL treatment and the least under the NH treatment (Table 3.3). There were no significant differences in SOC among root-C treatments (p = 0.078). The TN concentration and the C/N ratio were not significantly influenced by the addition of either root-C or N.

In a mass balance, the net change in SOC concentration in root-C treatments

(SOC after incubation - SOC before incubation) was equal to the C input from root substrates minus loss of C from SOC decomposition (Table 3.4). The SOC concentrations in all samples decreased after incubation compared to those before incubation (p < 0.05, matched pairs t-test). Thus, the input of root-C in this study could not compensate for the loss of SOC through decomposition.

Although the SOC concentration was not influenced by the addition of root-C, the isotopic composition showed that the CH treatment contained higher amount of C4 (root-C)-derived SOC and the lower amount of C3 (inherent SOC)-derived SOC. However, there was no interaction between root-C and N, and the addition of N did not influence C4-derived SOC and C3-derived SOC concentrations.

3.5 Discussion

The CO₂ production and remaining SOC concentration after 200 days of incubation showed a negative effect of inorganic N on mineralization of OM. These results were in accord with many studies which also observed a negative effect of N on OM decomposition. For example, Fog (1988) concluded that N addition retarded decomposition especially when decomposable materials were more recalcitrant (e.g., straw, wood). The C sources used in this study, SOM and switchgrass root, were not easily decomposable C. Switchgrass roots in this study had a high C:N ratio (73:1). Moreover, swichgrass roots contain as much as 16.5% acid-insoluble and soluble lignin (Johnson et al., 2007), and suberin is one of the most recalcitrant materials comprising root tissues (Rasse et al., 2005). A similar study conducted with *Miscanthus* roots showed

the negative effect of inorganic N addition on OM decomposition; inorganic N only decreased mineralization of C in SOM, but not that of C in roots (Foeried et al., 2004).

There are several possible mechanisms to explain negative effects of N on OM decomposition. First, the N mining theory predicts the inhibition of SOC decomposition by N addition. The characteristics of SOM in which C and N are bound together explain the coupled responses for C and N mineralization (McGill and Cole, 1981). The N mining theory is based on a guild of microbes which can decompose recalcitrant SOM to obtain N despite the high cost for the N. Thus, the addition of available N could prevent the N mining from the recalcitrant SOM, therefore, reducing the rate of SOM decomposition. The N mining would be the greatest under the circumstance of limited N with available C substrates. Although the remaining C3-derived SOC was the lowest in the CH+N0 and CH+NL treatments, this theory does not adequately explain the negative effect of N observed in this study, because N was not a limiting factor to control SOM decomposition in this experiment. The inorganic N concentration in soil increased over the course of incubation, and net N mineralization occurred even in the highest C treatment from the beginning of the incubation. The lack of N immobilization resulted in increased inorganic N concentration in soil. Less immobilization of inorganic N under the high N treatment was also reflected by the soil pH changes (Fig. 3.2 c, d). When OM is mineralized, organic N is transformed into inorganic N through ammonification and nitrification. When microorganisms actively take up ammonium ions, the rate of nitrification may be retarded and less protons are released into the soil environment. In this study, CO_2 -C efflux rates were negatively correlated with inorganic N levels (r = -0.73) and positively with soil pH (r = 0.68) (Appendix A1).

Another possible mechanism for the negative effect of N addition is the suppression of extracellular oxidative enzymes. Carrio et al. (2000) showed that the continuous N addition in forest ecosystems resulted in the lower activity of oxidative enzymes. Those enzymes are related to C cycling because they can decompose recalcitrant SOM as well as lignin-containing materials (Sinsabaugh, 2010). Therefore, decomposition can be decreased by the change in oxidative enzyme activities which might come from the reduced production of extracellular enzymes or from changes in microbial community composition induced by N addition. However, no strong correlation was observed between oxidative enzyme activities and CO_2 -C efflux rates (r = 0.39) (Appendix A1), and phenol oxidase and peroxidase enzyme activities did not show any differences among N treatments at three destructive sampling dates. On the other hand, Freeman et al., (2001) explained a correlation between oxidative enzyme and hydrolase enzyme activities; high activities of oxidative enzymes could stimulate the activities of hydrolase enzymes by mitigating the inhibition from phenolic compounds. In this present study, β -glucosidase was responsive to the root-C addition but not with the N addition, and the correlation between oxidative enzymes and β -glucosidase was very low and was not statistically not significant (r = 0.14, p = 0.20) (Appendix A1). Thus, the change in enzyme activities was not a likely cause of the reduced decomposition of OM by N addition.

Oxidative enzyme activities are related to microbial community composition (Sinsabaugh, 2010). Basidiomycetes are the dominant fungi in mostly temperate and boreal forests (Allison et al., 2008). In contrast, Glomeromycota and Ascomycota are dominant microbial communities in grasslands (Porras-Alfaro et al., 2010). Thus,

oxidative enzymes might exist in a small amount and do not play an important role in controlling SOM dynamics in grassland systems. Yao et al. (2009) reported that 60 mg N kg⁻¹ soil input in turf ecosystems had a minor impact on phenol oxidase activity. Yao and colleagues (2009) suggested that increased productivity by N fertilization could indirectly affect decomposition. Long-term N addition experiments conducted in forests and grasslands also showed that there was no particular response of lignin degrading enzymes, but cellulose degrading enzyme activity was stimulated by N addition (Keeler et al., 2009). Therefore, oxidative enzyme activities might not be very responsive to N addition in grassland systems in contrast to forest ecosystems (Sinsabaugh, 2010).

Another theory to explain the negative effect of N on OM decomposition is the formation of recalcitrant compounds. Greater N availability increases condensation reactions and can decrease the degradability of OM (Fog, 1988; Shevtsova et al., 2003). The potentially mineralizable C pool (C_0) decreased with N addition in the present study, and the size of the estimated Co is related to a labile C pool (Alvarez and Alvarez, 2000). Thus, in the NH treatment, the reduced labile C pool coupled with a greater total SOC pool indirectly indicates the presence of a greater proportion of a less readily available C pool (Table 3.1). Such a trend would partially support the theory that N addition accentuates the formation of recalcitrant compounds. Several studies have shown that mineral N facilitated the transformation of residue C into more stable forms of SOM such as brown and recalcitrant substances (melanoidins), humin, etc. (Fog 1988; Ågren et al., 2001; Moran et al., 2005). Nitrogenous compounds bonded to or in aromatic rings led to chemical stabilization and reduced the bioavailability of N (Schmidt-Rohr et al., 2004). Himes (1988) calculated the N requirement for increasing the SOC pools and explained

the important roles of N for accelerating the rate of SOC humification of biomass C. However, the size of C_0 estimated through regression can only indirectly imply the possibility for the recalcitrant compound formation. More direct measurements through fractionation of SOM pools (Moran et al., 2005), NMR spectra analysis (Schmidt-Rohr et al., 2004), etc., are needed to confirm such a mechanism.

When an exponential plus linear model was used to estimate the decomposition constants of labile (k_l) and recalcitrant C pools (k_r), there were several cases showing negative numbers of estimated k_r (Appendix A2). This trend implies that the incubation period was not long enough to estimate the decomposition constant for a resistant C pool (Alvarez and Alvarez, 2000). Nevertheless, there was a strong positive correlation (r =0.93) between k_l and k_r (Appendix A2) from the exponential plus linear model. Thus, this trend may support the argument that one mineralization constant from the exponential model in this study can represent the characteristics of the OM mineralization rather well in this study.

Decline in soil pH could cause a decrease in CO_2 efflux following N addition. The soil pH and CO_2 efflux rates were closely correlated (r = 0.68) (Appendix A1). Moreover, when a linear regression was used to establish the relationship between CO_2 efflux rate and pH among three N treatments under the same C level, three regression lines coincided (Appendix A3). Although soil pH varied narrowly in a neutral pH range, the CO_2 efflux rate was linearly correlated with the change in soil pH under the same C level regardless of N levels. Moreover, the effect of N on CO_2 efflux was mostly significant at the beginning but did not differ at the end of the incubation among N treatments. The change in soil pH also followed a similar pattern, that is, the pH in the high N treatment was the lowest from the beginning until 100 days and was not different at the end of the incubation. The relationship between soil pH and CO_2 efflux rates is also supported by numerous field studies that showed the importance of soil pH on microbial activities (Helyar and Porter, 1989; Högberg et al., 2007). However, Allsion and Murphy (1962, 1963) adjusted soil pH by adding CaCO₃ or Ca(OH)₂, but it did not completely counterbalance the decrease of CO₂ efflux with N addition in a lab incubation study. In addition, a causal relationship is not certain, whether the drop in soil pH reduced the CO_2 efflux rate, or a reduced microbial activity led to the drop in soil pH. Thus, a more sensitive experimental design is needed to avoid the confounding effects caused by change in soil pH. Besides the possibilities discussed herein, inorganic N addition could have a negative effect on microbial activities by altering soil environments such as increasing salt or NO_3^- mobility, or loss of base cations, etc. (Aber et al., 1995; Fog. 1998). Those possibilities were not examined in this study. Overall, the first hypothesis about the negative effect of N on decomposition was supported by this study. However, the mechanisms might also be the recalcitrant material formation or lowered pH rather than an N mining theory.

Theoretically, the change in SOC concentration under no root-C addition should be equal to the total cumulative CO_2 -C evolved in a closed system like this study. However, there was some discrepancy (average 1.1 mg C/g soil) between these two values (Table 3.4). The total cumulative CO_2 -C was higher than the loss of SOC observed in soils. This might come from a loss of C during acid washing, over-estimation from interpolating CO_2 -C efflux rates to the cumulative CO_2 -C calculation, and/or the relatively rougher estimation for the SOC with a very small fraction of soil compared to CO_2 measurements from the whole soil. However, the amount of this discrepancy among N treatments was not different (p = 0.133 in one-way ANOVA) in the C0 treatments.

Total CO₂ production increased with root C addition. The decrease in C3-derived SOC remaining at the end of the experiment with an increase in C addition implied a priming effect induced by added root C. Steinbeiss et al. (2008) showed that litter addition led to SOC losses because the rate of native SOC decomposition was greater than the formation of new SOC from litter. The priming effect is defined as a short-term change of SOM decomposition rates induced by an increase in easily degradable C sources (Kuzyakov et al., 2000). Most studies examined the priming effect by the isotopic discrimination of CO₂ from soil respiration. Thus, it should be checked whether additional CO₂ production is caused by increased microbial turnover or metabolism changes, which is an apparent priming effect (Kuzyakov et al., 2000; Blagodatskay et al., 2007). In this study, however, the real priming effect was confirmed directly from the loss of C3-derived SOC. Even though the natural abundance of ¹³C in swichgrass root which has less differences in ¹³C quantity compared to enriched ¹³C labeled materials was used in most studies, it was possible to verify the real priming effect from SOC due to the controlled experimental conditions, uniformly mixed soil, and a relatively long incubation period.

The amount and types of priming effects are regulated by the availability, composition, and amount of substrate (Blagodatskay et al., 2007). Blagodatskaya and Kuzyakov (2008) concluded that easily available C substrates could lead to a greater priming effect than low available C. On the other hand, some studies that view priming effects as a result of microbial competition argue that easily degradable C can not

influence decomposition of SOM since the guild of microbes which can degrade the labile C cannot play a role of degrading recalcitrant C. This study seemingly follows the second view of the priming effect. Growth of fungi was observed on the roots in the CL and CH treatments from the beginning of the incubation. Fungi are considered as decomposers which can break down the complicated structure of substrates (Swift, 1979). Thus, the proliferation of fungi over bacteria in the root addition treatments (CL and CH) might accentuate the decomposition of SOM. The change in microbial community with C addition was not quantified so that the mechanisms of the priming effect can not be corroborated in this study. Although the degree of the priming effect by roots cannot be compared with easily available C, the present study supports the idea that less available C (root C) could induce the priming effect.

The change in enzyme activities could partially explain the decomposition of C3derived SOC. A positive correlation between CO₂ efflux rates and oxidative enzymes was only observed on day 10, and it might be insufficient to explain the priming effect by the change in oxidative enzyme activities. On the other hand, β -glucosidase activity increased with root C addition, and it could facilitate the decomposition of the labile portion of SOC during 200 days of incubation. Manning et al. (2008) showed that facilitated litter decomposition was associated with a higher level of N (44 kg N ha⁻¹), and enzyme activity was also higher due to the combination of increased N availability and litter inputs. Nevertheless, the CO₂ from root C and native SOC was not differentiated in this study, and the isotopic C analysis was only conducted with the samples at the end of incubation in contrast to the enzyme activities measured 4 times. This limited the estimation of the initiation and duration of the priming effect and the relationship with β -glucosidase activity.

The increase in root-C addition contributed to the newly formed SOC (p = 0.008) but did not significantly affect the degree of the priming effect (ANOVA for the C3derived SOC, p > 0.05). Thus, the hypothesis about the degree of the priming effect depending on the amount of added root-C was not supported by this study. However, Blagodatskaya and Kuzyakov (2008) showed contrasting trends of priming effects depending on the amount of substrates. When added substrate C is much less than microbial biomass C (< 15%), primed CO₂ increased linearly with substrate-C. However, when too much C substrate was added (> 50% compared to microbial biomass C), CO₂ production decreased with addition of substrates. Although the present study showed no statistical differences, there seemed to be more SOC lost in the higher C addition treatment. Thus, a broad range for the amount of root-C addition needs to be tested to check whether any specific trends can be found with the low-available C substrates. Overall, the second hypothesis about the priming effect by the addition of root was supported by the present study, however, the amount of root-C addition did not affect the degree of the priming effect.

The C addition treatments also showed slow increase in inorganic N and slow decrease in pH compared to no C addition treatment. Even though C mineralization was higher, N mineralization was lower with the addition of root-C. This trend may suggest that root C addition stimulated microbial activities so that inorganic N was taken up by microbes. In this case, the retardation of nitrification could prevent the drop in soil pH. Moreover, the change in soil pH could be delayed by released organic anions from root materials (Yan et al., 1996).

The data presented suggest that N could have a positive effect on SOC sequestration by reducing decomposition. However, the results of this study have some limitations for the direct application under field conditions. Several other mechanisms for protecting against SOM decomposition simultaneously act together in the field. The SOM protection by soil structure (aggregation, pore occlusion, etc.) is one of the most important mechanisms to control SOM dynamics (Six et al., 2002; Strong et al., 2004; Jastrow et al., 2007), but it was not tested with the disturbed soil in a short-term lab incubation. Moreover, the form and duration of C input in the field would be different. In the field, C substrates would be continuously added into soil as a form of easily degradable C, i.e. rhizodeposition and very fine roots turnover (Kuzyakov, 2002; Personeni and Loiseau, 2004). These easily degradable compounds might play more important roles in controlling SOC dynamics than fine and coarse roots used in this study. Environmental constraints, such as, moisture, temperature, soil pH, accessibility for the C sources, etc., may be more influential for SOC dynamics than effect of N itself as a nutrient (Bontti et al., 2009; Yao et al., 2009).

3.6 Conclusion

Soil is the largest terrestrial C pool, and increasing the SOC pool can improve ecosystem functions and bring benefits from SOC sequestration. It is particularly important to understand SOC dynamics by considering other elements under rapidly changing environments (magnified N fertilization, N deposition, etc.). The data presented shows the negative effect of N addition on OM decomposition, and it was suggested that the recalcitrant material formation by N addition could be a likely cause. Thus, when N fertilizer is applied, an increase in SOC pools can be expected by reducing SOC mineralization. Many field experiments showed that the increased SOC pool can be attributed to the increased residue returns associated with higher biomass production under N fertilization. However, this study suggested a possibility that the increased SOC pool could also come from a decrease in SOC decomposition. Therefore, considering the contribution from the increased new SOC formation and from the decreased old SOC decomposition by the fractionations of the SOC pool will be very helpful to understand the SOC dynamics associated with N. In this study, possible mechanisms including biological factors were examined, but a decrease in soil pH and a possibility of recalcitrant compound formation are considered the most probable causes of the negative effect of N addition on OM decomposition. Induced changes in physical and or chemical properties by N addition might play a more important role in SOC decomposition than nutrient demands for microorganisms or enzyme activities. However, the negative effect of N might not be very significant under field conditions, since plants would absorb available N, and several environmental factors along with SOM protection by soil structure would function simultaneously. This study also corroborated that the addition of root-C induced a priming effect but that the loss of C was counterbalanced by the added C; formation of new SOC can offset the loss of SOC due to decomposition. Priming effects need to be considered as one of the important mechanisms to understand SOC dynamics. The stimulation of the activity of microbial groups that can decompose recalcitrant C by adding C substrates might be more effective on decomposition of recalcitrant SOC. More thorough investigation of priming effects about magnitudes and mechanisms is needed to understand SOC turnover. In addition to the relatively recalcitrant root-C, priming effects induced by rhizodeposits which are easily decomposable forms of C and continuous input of C into soil would be relevant to studies of SOC dynamics in the field.

3.7 References

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		potentially mineralizable C (C _o), mg C g ⁻¹		less readily available C (initial C – Co), mg C g ₋₁		decomposition constant (k), day ⁻¹		Mean Residence Time (1/k), day	
		Mean	SE	Mean	an SE Me		SE	Mean	SE
C level									
C0		2.28	0.05 ^c	19.95	5 0.05 ^c	0.010	0.0002	98.94 1.94	
CL		3.38	30.08 ^b	21.05 0.08 ^b		0.011 0.0003 ^{ns}		95.63 2.95 ^{ns}	
CH		4.29 0.16 ^a		22.34 0.16 ^a		0.011 0.0003		92.06 3.21	
N level N0 NL NH		3.58 0.34 ^A 3.30 0.29 ^B 3.07 0.26 ^B		20.85 0.31 ^B 21.13 0.35 ^A 21.36 0.40 ^A		0.010 0.0003 0.011 0.0003 ^{ns} 0.010 0.0003		97.18 2.95 91.62 2.66 ^{ns} 97.83 2.65	
Source	٦f	Б	р	Б	р	Б	п	Б	р
C		Г 1766	r ~0.001	г Эло 7	r <0.001	г 20	r 0 165	Г 167	r 0.22
U N	∠ 2	1/0.0	~0.001 0.001	240./ 11.2	~0.001	2.0 1.9	0.103	1.07	0.22
IN C*N	∠ 1	11.5	0.001	11.5	~0.001 0.26	1.0	0.199	1.03	0.22
U·N	4	1.4	0.203	1.4	0.20	1.1	0.393	1.11	0.38

Table 3. 1 Potentially mineralizable C pool, recalcitrant C pool, decomposition constant, and mean residence time among root-C and N treatments

Different lower case letters among C levels indicate significant differences at p < 0.05. Different capital letters among N levels indicate significant differences at p < 0.05. ns: not significantly different.

			Inorgani	ic N	pН	
Day		DF	F	Р	F	Р
0	С	2	2.3	0.130	10.4	0.001
	Ν	2	133.8	< 0.001	6.0	0.010
	C*N	4	1.5	0.244	1.0	0.447
10	С	2	3.4	0.056	1.4	0.262
	Ν	2	246.7	< 0.001	71.7	< 0.001
	C*N	4	0.1	0.973	1.9	0.152
45	С	2	7.4	0.005	30.5	< 0.001
	Ν	2	54.3	< 0.001	31.1	< 0.001
	C*N	4	2.9	0.053	1.7	0.187
100	С	2	6.8	0.006	29.6	< 0.001
	Ν	2	1.4	0.280	11.1	0.001
	C*N	4	0.4	0.817	1.3	0.306
200	С	2	16.0	< 0.001	70.3	< 0.001
	Ν	2	28.9	< 0.001	1.9	0.185
	C*N	4	1.4	0.272	2.1	0.117

Table 3. 2 Two-way ANOVA table to test the effects of root-C and N addition on the inorganic N levels and soil pH at each destructive sampling date.

		SOC		na	ative SOC	2	root-C-derived SOC			
	(m	ig C/g soi	il)	(m	ig C/g soi	il)	(mg C/g soil)			
	Mean	Mean SE		Mean	Mean SE			Mean SE		
C level										
C0	19.16	0.05		19.16	0.05	а				
CL	19.21	0.07	ns	18.80	0.15	ab	0.41	0.16	b	
СН	19.39	0.13	0.13		0.20	b	1.09	0.16	а	
N level										
N0	19.25	0.08	AB	18.83	0.22		0.63	0.33		
NL	19.06	0.03	В	18.60	0.16	ns	0.69	0.17	ns	
NH	19.44	0.10	А	18.83	0.19		0.93	0.21		
ANOVA -										
Source	df	F	Р	df	F	Р	df	F	Р	
С	2	2.947	0.078	2	9.195	0.002	1	10.173	0.008	
Ν	2	7.722	0.004	2	0.801	0.464	2	0.729	0.503	
C*N	4	1.621	0.212	4	1.516	0.240	2	2.307	0.142	

Table 3. 3 The concentrations of soil organic carbon (SOC), native SOC, and root-C derived SOC at the end of incubation (200 days)

Different lower case letters among C levels indicate significant differences at p < 0.05. Different capital letters among N levels indicate significant differences at p < 0.05. ns: not significantly different.

C level	N level	Initial soil C			Root-C		Total C before incubatio n	Final SOC after acid washing		Total CO ₂ producti on	Final SOC+ CO ₂	[†] Differ- ence in C
		before acid washing	after acid washing	mean ¹³ δ (‰)	amount	mean ¹³ δ (‰)		amount	mean ¹³ δ (‰)			
		(i)	(ii)		(iii)		(i+iii)	(I)		(II)	(I+II)	(i+iii)- (I+II)
C0	N0	22.23	20.53	-26.40	0.00		22.23	19.22	-26.38	2.08	21.30	0.93
	NL	22.23	20.53	-26.40	0.00		22.23	19.08	-26.43	1.96	21.04	1.19
	NH	22.23	20.53	-26.40	0.00		22.23	19.19	-26.69	1.85	21.04	1.20
CL	N0	22.23	20.53	-26.40	2.20	-15.00	24.43	19.14	-26.64	3.11	22.25	2.18
	NL	22.23	20.53	-26.40	2.20	-15.00	24.43	19.06	-26.18	2.98	22.04	2.39
	NH	22.23	20.53	-26.40	2.20	-15.00	24.43	19.42	-25.90	2.74	22.17	2.27
СН	N0	22.23	20.53	-26.40	4.40	-15.00	26.63	19.38	-25.66	4.11	23.49	3.14
	NL	22.23	20.53	-26.40	4.40	-15.00	26.63	19.05	-25.80	3.87	22.93	3.71
	NH	22.23	20.53	-26.40	4.40	-15.00	26.63	19.72	-25.82	3.37	23.09	3.54

Table 3. 4 Mass balance for the amount of C before and after incubation

[†] Difference in SOC before and after incubation. While this difference was attributed to acid washing in the C0 treatment, these differences in the CL and CH treatments included the root-C remained and the soil C loss from acid washing. Note: All the amount of C were presented as mg C g⁻¹ soil



Fig. 3. 1 CO₂-C efflux rates in (a) C0 treatments (c) CL treatments, and (e) CH treatments and the cumulative CO₂-C production in (b) C0 treatments, (d) CL treatments, and (f) CH treatments in a course of 200-day incubation.



Fig. 3. 2 Changes in inorganic N levels (a), (b) and in soil pH (c), (d) in a course of the incubation.

Different capital letters indicate significant differences at each destructive sampling date at p < 0.05. NS: not significantly different



Fig. 3. 3 Changes in β -glucosidase activities among C treatments (a) and those among N treatments (b) and changes in oxidative enzyme activities among C treatments (c) and those among N treatments (d) during the incubation.

Different lower case letters in (a) and (b) indicate significant differences among C or N treatments in β -glucosidase activities at each destructive sampling date at p < 0.05. In (c) and (d), lower case letters inside bars compare the effect of C or N on either peroxidase or phenol oxidase in each destructive sampling date. The different capital letters outside of bars denote significant differences (p<0.05) in oxidative enzyme activities (sum of phenol oxidase and peroxidase activities) among C or N treatments. ns: not significantly different.

CHAPTER 4

NITROGENOUS FERTILIZER EFFECTS ON SOIL STRUCTURAL PROPERTIES UNDER SWITCHGRASS

4.1 Abstract

Nitrogen (N) fertilization is needed to sustain the biomass yield of switchgrass (*Panicum virgatum* L., Poaceae) as a biofuel feedstock and, consequently, may influence the potential for soil quality improvements through soil organic carbon (SOC) sequestration. Because changes in soil physical quality can feed back to affect the sustainability of biomass production, the impacts of N application on switchgrass biomass production and soil quality need to be evaluated together. Therefore, the objective of this study was to assess how inorganic N application to switchgrass affects soil structural properties. Soil was sampled at 0-5, 5-10, and 10-15 cm depths in April and November 2008 during the fifth year of switchgrass growth in Milan, TN. Nitrogenous fertilizer was applied as NH₄NO₃ at 0, 67, and 202 kg N ha⁻¹ yr⁻¹ beginning in the second year. Root weight density (RWD), root length density (RLD) and SOC concentration were measured under the three N treatments as factors potentially influencing soil structural properties. The soil structural parameters that were measured included bulk density (ρ_b), soil moisture characteristic curve (SMCC), and aggregate

stability through wet-sieving. At 0-5 cm depth, spring RWD (3.8 mg cm⁻³) was significantly lower with 202 kg N ha⁻¹ application compared to 0 and 67 kg N ha⁻¹ (14.1 and 17.0 mg cm⁻³, respectively). Although fall RWD did not vary among N treatments, RLD under 202 kg N ha⁻¹ (7.1 cm cm⁻³) was less than half of that at 0 kg N ha⁻¹ (15.7 cm cm⁻³). The SOC concentration was more in both fertilized (average 1.96%) than in the unfertilized treatment (1.65%) for 0-5 cm depth. Although SMCC varied somewhat between seasons, it did not exhibit any consistent trends attributable to N application. The ratio of mean weight diameter (MWD) after to that before wet-sieving for 0-15 cm depth and the proportion of macroaggregates for 0-10 cm depth were significantly higher in the 0 and 67 kg N ha⁻¹ than in the 202 kg N ha⁻¹ treatment. These data suggest that high N application to switchgrass could have negative impacts on soil structural properties by reducing root biomass and length, crucial determinants of soil structure, despite an increase in SOC.

Keywords: switchgrass, nitrogen fertilizer, soil structure, aggregate stability, root, soil organic carbon (SOC)

4.2 Introduction

Increasing atmospheric carbon dioxide (CO₂) concentrations and anthropogenic nitrogen (N) depositon are major environmental concerns (Vitousek et al., 1997;

Abbreviation: SOC: soil organic carbon; TC: total carbon; TN: total nitrogen; RWD: root weight density; RWD: root length density; WSA: water stable aggregate; MWDpre: mean weight diameter before wetsieving; MWDpost: mean weight diameter after wet-sieving; TS: tensile strength Heimann and Reichstein, 2008). Cultivation of bioenergy crops can be used to off-set increases in CO₂ in two ways: production of energy from above-ground biomass to replace fossil fuels, and the return of assimilated CO₂ to the soil organic carbon (SOC) pool through the production and turnover of below-ground biomass. Switchgrass (*Panicum virgatum* L., Poaceae) was selected by the United States Department of Energy (U.S. DOE) as an herbaceous bioenergy crop due to its high potential for biomass production and its cost-effective growth characteristics (McLaughlin, 1992; Bransby et al., 1998).

Sequestration of SOC by cultivation of switchgrass is primarily through belowground carbon (C) inputs. The aboveground biomass is harvested, leaving the plants' perennial root system intact in the soil. Application of N can change biomass allocation (Heggenstaller et al., 2009), because alleviation of nutrient constraints achieved through fertilization can allocate more C into the aboveground biomass (Tilman, 1987). However, the effects may vary depending on plant functional groups (Xia and Wan, 2008). Although many switchgrass studies have examined aboveground productivity and C sequestration, the probable shift of switchgrass biomass allocation with N application and resultant effects on the role of roots in SOC sequestration have not been very thoroughly investigated (Ma et al., 2000; Frank et al., 2004; Lee et al., 2007; Lemus et al., 2008).

Application of inorganic N may influence soil structural properties through changes in root development, microbial community composition and activity, SOC concentration, and soil chemical properties (e.g., flocculation, zeta potential, etc.) (Haynes and Naidu, 1998). If N application reduces the production of roots and fungal hyphae (Nadelhoffer, 2000; Bradley et al, 2005), which are temporary binding agents of aggregates, then aggregate size distribution and stability could be negatively affected (Abiven et al., 2007). When N addition affects the level of SOC, it could eventually influence soil physical properties related to SOC concentration such as bulk density (ρ_b), soil moisture characteristic curves (SMCC), tensile strength (TS), etc. (Tate, 1992; Kay, 1998; Blanco-Canqui et al., 2005). Many studies have investigated the feasibility of bioenergy crop cultivation with a focus on economic consideration, but attendant changes to soil quality are rarely evaluated. Thus, it is necessary to determine appropriate levels of N application that can achieve high production of harvestable biomass while maintaining or improving soil structural properties.

The overall goal of this study was to investigate the effects of inorganic N application on switchgrass rooting characteristics, SOC concentration, and soil structural properties. It is hypothesized that root biomass and length would decrease with increase in rate of N application. Decreased root C input with high N application would result in the lower level of SOC. As a temporary binding agent of aggregates and a source of C inputs to soil, decreases in root growth could lead to attendant decreases in the size distribution of stable aggregates, TS of aggregates, and SOC concentration at high N application rates.

4.3 Materials and Methods

4.3.1 Study site and soil sampling

This study was conducted in Milan, TN (35°56' N, 88°43' W) on a Grenada silt loam (fine-silty, mixed, active, thermic, Oxyaquic Fraglossudalfs). Site soil had 7.0% sand, 82.6% silt, and 10.4% clay at 0-5 cm depth; 4.2% sand, 82.2% silt, and 13.6% clay at 5-10 cm depth; and 3.3% sand, 80.7% silt, and 16.0% clay at 10-15 cm depth based on the pipette method without destruction of soil organic matter (Kilmer and Alexander, 1949). The experiment was a randomized complete block design involving four replicates and three N treatments: 0, 67, and 202 kg N ha⁻¹. Each treatment plot was 7.3 m long and 4.6 m wide (Mooney et al., 2009). Switchgrass (cultivar: Alamo) was planted in spring 2004. Seeding rate was 8.4 kg ha⁻¹ pure live seed (PLS). Fertilizer was not applied in the first season, but after that it was applied as NH₄NO₃ at rates of 0, 67, and 202 kg N ha⁻¹ annually in the spring. The experimental design is described in detail in Mooney et al. (2009). Soil samples were obtained in April and November 2008 from four randomly placed 0.1-m² circular quadrats per plot from which aboveground biomass and surface litter were removed. Within each quadrat, one 4.8 cm diameter core was randomly taken to a 15 cm depth and cut into 0-5, 5-10, and 10-15 cm depth increments. Samples from each plot were pooled by depth increment prior to analyses, but were handled in a manner that allowed individual cores to remain intact.

4.3.2 Root, soil, and aggregate analyses

One core per plot was used to determine the SMCC for each depth increment. The other core samples were manually broken apart along natural planes of weakness for aggregate analysis. During soil preparation, roots between aggregates were removed, washed, and dried at 45° C for 48 hrs, and then weighed. Thus, the root data were limited to roots removed during soil preparation and excluded those occluded within aggregates of the 8 mm sieved soil. Rhizomes were separated from roots and were not included with the root biomass data presented here. Root weight density (RWD) was calculated as root weight divided by soil volume. Root length and average root diameter were determined for the fall sample by scanning and image analysis using the WinRhizo software program (Regent Instruments, Inc., Québec, Canada). Roots were classified on the basis of diameter classes as coarse root (> 2 mm diam.) and fine root (< 2 mm diam.). Root length density (RLD) was calculated as root length divided by soil volume. Specific root length (SRL) was calculated as total root length divided by root weight for each depth.

Soil ρ_b was calculated from the oven-dried (105°C) weight of soil divided by core volume (Topp and Ferré, 2002). The SMCC was determined by measuring moisture retention at 0.25, 1, 3, 5, 7.5, 10, 33, 100, and 300 kPa suction using intact core samples and at 1500 kPa suction using 2 mm sieved soils (Dane and Hopmans, 2002). High energy SMCC (0.25 ~ 7.5 kPa) were measured on a tension table equipped with a capillary outflow tube. A pressure plate apparatus was used for low energy SMCC (10 ~ 1500 kPa). When water outflow ceased at each suction or pressure, the weights of the core samples were measured to calculate retained water content. The available water capacity (AWC) was calculated by subtracting volumetric moisture content (θ) at 1500 kPa (permanent wilting point) from that at 33 kPa (field capacity).

After removing roots and plant residues, soil was passed through an 8-mm sieve and air-dried. The size distribution of aggregates (< 8 mm) was determined with a nest of
sieves (4.75, 2, 1, 0.5, 0.25, and 0.053 mm) by manually dry-sieving 50 g of air-dried soil followed by wet-sieving with a Yoder (1936) apparatus. The mean weight diameter before wet-sieving (MWDpre) was calculated with dry sieved aggregates (> 0.25 mm) (Nimmo and Perkins, 2002)

$$\mathbf{MWD} = \sum m_i \times x_i \qquad \qquad \mathbf{Eq. [1]}$$

where m_i = mass of the aggregates within each measured size range (> 0.25 mm size) as a fraction of the total dry mass of the analyzed sample; x_i = the corresponding mean diameter of each aggregate size range.

After dry sieving, the aggregate size fractions were pooled together again and used in wet-sieving. The aggregates were immersed in water for 30 min on nested sieves (4.75, 2, 1, 0.5, and 0.25 mm) and wet-sieved for 30 min (oscillation rate 25 cycles min⁻¹). The water in the wet-sieving apparatus was decanted through a 53-µm sieve after wet-sieving, allowing for separation of the 0.25-0.053 mm (microaggregates) and <0.053 mm (silt and clay fraction) size classes. Every size fraction after wet-sieving was dried at 45°C and weighed. As with dry-sieved aggregates, only the water stable aggregates (WSA) classified as macroaggregates (> 0.25 mm) were used for the calculation of MWD after wet-sieving (MWDpost) (Eq. [1]). Because of initial differences in the size distribution of dry aggregates before wet-sieving, MWD ratio (Eq. [2]) was used to standardize these differences and, thereby, improve assessments of aggregate stability and treatment comparisons. The MWD ratio was calculated as

MWD ratio = MWDpost/MWDpre Eq. [2]

where MWDpost = mean weight diameter of WSA after wet-sieving; MWDpre = mean weight diameter of aggregates obtained by dry-sieving (before wet-sieving).

Wet-sieved aggregates (4.75-8 and 2-4.75 mm) were air-dried on the sieve at first to avoid deformation of wet aggregates and then dried at 45°C for 24 hrs. The TS of dried water-stable aggregates was measured with a simple crushing apparatus designed by Horn and Dexter (1989). When cracks were visible on an aggregate, the force was recorded (Dexter and Watts, 2001). Before crushing aggregates, the diameters of each aggregate in the 4.75-8 mm size class were measured with calipers. The TS of aggregates was calculated by using Eq. [3], [4] and [5]:

$$TS = 0.576 (F/d_{agg}^2)$$
 Eq.[3]

where F is the vertical breaking force, and d_{agg} is the mean aggregate diameter.

d_{agg} of aggregates in 4.75-8 mm size class = $(d_1 + d_2 + d_3)/3$ Eq.[4]

where d_1 is the longest, d_2 is the mediate, and d_3 is the smallest diameter of each aggregate,

$$d_{agg}$$
 of aggregates in 2-4.75 mm size class = $(2+4.75)/2$ Eq.[5]

For the analysis of total C (TC) and total N (TN) concentrations, soil was 2-mm sieved, ground in a roller-mill grinder (Sampletek 200 Vial Rotator, USA) for 2 days, and passed through a 0.25-mm sieve. Soil TC and TN concentrations were determined by dry combustion (900°C) using a CN analyzer (Vario Max Elementar Americas, Inc., Germany) (Nelson and Sommers, 1996). Because no carbonates were present, TC was equivalent to SOC.

4.3.3 Statistical analyses

The normality of data by Kolmogorov-Smirnov test and constant variance of errors by Bartlett's Test were checked prior to conducting statistical analysis. When the data distribution was not normal, or the error assumption was violated, the data were log-transformed. Data were analyzed by split-plot ANOVA with a randomized complete block (RCB) design. Fertilizer N rates were considered to be the whole plot treatment, with seasons as a split plot. This model produces the identical results as a repeated-measures (seasons) RCB design ANOVA (factor = N rates).

The model used for this experiment was

$$Y_{ijk} = \mu + \beta_i + \tau_j + \gamma_k + (\beta \tau)_{ij} + (\tau \gamma)_{jk} + \varepsilon_{ijk}$$
 Eq. [6]

where Y_{ijk} was the observed values for the *i*th block assigned to the *j*th N rates in season k; μ was the overall mean; β_i was the *i*th block effect; τ_j was the *j*th N main effect; γ_k was the *k*th season main effect; $(\beta \tau)_{ij}$ represented the random effects of the whole plots; $(\tau \gamma)_{jk}$ was the interaction effect between N rates and seasons; and ε_{ijk} represented the random effects of the split plots.

The GLM procedure in SAS release 9.2 (SAS Institute, 2002) was used to estimate the split-plot ANOVA as specified in Eq. [6] on RWD, SOC, TN, C/N ratio, ρ_b , θ at different suctions, AWC, the proportions of macroaggregate, microaggregate, and silt-clay size fractions after wet-sieving, MWDpost, MWD ratio, and TS for each depth. The data collected only in fall (RLD, SRL, and root diameter) were analyzed with a one-way ANOVA model (JMP 7.0) to evaluate the treatment effect (N rates) for each depth. When significant differences were observed (p < 0.1) in the ANOVA, post-hoc mean comparisons were evaluated by LSD (p = 0.1) To check the correlations between

measured parameters, all data was pooled together by depth regardless of N rates and seasons, and the Pearson product-moment correlation coefficients were calculated. Non-linear regression curve fitting was used to examine the relationships between MWD ratio and RWD and between MWD ratio and RLD after pooling data across depths, N rates, and seasons (Origin 8.0).

4.4 Results

4.4.1 N fertilization effects on root distribution

Root biomass at 0-5 cm depth was significantly influenced by the season x N interaction (p<0.01). A significant decrease in RWD for the 202 kg N ha⁻¹ treatment was observed at 0-5 cm depth (Fig. 4.1(a)) in spring. However, RWD in fall did not vary with N application rates. RWD in the 67 kg N ha⁻¹ treatment in spring was significantly higher than all measurements in fall and RWD in the 202 kg N ha⁻¹ treatment in spring. Root biomass was not statistically different at 5-15 cm depth, but a tendency for lower root biomass in the 202 kg N ha⁻¹ treatment occurred in spring. Most root biomass was found in the surface 5 cm, and decreased with soil depth. In the fall, total RLD decreased with increasing N application for 0-10 cm depth (Fig. 4.2(a)). The relationship between the N application rate and RLD was driven by fine roots (< 2 mm) as coarse roots (> 2 mm) accounted for only 1~6 % of total RLD, and coarse roots were not significantly affected by N treatments (Fig. 4.2(b), (c)). The lower RLD under the highest N treatment, coupled with no N treatment effect on RWD, was reflected also in lower specific root length (SRL) and higher average root diameter for 0-5 cm depth under the high N treatment in the fall (Fig. 4.3).

4.4.2 N fertilization effects on soil organic carbon (SOC) and nitrogen (TN) concentration

The SOC and TN concentrations were significantly influenced either by N treatments or by season in each depth but no interaction occurred between season and N treatments (Table 4.1). The unfertilized control had lower SOC and TN concentrations than the fertilized treatments (Table 4.1), and this difference was evident at 0-10 cm depth for SOC and at 0-5 cm depth for TN. Differences in the SOC concentration among N rates were the highest for 0-5 cm depth and decreased with depth. Fertilization with N did not change the C/N ratio for 0-5 cm depth because the significant changes in both C and N balanced each other, but at 5-10 cm the C/N ratio increased with N fertilization. The C/N ratio was higher in fall than in spring, in general, because TN was lower in the fall, although SOC was also higher in the fall at 5-15 cm depth.

4.4.3 N fertilization effects on soil structure

Soil ρ_b did not differ significantly among N treatments at any depth, although at 0-5 cm ρ_b tended to be lowest in the 67 kg N ha⁻¹ treatment and highest in the 202 kg N ha⁻¹ treatment (Tables 2 and 3). In the surface 5 cm, ρ_b decreased significantly between spring and fall. However, at 5-15 cm depth, the variations in ρ_b among N treatments or between seasons did not show consistent patterns. As fertilizer rate increased, ρ_b tended to increase in spring and decrease in fall at 5-10 cm depth. But at 10-15 cm depth, the 67 kg N ha⁻¹ treatment had the highest in spring and the lowest ρ_b in fall.

Volumetric water content (θ) at various suctions was significantly influenced by season and the interaction (season x N) for 0-5 cm depth (Table 4.2). At 0-5 cm depth, soil core samples collected in spring generally retained higher amounts of water than those collected in fall (Fig. 4.4). Soil receiving 67 kg N ha⁻¹ contained a higher volume of water than soil receiving 202 kg N ha⁻¹ in spring. However, θ was significantly higher in the 202 kg N ha⁻¹ treatment than in the 67 kg N ha⁻¹ treatment in fall. Because of these opposite responses between the 67 and 202 kg N ha⁻¹ treatments, the interaction of season and N treatment was significant for all suctions except 100 kPa at 0-5 cm depth. At 5-10 cm depth, N treatments affected θ from 1 to 33 kPa, with significantly higher amount of water retained by the soil receiving 67 kg N ha⁻¹ than that receiving 202 kg N ha⁻¹. In addition, seasonal differences were observed at 33 and 300 kPa. Although spring samples retained more water than fall samples at 33 kPa, the reverse was true for 300 kPa. At 10-15 cm depth, seasonal differences were observed at 0.25, 1, 3, 300, and 1500 kPa (θ in fall > θ in spring at the same suction), and differences in θ among N treatments were observed at 100, 300, and 1500 kPa (θ in the 202 kg N ha⁻¹ treatment > θ in the 67 kg N ha⁻¹ treatment). However, these observed differences in θ at different suctions among N treatments did not influence the AWC (Tables 2 and 4). The measured AWC was significantly higher in spring than in fall at all depths.

The N treatments significantly affected the size distribution of aggregates after wet-sieving at 0-10 cm depth, and a seasonal difference was observed at 0-15 cm depth in different size fractions (Table 4.5). The proportion of macroaggregates under the control treatment and the 67 kg N ha⁻¹ treatment was significantly higher than that for the 202 kg N ha⁻¹ treatment for 0-10 cm depth (Fig. 4.5). The microaggregate size fraction at 5-10

cm depth was the highest in the 202 kg N ha⁻¹ treatment. At 5-15 cm depth, fall samples had more macroaggregates and fewer microaggregates than spring samples (Fig. 4.5). The MWDpost among N treatments was not statistically different at 0-5 cm depth due to the initial differences in aggregate size distribution, but the MWD ratio declined with increasing N fertilization rates, and was significantly lower in the 202 kg N ha⁻¹ treatment (Table 4.6). The MWD ratio was significantly higher in fall than in spring for all depths.

Because the strength of wet-sieved aggregates in the same size class did not appear to be equal across sampling dates, treatments or depths, TS measurements were made to further assess the degree of stability of WSA. At 0-5 cm depth, the interaction between seasons and N treatments was significant (Table 4.7) for the largest WSAs (4.75-8 mm); the TS decreased with increasing N application in spring, but the TS was the lowest in the 67 kg N ha⁻¹ treatment in fall. In smaller-sized WSAs (2-4.75 mm), TS did not differ among N treatments. In contrast to the evaluation of aggregate stability as indicated by the MWD ratio, the TS of the two largest WSA size classes was higher in spring than in fall. The TS of the 4.75-8 mm size of WSAs was correlated with RWD (r = (0.56) and WSA (r = 0.46) at 0-5 cm depth, but it was also negatively correlated with the percentage of silt (r = -0.74) (Appendix B1). The TS of the 2-4.75 mm size of WSAs was negatively correlated to the sand percentage in all depths (r = -0.65, -0.60, and -0.64 at 0-5, 5-10, and 10-15 cm depth, respectively). At 10-15 cm depth, TS in both size classes were positively correlated with the proportion of microaggregates (0.053-0.25 mm) after wet-sieving (r = 0.54 and 0.63 for 4.75-8 and 2-475 mm size of WSAs respectively).

At 0-5 cm depth, RWD and RLD were correlated with the measurements of soil structure, including MWD ratio, macroaggregate, and microaggregate fractions. Similar

correlations between RWD and several structural parameters were also observed for 5-10 cm but not for 10-15 cm depth. At 10-15 cm depth, the SOC concentration was correlated with the MWD ratio (r = 0.53) and macroaggregate fraction (r = 0.55). When the relationship between MWD ratio and root measurements (RWD and RLD) was examined by pooling all the data points, a strong non-linear relationship was observed (Fig. 4.6). When the RWD and RLD were less than 7 mg cm⁻³ and 8 cm cm⁻³ respectively, MWD ratio increased almost linearly as the RWD and RLD increased. After that, MWD ratio approached a maximum value and was far less responsive to further increases in RWD or RLD.

4.5 Discussion

The hypothesis that root biomass and length would decrease with increasing N application was supported by reductions in root biomass in spring and root length in fall. The highest N application rate decreased the standing root biomass of switchgrass for the 0-5 cm depth in spring (Fig. 4.1(a)). However, it was not clear whether less root biomass in the high N treatment was caused by less root production or by an enhanced turnover of roots. Several studies have reported reductions in root biomass due to increasing N input in prairies, grasslands, and forests (Rice et al., 1998; Bardgett et al., 1999; Magill et al., 2004; Högberg, 2007). Elimination of nutrient deficiencies by fertilization can increase the allocation of C to aboveground biomass. This optimal partitioning theory has been supported by several experiments on the adjustment of biomass allocation after manipulation of limiting resources (Thornley, 1972).

Although N application was associated with a significant decrease in root biomass only in the spring for 0-5 cm depth in the present study, the decrease in root length was very evident for the high N application in fall (Fig. 4.2). The change in RLD with N fertilization observed in this study could be related to differential responses of fine and coarse roots to N fertilization. Also working with grassland species, Craine et al. (2002) reported that fine root biomass was negatively correlated with the amount of available N but found no relationship between coarse belowground biomass and N. Studies conducted in forests showed that the productions of leaves, wood, and small roots were enhanced, but that of fine roots decreased with N fertilization (Oren et al., 2001; Iivonen et al., 2006). The apparent inconsistency between observed fall root biomass and root length responses to N fertilization could be accounted for by small changes in root size distribution. Since more than 90% of root biomass was comprised of fine roots, a shift to slightly coarser fine roots could reduce root length without affecting root biomass.

Another possible explanation for low root biomass at high N fertilization rates is an increase in root turnover rate. Nutrient availability can affect both root production and its turnover or dynamics. Standing root biomass stocks could be reduced if root turnover was faster under higher nutrient availability, despite high production of root biomass (Nadelhoffer, 2000). Several studies of C and N budgets conducted in forests have shown that increased fine root turnover coincides with increases in N availability and that a relatively constant fraction of net primary production (NPP) is allocated to roots (Raich and Nadelhoffer, 1989; Burton et al., 2000). Thus, lower root biomass in the 202 kg N ha⁻¹ treatment may also be attributed to faster turnover of roots, despite more production of photosynthate. However, the slightly greater length of coarse roots (> 2 mm) and larger average root diameter (Fig. 4.2, Fig. 4.3) might imply slower root turnover under highly fertilized than in unfertilized or in plots receiving less fertilizer. Thus, the effect of the 202 kg N ha⁻¹ treatment on root morphology could weaken the argument for low root biomass due to high turnover rates. Nevertheless, root biomass, length, and diameter measured at one or two points in time provide only a snap-shot of root growth. More coarse roots might be present in the highly fertilized treatment during the fall sampling because fine roots have already turned over due to altered root phenology leading to earlier senescence of the finest roots under the highly fertilized treatment.

A field study investigating the effects of N application (0, 112, and 224 kg N ha⁻¹) on switchgrass roots (Ma et al., 2000) found no effect on RWD. However, there were several differences between the experiment conducted by Ma et al. (2000) and the present study. While the present study intensively focused on the surface 15 cm of soil at 5-cm depth intervals for two seasons, Ma et al. (2000) reported root biomass for 15-cm intervals up to 30 cm depth, and for 30-cm intervals up to 120-cm depth at the end of growing season. Ma et al. (2000) showed that less root biomass was distributed in the upper soil profile with a higher level of N application than in the control, but this was counterbalanced by more root biomass observed in the sub-soil. Nevertheless, the present study and Ma et al. (2002) were comparable in that N fertilization rate had no significant effect on RWD at the end of the growing season. In another study, Heggenstaller et al. (2009) showed a quadratic response of switchgrass root biomass to an increase in N application rates; the highest root biomass was achieved at 140 kg N ha-1, but root biomass decreased at 220 kg N ha⁻¹. The present study found a similar response of switchgrass root growth to that of Heggenstaller et al. (2009) during the spring sampling,

although the effect of N application rates similar to 140 kg N ha⁻¹ could not be compared. Measurements of root length and root biomass for one year in the present study do not completely characterize the influence of high N application rates on root growth and dynamics. Thus, further studies are needed to determine how root production, turnover rates, biomass allocation, and root size distribution and other morphological characteristics are affected by high application rates of N fertilizer for a longer time period.

A higher SOC concentration was associated with higher rates of N fertilization (Table 4.1). Although the main source of C input to soil under switchgrass could be root derived-C, measured root biomass was not related to SOC concentration (r = 0.11, 0.002, and 0.08 at 0-5, 5-10, and 10-15 cm depth, respectively). The present study indicated higher root biomass but lower SOC concentration in 0 kg N ha⁻¹ and lower root biomass with higher SOC concentration in 202 kg N ha⁻¹, particularly for 0-5 cm depth. Differing root productivity and turnover with varying N availability might partially explain this discrepancy. Fast turnover of roots associated with highly fertilized soil might increase C inputs to soil but result in low standing root biomass. Moreover, litter inputs from aboveground or organic matter decomposition in response to increased N addition (Fog, 1998) might influence SOC concentration, but the potential for these effects were not investigated in this study.

Soil physical parameters are important determinants of soil quality and agricultural sustainability (Subbian et al., 2000). Soil ρ_b and AWC did not show any clear trend among N treatments. The volumetric water retention at low suction (< 100 kPa) was higher for the 67 kg N ha⁻¹ than in the 202 kg N ha⁻¹ treatment for 0-5 cm depth in spring

and for 5-10 cm depth in both seasons (Fig. 4.4). In contrast, soil in the 202 kg N ha⁻¹ treatment contained higher amount of water at low suction (< 33 kPa) than that in the 67 kg N ha⁻¹ treatment for 0-5 cm depth in fall. Water retention at lower suction (< 100 kPa) is determined more by soil structure (pore size distribution) than by texture (Brady and Weil, 1998). It is widely known that plant roots can influence soil structure via processes such as root penetration, localized drying due to water uptake, and aggregate formation and stabilization (Angers and Caron, 1998). Root growth and function are often both affected by and contribute to the development of soil pore structure, which influences air and water flow in soil. Greater amounts of fine roots may enhance aggregation and increase AWC. Moreover, root exudates can enhance the activities of soil biota in the rhizosphere and facilitate aggregation (Ehrenfeld et al., 2005). Such interactions between soil structure and biota might explain the higher water retention in accord with higher root biomass under the 67 kg N ha⁻¹ treatment in spring.

Roots can also have opposite effects on soil structure. Growing roots can deform soil structure, applying compressive and shear stresses of up to 2 MPa (Goss, 1991). Such radial forces due to root growth can decrease porosity around roots (Guidi et al., 1985, Bruand et al. 1996), and might be a cause of lower amounts of water retention under the 67 kg N ha⁻¹ treatment in fall. However, with data only for root biomass and length, it is difficult to draw valid conclusions for two opposite trends in SMCC between spring and fall during the same year. The most remarkable differences in SMCC between seasons occurred in the surface 5 cm. These findings suggested that abiotic factors, such as drying/wetting cycles, might contribute as much (or more) to soil structure formation (especially for macropores) than biotic factors in the soils at this site. Moreover, the small size of the cores (diameter 4.8 cm and height 5 cm) and small number of replicates (n = 4) might be insufficient to adequately separate spatial variability from true treatment effects.

Aggregates are basic units of soil structure, and porosity within and between these units determine air and water flow and influence plant growth and microbial activity. Well-fertilized agricultural soils are characterized by more WSA than poorly fertilized soils (Campbell et al., 1993). Increases in the return of crop residues to soil due to an increase in productivity with fertilization also increases SOC concentration (Alvarez, 2005) and improves aggregation. However, the present study revealed that application of 202 kg N ha⁻¹ decreased both root biomass and length as well as aggregate stability despite an increase in SOC concentration for 0-10 cm depth. Jastrow et al. (1998) showed that the contribution from C pools to aggregation was less than that of roots and fungal hyphae during the first decade of restoring native prairie plants on long-term agricultural soils. This study also showed that the increase in SOC did not play an important role in aggregate stability in the highly fertilized treatment.

The decline in aggregate stability for the 202 kg N ha⁻¹ treatment (Tables 4 and 5) can be explained by the decrease in RWD and RLD (Figs. 1 and 2). Further, the positive relationship between MWD ratio and root parameters (RWD and RLD) is clearly illustrated by Fig. 4.6. Roots form and stabilize aggregates either directly by enmeshing soil particles, or indirectly by stimulating microbial activity via the production of exudates, rhizodeposits, and root turnover (Jastrow et al., 1998; Gale et al., 2000). In general, the length of fine roots affected aggregate stability more than did the biomass of

fine roots. Thus, decreases in RWD in spring and the decline in RLD without decreases in RWD in fall could result in reduced aggregate stability at high N application rates.

Another plausible explanation for decreased aggregate stability with high N application may be an N-induced shift in the microbial community (Compton et al., 2004; Bradley et al., 2006; Chu et al., 2007). High N application rates can affect soil microbial populations causing shifts from fungal- to bacterial-dominated communities (Bradley et al, 2006). If the high rate of N fertilization was associated with a decrease in fungal populations (Bradley et al., 2006), it could contribute to reductions in aggregate stability, because fungal hyphae are important binding agents of aggregates (Tisdall and Oades, 1982; Degens et al., 1996). In grasslands, vesicular-arbuscular mycorrhizas (VAM) play a critical role in aggregation along with fine roots by enmeshing soil particles (Jastrow et al., 1998). If high N fertilization decreased the abundance of VAM or saprophytic fungi, it could degrade aggregate formation and stability. However, the potential for N additions to induce changes in the microbial community was not tested in the present study.

In the present study, aggregate stability was related more to a decrease in root biomass and length than to an increase in SOC concentration. Finally, the second hypothesis regarding aggregate stability and SOC concentration was partially supported by the finding of decreased MWD ratio and macroaggregate fraction with the highest N application. However, despite the decreased root biomass and length under higher N fertilization, the SOC concentration was higher than that of the control treatment which contradicts our second hypothesis.

4.6 Conclusions

This study showed that N application influenced root growth, SOC concentration, and consequently soil structural properties. The N application rate of 67 kg N ha⁻¹: (i) did not reduce root biomass or length and increased SOC concentration, and (ii) did not lessen soil physical quality as measured by aggregate stability compared to no fertilization. However, N application at the highest rate (202 kg N ha⁻¹) decreased root biomass and length and adversely affected soil structural properties. Although N fertilization of switchgrass increased SOC concentration on the soil surface layer, the negative impact on root growth was more crucial for controlling soil structural properties. Determining an optimal rate of N application for soil-specific conditions is essential to produce biomass sustainably, increase the SOC pool, and improve soil quality.

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		C (%)			N (%)		C/N				
Depth (cm)	0-5	5-10	10-15	0-5	5-10	10-15	0-5	5-10	10-15		
N rate (kg N	√ ha⁻¹)										
0	1.65 b (0.06)	0.88 b (0.04)	0.60 (0.02)	0.16 b (0.01)	0.10 (0.004)	0.07 (0.005)	10.28 (0.24)	8.97 b (0.36)	8.22 (0.46)		
67	1.95 _a (0.11)	0.91 b (0.04)	0.65 _{ns} (0.03)	0.18 _a (0.01)	0.10 _{ns} (0.004)	0.08 _{ns} (0.002)	10.53 _{ns} (0.12)	9.13 _{ab} (0.34)	8.58 _{ns} (0.38)		
202	1.97 a (0.08)	0.98 a (0.06)	0.63 (0.03)	0.19 _a (0.01)	0.10 (0.01)	0.07 (0.003)	10.48 (0.15)	9.39 _a (0.30)	8.63 (0.34)		
Season											
Spring	1.95 _A (0.09)	0.90 (0.03) _{ns}	0.60 _в (0.02)	0.19 _A (0.01)	0.11 _A (0.003)	0.08 A (0.003)	10.24 _в (0.15)	8.34 _в (0.11)	7.52 _в (0.16)		
Fall	1.75 в (0.06)	0.96 (0.04)	0.65 A (0.02)	0.16 в (0.01)	0.10 _в (0.003)	0.07 _В (0.002)	10.62 _А (0.11)	9.99 _A (0.11)	9.44 A (0.10)		
ANOVA											
Source d	f ———				p-value						
Block 3 N 2 Season 1 Season*	0.208 0.099 0.020	0.004 0.033 0.136	0.014 0.585 0.019	0.288 0.051 0.002	0.010 0.707 0.007	0.167 0.842 0.005	0.072 0.580 0.017	0.532 0.078 <.0001	0.315 0.235 <.0001		
N 2	0.344	0.946	0.220	0.515	0.852	0.366	0.043	0.852	0.277		

Table 4.1. Concentration of C and N and C/N ratio in soils under switchgrass after 4 years of three rates of N application Different lower case letters among N rates for each depth indicate significant differences at p < 0.10. Different capital letters between seasons for each depth indicate significant differences at p < 0.10. Values in parentheses are standard errors (n = 8 for N rates and n = 12 for seasons). ns: not significantly different.

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Company	16		AWC	Volumetric water content (θ) under each suction (kPa)									
Sources	ai	$ ho_b$	AWC	0.25	1	3	5	7.5	10	33	100	300	1500
0-5 cm													
Block	3	0.766	0.204	0.610	0.752	0.727	0.688	0.718	0.620	0.628	0.408	0.004	0.006
Ν	2	0.108	0.118	0.374	0.411	0.461	0.564	0.529	0.563	0.565	0.046	0.338	0.070
Season	1	0.027	<.0001	0.007	0.005	0.004	0.001	0.001	<.0001	<.0001	<.0001	0.075	0.004
Season*N	2	0.917	0.722	0.002	0.003	0.004	0.007	0.007	0.009	0.012	0.272	0.018	0.003
5-10 cm													
Block	3	0.038	0.183	0.583	0.613	0.526	0.543	0.500	0.460	0.372	0.353	0.085	0.024
Ν	2	0.229	0.127	0.386	0.059	0.033	0.037	0.026	0.034	0.048	0.884	0.395	0.810
Season	1	0.287	0.001	0.157	0.372	0.606	0.949	0.888	0.738	0.033	0.407	0.031	0.252
Season*N	2	0.069	0.721	0.823	0.969	0.982	0.983	0.913	0.967	0.933	0.803	0.956	0.488
10-15 cm													
Block	3	0.180	0.003	0.161	0.652	0.464	0.331	0.475	0.199	0.106	0.029	0.022	0.002
Ν	2	0.500	0.179	0.688	0.750	0.663	0.536	0.533	0.593	0.395	0.039	0.018	0.039
Season	1	0.106	0.002	0.001	0.011	0.042	0.245	0.110	0.250	0.186	0.665	0.008	0.082
Season*N	2	0.032	0.222	0.298	0.486	0.349	0.488	0.448	0.261	0.697	0.469	0.710	0.547

Table 4.2. The results of analysis of variance (p-values) for soil structural properties in each depth

 $\rho_{b}\!\!:$ bulk density; AWC: available water content

		Spring		Fall						
Depth (cm)		N rate (kg N ha ⁻¹)		N rate (kg N ha ⁻¹)						
(em)	0	67	202	0	67	202				
0-5	1.29 (0.01) ^{ab}	$1.22(0.07)^{bc}$	1.37 (0.04) ^a	$1.21(0.03)^{bc}$	1.15 (0.02) ^c	1.26 (0.04)	b			
5-10	1.48(0.01) bc	$1.49(0.01)^{abc}$	$1.51(0.02)^{abc}$	1.55 (0.02) ^a	1.53 (0.03) ^{ab}	1.47 (0.04)	c			
10-15	1.48 (0.03) ^b	$1.64(0.07)^{a}$	1.53 (0.01) ^b	1.52 (0.05) ^b	1.44 (0.05) ^b	1.52 (0.01)	b			

Table 4.3. Bulk density (Mg m⁻³) for 0-5, 5-10, and 10-15 cm depths under 0, 67, and 202 kg N ha⁻¹.

Values in parentheses are standard errors (n=4). Different letters for each depth indicate significant difference within a row (p < 0.10).

Depth]	N rate (kg N ha ⁻¹)	Season			
(cm)	0	67	202	Spring	Fall	
0-5	0.18 (0.02)	0.18 (0.02)	$0.20 (0.01)^{\rm ns}$	$0.22 (0.01)^{A}$	$0.15 (0.01)^{\rm B}$	
5-10	0.17 (0.01)	0.17 (0.01)	$0.15 (0.01)^{\rm ns}$	$0.19(0.01)^{A}$	$0.15 (0.01)^{\rm B}$	
10-15	0.14 (0.01)	0.14 (0.01)	$0.13 (0.01)^{ns}$	$0.15(0.01)^{A}$	$0.13(0.01)^{\rm B}$	

Table 4.4. Available water content (θ_{FC} - θ_{PWP}) for 0-5, 5-10, and 10-15 cm depths under 0, 67, and 202 kg N ha⁻¹. Mean differences among N treatments and between seasons were compared in each depth.

 θ_{FC} : Volumetric water content at field capacity (at 33 kPa); θ_{PWP} : Volumetric water content at permanent wilting point (at 1500 kPa). Different capital letters between seasons for each depth indicate significant difference (p < 0.10). Values in parentheses are standard errors (n = 8 for N rates and n = 12 for seasons). ns: not significantly different.

Depth (cm)	Source	df	Macroaggregate >.25 mm	Microaggregate .25053 mm	Silt+clay size fraction <.053 mm
0-5	Block	3	0.015	0.090	0.100
	Ν	2	0.014	0.130	0.529
	Season	1	0.355	0.075	0.279
	Season*N	2	0.267	0.351	0.337
5-10	Block	3	0.011	0.067	0.025
	Ν	2	0.015	0.048	0.213
	Season	1	0.095	0.186	0.686
	Season*N	2	0.800	0.804	0.576
10-15	Block	3	0.019	0.038	0.001
	Ν	2	0.473	0.679	0.227
	Season	1	0.013	0.001	0.307
	Season*N	2	0.791	0.645	0.380

 Table 4.5. The results of analysis of variance (p-values) for aggregate size distribution after wet –sieving in each depth

		MWD post						MWD ratio					
Depth (cm)		0-5		5-10		10-15		0-5		5-10		10-15	
N rate (kg N h	a ⁻¹)												
0		2.37 (0.21)		2.21 (0.17)	а	0.75 (0.09)	ab	0.88 (0.02)	а	0.60 (0.03)	а	0.21 (0.03)	а
67		2.62 (0.29)	ns	1.99 (0.13)	а	0.84 (0.06)	а	0.82 (0.04)	а	0.53 (0.02)	а	0.22 (0.02)	а
202		2.26 (0.21)		1.43 (0.25)	b	0.64 (0.10)	b	0.65 (0.07)	b	0.37 (0.07)	b	0.16 (0.03)	b
Season													
Spring		2.85 (0.17)	А	2.07 (0.19)	А	0.73 (0.06)	ns	0.72 (0.05)	В	0.46 (0.04)	В	0.16 (0.02)	В
Fall		1.99 (0.13)	В	1.69 (0.16)	В	0.76 (0.08)		0.85 (0.03)	A	0.55 (0.04)	А	0.23 (0.02)	А
ANOVA													
Source	df					I	o-valı	ie ———					
Block	3	0.008		0.085		0.038		0.008		0.019)	0.054	
Ν	2	0.165		0.046		0.067		0.023		0.022	2	0.028	
Season	1	0.000		0.060		0.741		0.002		0.027	7	0.020)
Season*N	2	0.234		0.479		0.958		0.215		0.600)	0.894	

Table 4.6. Mean weight diameter of aggregates after wet sieving (MWDpost) and the ratio of MWD after to before wet sieving among 0, 67, and 202 kg N ha⁻¹ and between two seasons

Different lower case letters among N rates for each depth indicate significant difference (p < 0.10). Different capital letters between seasons for each depth indicate significant difference (p < 0.10). Values in parentheses under mean are standard errors (n = 8 for N rates and n = 12 for seasons). ns: not significantly different.

		TS 4.75-8 mm					TS 2-4.75 mm				
Depth (cm)		0-5	5-10		10-15		0-5	5-10	10-15		
N rate (kg N	ha ⁻¹)										
0		39.46 (2.40)	25.45 (2.89)		21.51 (3.82)	b	71.21 (5.43)	47.35 (4.59)	40.96 (5.70)		
67		34.66 (3.78)	^{ns} 20.95 (2.89)	ns	18.35 (2.47)	с	60.53 (6.91) ^{ns}	44.21 (3.50) ⁿ	^s 40.34 (5.29) ^{ns}		
202		32.65 (3.73)	19.71 (3.42)		24.35 (2.55)	a	61.49 (7.37)	56.18 (7.10)	34.25 (4.96)		
Season											
Spring		35.75 (3.26)	ns 27.69 (2.25)	A	25.88 (1.38)	A	66.34 (4.88) _{ns}	55.87 (4.28) ^A	50.10 (2.84) ^A		
Fall		35.43 (2.29)	16.38 (1.52)	В	16.31 (2.28)	В	62.48 (5.96)	42.62 (3.72) ^E	^B 26.93 (2.31) ^B		
ANOVA											
Source	df				p	-val	ue <u></u>				
Block	3	0.021	0.136		0.435		0.003	0.173	0.201		
Ν	2	0.307	0.243		0.012		0.425	0.402	0.129		
Season	1	0.913	0.001		0.048		0.433	0.015	< 0.001		
Season*N	2	0.092	0.673		0.742		0.341	0.704	0.907		

Table 4.7. Tensile strength (kPa) of wet-sieved aggregates in the 4.75-8 mm and 2-4.75 mm size classes

Different lower case letters among N rates for each depth indicate significant differences at p < 0.10. Different capital letters between seasons for each depth indicate significant differences at p < 0.10. Values in parentheses are standard errors (n = 8 for N rates and n = 12 for seasons). ns: not significantly different.



Fig.4. 1 Root weight density (RWD, mg cm⁻³) for (a) 0-5, (b) 5-10, and (c) 10-15 cm depths under 0, 67, and 202 kg N ha-1 in spring and fall. Bars represent standard errors (n=4). Different letters for 0-5 cm depth indicate significant difference (p < 0.10) by mean comparison. ns: not significantly different



Fig. 4.2 (a) Total root length density (RLD, cm cm⁻³), (b) fine root length density, and (c) coarse root density for 0-5, 5-10, and 10-15 cm depths under 0, 67, and 202 kg N ha⁻¹ in fall. Bars represent standard errors (n=4). For statistical analysis, coarse root (2-5 mm) length density data for the 5-10 cm depth was log transformed. Different letters for each depth indicate significant difference (p < 0.10) among N treatments. ns: not significantly different.



Fig. 4.3 (a) Specific root length (SRL, cm g^{-1}) and (b) average root diameter for 0-5, 5-10, and 10-15 cm depths under 0, 67, and 202 kg N ha⁻¹ in fall. Bars represent standard errors (n=4). Different letters for each depth indicate significant difference (p < 0.10) among N treatments. For statistical analysis, SRL for 5-10 cm depth was log-transformed. NS: not significantly different.



Fig. 4.4. Soil moisture characteristic curves for different depths (a) 0-5, (b) 5-10, and (c) 10-15 cm depth under 0, 67, and 202 kg N ha⁻¹. Bars represent LSD values (p = 0.10) at each suction pressure.



Fig. 4.5. Size distribution of aggregates (a) among N rates and (b) between seasons after wet-sieving for different depths. Bars represent standard errors. Different letters for each size fraction indicate significant difference (p < 0.10). MacroAgg: macroaggregate size fraction (> 0.25 mm); MicroAgg: microaggregate size fraction (0.25 - 0.053 mm); Silt+Clay: silt and clay size fraction (< 0.053 mm); ns: not significantly different



Fig. 4.6. Relationship (a) between MWD ratio and root weight density and (b) between MWD ratio and root length density. All data was pooled for the non-linear curve fit.

CHAPTER 5

ASSESSMENT OF SOIL STRUCTURAL PROPERTIES UNDER FOUR DIFFERENT CULTIVARS OF SWITCHGRASS (*Panicum virgatum*)

5.1 Abstract

Switchgrass (*Panicum virgatum*) is a promising bioenergy crop which has the potential to reduce anthropogenic carbon dioxide (CO₂) emissions as well as to improve soil quality through soil organic carbon (SOC) sequestration. Varieties of switchgrass may have different rooting characteristics and C inputs which consequently affect soil structural properties. Therefore, the objective of this study was to examine aggregate properties under four cultivars of switchgrass: Alamo, GA992, GA993, and SL-93-3. Soil was obtained at 0-5, 5-10, and 10-15 cm depth in April, July, and October 2007 during the 4th year of switchgrass growth in Milan, Tennessee. Samples were analyzed for root weight density (RWD), carbon (C) and nitrogen (N) concentration in root samples, moisture content (MC), bulk density (ρ_b), aggregate stability through wet-sieving, C concentration in dry sieved aggregates (C-dry) and that in wet sieved aggregates (C-wet) from the 4.75-8 mm size fraction of aggregates, and total porosity (f_t) and pore size distribution of aggregates (4.75-8 mm size fraction). The RWD decreased with increasing soil depth but did not vary among cultivars. Root C and N concentrations and C/N ratio

in October were not significantly different among cultivars, and the average values were 41% C, 0.48% N, and 88:1 for the C/N ratio. The measured aggregate properties did not differ among cultivars but seasonal differences were observed. The ratio of mean weight diameter (MWD), i.e., a MWD after wet-sieving to that before wet-sieving, and tensile strength (TS) of wet-sieved aggregates (4.75-8 mm fraction) were significantly higher in July than in April or October for 0-5 cm depth. The C-dry was not different across cultivars or months, but the C-wet in April was higher than that in other two months for 0-5 cm depth. Generally, the C-wet was higher than the C-dry. The f_t and pore size distribution also did not differ among cultivars or among months. The ratios of MWD were positively correlated to TS measurements for 0-5 and 5-10 cm depth. Those TS measurements were negatively correlated to MC. These results suggested that the seasonal differences in aggregate properties could be greater than treatment effects when the antecedent moisture content varied greatly.

Key words: switchgrass, cultivar, soil structure, aggregate properties, seasonal variances

5.2 Introduction

The concentration of carbon dioxide (CO₂) in the atmosphere has increased rapidly due to anthropogenic activities. Switchgrass (*Panicum virgatum* L., Poaceae) has been proposed as an alternative energy crop that can potentially reduce CO₂ emission from fossil fuels combustion by recycling photosynthates (McLaughlin et al., 2002). Furthermore, the deep and extensive rooting characteristics of switchgrass may sequester soil organic carbon (SOC) while simultaneously improving soil quality (McLaughlin and Walsh, 1998; Tolbert et al., 2002; Frank et al., 2004; Cowie et al., 2006).

Soil structure refers to an arrangement of mineral particles and voids. It impacts plant growth by controlling the transport of air and water (Brussaard, 1997). Additionally, it influences decomposition of SOC and nutrient cycling (Hassink et al., 1997; Strong et al., 2004). Thus, good soil structure is a critical factor in determining soil functions. Soil aggregates are the basic soil structural units, and aggregate stability is an important indicator of soil structure and tilth (Amézketa, 1999; Bronick and Lal, 2005). Dexter (1988) suggested that the instability of the lower hierarchical structural order simultaneously destroyed the stability of the upper hierarchical orders. Therefore, studying aggregate properties which represent a lower hierarchy could reflect the properties of the upper hierarchical order of soil structure and its response to management.

Plant-soil interactions play an essential role in aggregate formation and stabilization (Oades, 1993; Angers and Caron, 1998; Ehrenfel et al., 2005). Plant roots and fungal hyphae, as temporary binding agents, can directly enmesh soil mineral particles and microaggregates into macroaggregates (Tisdall and Oades, 1982). Root exudates stimulate microbial activities in rhizospheres, thereby producing microbial mucilage which acts as a binding agent for mineral particles. Moreover, SOC, an important binding agent in aggregation, is largely plant-derived C. Several studies have shown significant changes in soil structure and SOC dynamics under different plant species and agricultural management practices (Burke et al., 1998; Rahimi et al., 2000; Cheng et al., 2003; Blanco-Canqui and Lal, 2004; Márquez et al., 2004; Dijkstra et al., 2006). Although Scott (1988) did not observe differences in aggregate stability among grass species, aggregate size distribution varied among tree species. In addition, quality
of plant residue can alter the SOC dynamics associated with aggregation, and white clover (*Trifolium repens*) residue (low C:N ratio) led to a faster turnover of macroaggregates and contained a higher proportion of new C in macroaggregates than those under perennial ryegrass (*Lolium perenne*) under elevated CO₂ conditions (Six et al., 2001). Several other changes in physical properties beyond aggregation have also been reported under different plant species and management systems. Rachman et al. (2004) reported that higher total porosity and a larger fraction of macropores occurred in soil under grass hedges than that under row crops. Accordingly, different traits among species can directly and indirectly induce changes in soil structure.

Extensive breeding and other genetic approaches have been used to improve biomass production and feedstock quality of switchgrass for cellulosic ethanol (McLaughlin and Kszos, 2005; Parrish and Fike, 2005). These developed cultivars could have different traits including root characteristics. Jiang et al. (2000) showed the differences in N use efficiency and nitrate uptake rate among Kentucky bluegrass (*Poa pratensis*) cultivars, and these differences were attributed to belowground characteristics (fibrous roots and rhizomes) and leaf sheaths. Root morphological characteristics (root length, branching or number of seminal roots) were different among four wheatgrass cultivars during growing periods (Aguirre and Johnson, 1991). A soybean (*Glycine max*) cultivar which was susceptible to soybean cyst nematode (SCN) had higher root length density and root surface area than in a resistant cultivar (Murillo-Willianms et al., 2010). The yield for the SCN-susceptible cultivar was less than from the resistant one. When the switchgrass cultivars have different root traits (biomass, length, and architecture) like these previous examples or residue quality, it can differently impact soil structure and SOC sequestration potentials.

Little published documentation, however, is available regarding the synthetic varieties which may have different root characteristics and thus C inputs to soil and soil structural properties. Even there are a few field-based studies on aggregate properties under switchgrass (Blanco-Canqui et al., 2005; Bharati et al., 2002; Rachman et al., 2004). Yet, information about these properties is essential to identify techniques for sustainable management of biomass plantations. Therefore, this study was conducted to examine aggregate properties under four different switchgrass cultivars during a growing season. It is hypothesized that cultivars with different rooting characteristics induce soil structural changes and affect SOC concentrations, and that cultivars which produce higher root biomass generally result in higher SOC concentration and higher aggregate stability.

5.3 Materials and Methods

5.3.1 Study site and soil sampling

The experiment was conducted at the University of Tennessee's Research and Education Center in Milan, TN (35°56′ N, 88°43′ W). The soil series is a Grenada silt loam (fine-silty, mixed, active, thermic, Oxyaquic Fraglossudalfs). The experiment was a randomized complete block design with three replicates and four lowland cultivars: Alamo, GA992 (origin: Kanlow, Georgia), GA993 (origin: Alamo, Georgia), and SL-93-3 (origin: Alamo, Oklahoma). In spring 2004, four switchgrass cultivars were planted in each plot (8 m x 5 m). Ammonium nitrate (NH₄NO₃) was applied at 67 kg N ha⁻¹ annually in the spring from the second growing season in 2005. The experimental design

and field layout are described by Garten Jr. et al. (2010).

Soil samples were obtained in April, July, and October 2007 from four randomly placed 0.1-m² circular quadrats per plot after removing aboveground biomass and surface litter. Within each quadrat, one core (4.8 cm diameter) was randomly sampled to a 15 cm depth and cut into 0-5, 5-10, and 10-15 cm depth increments. Four cores in each plot were pooled by depth increments but were carefully handled not to impair soil structure.

5.3.2 Root, soil, and aggregate analyses

Field moist soil samples were broken into aggregates along planes of weakness by gentle hand manipulation. During aggregates preparation, roots between aggregates were manually removed, washed, dried at 45°C for 48 hours, and then weighed. Rhizomes were separated from roots and were not included in estimating the root biomass. The root data in this study did not include root pieces occluded within aggregates that passed through an 8-mm sieve but the roots between aggregates were included. Root biomass was computed as root weight density (RWD), defined as root weight per unit of soil weight. For C and N analysis in root tissues, root samples in October were ground with a Wiley Mini Mill (Thomas Scientific, USA) after biomass measurements, and were passed through a 0.64 mm-sieve. The C and N in root tissues were measured by dry combustion (900°C) with a CN analyzer (Vario Max Elementar Americas, Inc., Germany).

Soil moisture content (MC) was calculated after drying soil at 105°C. Soil bulk density (ρ_b) was calculated as moisture corrected soil weight divided by the core volume (Topp and Ferré, 2002).

Dry aggregates size distribution was determined from 50 g of air-dried soil (< 8 mm) with a nest of sieves (4.75, 2, 1, 0.5, 0.25, and 0.053 mm) by manual dry-sieving. The mean weight diameter before wet-sieving (MWDpre) (Eq. [1]) was calculated with dry sieved aggregates (> 0.25 mm) (Nimmo and Perkins, 2002)

$$\mathbf{MWD} = \sum m_i \times x_i \qquad \qquad \mathbf{Eq. [1]}$$

where m_i = mass of the aggregates within each measured size range (> 0.25 mm size) as a fraction of the total dry mass of the analyzed sample; x_i = the corresponding mean diameter of each aggregate size range.

Dry sieved aggregates were combined together again and used for wet-sieving. Prior to the wet-sieving, the soil (50 g) samples were submerged for 30 minutes on nested sieves (4.75, 2, 1, 0.5, and 0.25 mm). The nest of sieves was shaken under water at 25 oscillations min⁻¹ for 30 minutes in a Yoder (1936) apparatus. Soils passed through a 0.25 mm sieve during the wet-sieving process were transferred to a 0.053 mm sieve to separate into microaggregates (0.25-0.053 mm) and silt and clay size fractions (< 0.053 mm). A jet of water stream was used to remove the silt and clay size fractions from the soils on the 0.053 mm-sieve until the water passing through the sieve became clear. Wet-sieved aggregates (4.75-8, 2-4.75, and 1-2 mm) were first air-dried on the sieve to avoid deformation, dried at 45°C for 24 hours, and weighed. The remainder of aggregates (> 0.25 mm) were used for calculation of MWD after wet-sieving (MWDpost) (Eq. [1]). Since the size distribution of dry aggregates in each season was different, a MWD ratio was calculated to standardize these initial differences. The MWD ratio was calculated as (Eq.

MWD ratio = MWDpost/MWDpre Eq. [2]

where MWDpost = mean weight diameter of water stable aggregates (WSA) after wetsieving; MWDpre = mean weight diameter of aggregates obtained by dry-sieving (before wet-sieving).

After drying the wet-sieved aggregates (4.75-8, 2-4.75, and 1-2 mm) at 45°C, tensile strength (TS) was measured as another property of water stable aggregates. A simple crushing apparatus designed by Horn and Dexter (1989) was used to measure TS whereby force required for aggregate deformation was recorded (Dexter and Watts, 2001). Prior to the measurement of TS, the diameter of 4.75-8 mm size of wet-sieved aggregates was recorded by using a caliper. The TS from five aggregates per plot was examined as pseudo replicates. The TS of aggregates was calculated by Eq. [3], [4], [5], and [6]:

$$TS = 0.576 \ (F/d_{agg}^{2})$$
 Eq.[3]

where F is the vertical breaking force, and d_{agg} is the mean aggregate diameter.

$$d_{agg}$$
 of aggregates in 4.75-8 mm size class = $(d_1 + d_2 + d_3)/3$ Eq.[4]

where d_1 is the longest, d_2 is the mediate, and d_3 is the smallest diameter of each aggregate,

$$d_{agg}$$
 of aggregates in 2-4.75 mm size class = $(2+4.75)/2$ Eq.[5]

$$d_{agg}$$
 of aggregates in 1-2 mm size class = $(1+2)/2$ Eq.[6]

Total C (TC) concentration in aggregates (4.75-8 mm size fraction) after dry- and wet-sieving was determined by dry combustion (900°C) using a CN analyzer (Vario Max Elementar Americas, Inc., Germany) (Nelson and Sommers, 1996). Aggregates were

ground with a mortar and pestle and passed through a 0.25-mm sieve. Since there were no carbonates in the soil, the TC concentration was considered to be equal to the SOC concentration.

Total porosity (f_t) and pore size distribution in 4.75-8 mm aggregates were determined from the soil moisture retention characteristics (SMCC). The gravimetric water content (ω) of aggregates was measured at saturation, 6 and 600 kPa. The moisture retention of aggregates at saturation and 6 kPa was measured on a tension table equipped with a capillary outflow tube (Dane and Hopmans, 2002). A pressure plate apparatus was used for measuring moisture retention at 600 kPa. When the outflow of water stopped, the ω in each aggregate was measured. Five aggregates per plot were used as pseudo replicates. The saturated MC was used to calculate f_t , and it was assumed that particle density (ρ_s) is 2.7 g cm⁻³, and the density of water (ρ_w) is 1.0 g cm⁻³ (Eq. [7]).

where f_t is total porosity; ω_s is gravimetric water content at saturation (no air filled porosity); ρ_s is a particle density (2.7 g cm⁻³); ρ_w is density of water (1.0 g cm⁻³). The moisture retentions at 6 and 600 kPa suctions represent the water filled pores of less than 50 and 0.5 µm in equivalent cylindrical diameter (ECD), respectively. The ω was converted to volumetric water content (θ) by using aggregate density (ρ_{agg}) (Eq. [8] and Eq. [9]).

where ρ_{agg} is an aggregate density; f_t is total porosity; ρ_s is a particle density (2.7 g cm⁻³).

$$\theta_{i} = \omega_{i} \rho_{agg} / \rho_{w}$$
 Eq. [9]

where θ_i is the volumetric water content at suction *i*; ω_i is the gravimetric water content at

suction *i*; ρ_{agg} is an aggregate density calculated from Eq. [8]; ρ_w is density of water (1.0 g cm⁻³).

Pore classification was done according to the scheme proposed by Greenland (1977): transmission pores (> 50 µm), storage pores (0.5-50 µm)), and residual and bonding pores (< 0.5 µm). The porosity of transmission pores (f_{trans}) was calculated as the difference between f_t and θ_{6kPa} , that of storage pores ($f_{storage}$) as the difference between θ_{6kPa} and θ_{600} kPa, and θ_{600kPa} was considered as the porosity of residual and bonding pores ($f_{residual}$).

5.3.3 Statistical analyses

The normality of data and constant variance of errors were checked prior to conducting statistical analysis. The data of MWDpost and silt and clay size fractions were not normally distributed, and the error assumption was violated. Thus, the analysis of variance (ANOVA) was performed after log-transformation for these data.

One-way ANOVA model (JMP 7.0) was used to test differences among C, N, and C/N ratio in root tissues. A repeated measures (month) randomized complete block (RCB) design ANOVA (factor = cultivar and month) (Eq. [10]) was used to test the treatments effects on MC, ρ_b , RWD, MWDpre, MWDpost, MWDratio, macroaggregates, microaggregates, silt and clay size fractions, C in dry-sieved aggregates, and C in wet-sieved aggregates by each depth. A covariance structure chosen by AIC criteria was used in the model, and the model used for this study is shown in Eq. [10]:

$$Y_{ijk} = \mu + \beta_i + \tau_j + \gamma_k + (\tau\gamma)_{jk} + \varepsilon_{ijk}$$
 Eq. [10]

where Y_{ijk} is the observed values for the *i*th block assigned to the *j*th cultivar in month *k*; μ is the overall mean; β_i is the *i*th block effect (random effect); τ_i is the *j*th cultivar main effect; γ_k is the *k*th month main effect; $(\tau \gamma)_{jk}$ is the interaction effect between cultivars and months; and ε_{ijk} represents error terms.

The PROC MIXED in SAS release 9.2 (SAS Institute, 2002) was used for the repeated measures RCB design ANOVA. When significant differences were observed (p < 0.05) in the ANOVA, post-hoc mean comparisons were conducted by using the LSD method (p < 0.05).

The TS data showed a highly skewed distribution and included drastically outlying observations. The data distribution of f_t , f_{trans} , $f_{storage}$, and $f_{residual}$ were not skewed but double peaked. Transformation of data did not help these data fit to the ANOVA assumptions. Therefore, Kruskal-Wallis (rank sum) nonparametric ANOVA was used to test the effects of cultivars and months for these data sets (JMP 7.0). When significant differences were observed (p < 0.05), post-hoc mean comparisons were conducted by a Mann-Whitney Test (p < 0.05) (JMP 7.0).

The parameters measured in all three months were pooled together by depth, and principal component analysis (PCA) was conducted by using a correlation matrix (JMP 7.0). Five principal components which explained more than 80% of total variance were selected based on a scree plot and eigenvalues (> 1) to estimate the relationships among measured aggregate properties.

Since RWD were not measured in July, the Pearson product-moment correlation coefficients were calculated by using data sets in April and October to determine relationships between aggregate properties and RWD (JMP 7.0).

5.4 Results

The MC decreased, and the ρ_b increased with increase in depth across all cultivars and for both months (Table 5.1). The MC for 0-5 cm depth in Alamo was significantly lower than that under SL-93-2 (Table 5.1). There were no differences in ρ_b among cultivars for any of the three depths. The MC in July was significantly lower than other months across all three depths, and ρ_b in April for 5-10 cm depth was significantly lower then other months.

The RWD decreased with increase in soil depth (Table 5.1). The RWD for 0-5 cm depth (15.5 mg g⁻¹) was 2-3 times higher than that for the 5-10 cm layer (6.0 mg g⁻¹). The RWD in April tended to be higher than that in October, but the significant difference occurred only for the 10-15 cm depth. The mean RWD of GA-992 was the highest and that of SL-93-2 the lowest for 0-5 cm depth, but this difference was not statistically significant probably because of a high variability.

Concentrations of C and N, and C/N ratios in roots measured in October were not different among cultivars (Table 5.2). On average, roots of switchgrass were comprised of 41% of C and 0.48% of N, and had the C/N ratio of 88:1.

The measured aggregates properties after dry- and wet-sieving did not differ among cultivars across any of the three depths (Table 5.3, 5.4, 5.5). However, seasonal differences were observed and were specifically evident in the surface 0-5 cm depth. The MWDpre was the highest in April, and was linked to the highest macroaggregate fractions, the lowest microaggregates and silt and clay size fractions for 0-5 and 5-10 cm depths after wet-sieving (Table 5.3). Although aggregate size distribution from drysieving showed the lowest MWDpre in July, most of aggregates remained on the sieves after wet-sieving and resulted in the highest MWDratio. For 10-15 cm depth, the MWDpre differed significantly among three months (April > October > July), but the MWDpost did not.

Similar to the aggregate size distribution, the TS measurements did not differ among cultivars either (Table 5.4). For seasonal differences, the TS of WSAs was generally the highest in July although MWDpost and macroaggregates fractions were higher in April than in July. Specifically, the TS in all size fractions of WSAs was the highest in July for 0-5 cm depth. For 5-10 cm depth, the TS in July was significantly higher than that in October for 1-2 and 2-4.75 mm size fractions of WSAs. For 10-15 cm depth, the TS of 4.75-8 mm size fraction of WSAs in July was significantly higher than those for the other two months. The TS generally decreased with increase in aggregate size and soil depth. The arithmetic means of TS in July varied substantially because of a high variability among aggregates, but the median values seemed to less variable among treatments (Table 5.4).

Concentration of C in dry-sieved aggregates (4.75-8 mm) did not differ across cultivars or among months (Table 5.5). Concentration of C in WSAs (4.75-8 mm) was significantly higher in April than in other two seasons for 0-5 cm depth. In most cases, the C concentration in aggregates was higher after than before wet-sieving (Table 5.5). Geneally, differences in C concentration between wet-sieved and dry-sieved aggregates tended to increase with increase in soil depth.

The f_t and distribution of f_{trans} , $f_{storage}$, and $f_{residual}$ did not differ among cultivars and among months (Table 5.6). Although there were no significant differences among months, the mean values of f_t and f_{trans} were the lowest in July across all three depths. The f_{trans} , $f_{storage}$, and $f_{residual}$ were comprised of 16%, 50%, and 34% of total pore space, respectively.

The PCA was conducted after pooling the available data for all three months. The PCA results showed that the first five principal components accounted for ca. 80% of total variance for each depth (Table 5.7). The first component was related to the measurements from sieving, such as MWDpre, MWDpost, macroaggregates, and microaggregates for 0-5 cm depth. The second component was related to the pore size distribution and the silt and clay size fraction. The loading plot for first two components showed that the TS from three size fractions of WSAs was distributed in an opposite direction to the MC in all three depths (Fig. 5.1). The ft was closer to the C concentration in wet-sieved aggregates but not in the dry-sieved aggregates for 0-5 and 5-10 cm depths. Across all three depths, the ftrans and ft were located in a similar position in the coordinates (Fig. 5.1).

Correlation coefficients showed that the RWD was positively correlated with the C concentration in wet-sieved but not with that in dry-sieved aggregates for 0-5 cm depth (Table 5.8A). While the RWD for 5-10 cm depth was not significantly correlated with measured variables (Table 5.8B), the RWD for 10-15 cm depth was positively correlated with MWDpre and macroaggregates (Table 5.8C). The MC was correlated to several parameters measured through dry- and wet-sieving, and the MWDpre was correlated to parameters measured after wet-sieving for 0-5 and 5-10 cm depths (Table 5.8A, B). For 10-15 cm depth, the MC did not show strong correlations with measurements through wet-sieving, but there were still negative correlations with TSs of bigger size of aggregates.

For 0-5 cm depth, MWDpre was positively correlated with the MWDpost and macroaggregates size fractions, and negatively with microaggregates and silt and clay size fractions. The MWDratio was not correlated with any of the parameters measured but with the MWDpost. Macroaggregates size fractions were negatively correlated to microaggregates and silt and clay size fractions since those values represent the relative ratios. Overall, aggregates size distributions were closely correlated among each other. The data on TS of 4.75-8 mm WSAs were negatively correlated with MWDpre and macroaggregates, but positively with the TS of 2-4.75 mm WSAs. Despite a smaller contribution of f_{trans} (16%) to f_{t} , the correlation coefficient between two parameters was rather high (r = 0.78). For 5-10 cm depth, MWD measurements were closely related to each other. In accordance with the negative relationship of C concentration in WSAs (4.75-8 mm). For 10-15 cm depth, C concentration in drysieved aggregates (4.75-8 mm) was correlated with measurements after the wet-sieving.

5.5 Discussion

In general, no significant differences were observed in measured properties of aggregate among the cultivar treatments. It was initially hypothesized that different cultivar traits (especially root characteristics) would induce changes in soil structural properties. However, RWD and C and N concentrations in roots were not different among cultivars. Garten Jr. et al. (2010) evaluated plant traits among 4 different switchgrass cultivars for the same study site. Garten Jr. and colleagues measured aboveground and belowground biomass and C and N concentrations in both tissues

among switchgrass cultivars during a growing season. However, no statistical differences were observed among the cultivars. It is not surprising, therefore, that aggregate properties did not differ among cultivars in the present study either. Furthermore, high spatial variability in soil may also have contributed to the lack of any significant differences.

The observation of seasonal differences in aggregate properties from three seasonal measurements showed the importance of the timing of soil sampling and of the sample pretreatments. Soil was friable and the driest in July among three seasonal samplings, and the MC was the lowest. Thus, it could be expected that soil dryness in July has influenced the results of aggregate stability in this study. The temporal changes in structural properties due to differences in the field MC could have bigger impacts than those caused by treatments (Kay, 1998; Amézketa, 1999; Díaz-Xorita et al., 2002). Slaking, as a pre-wetting treatment, has more drastic impacts on aggregate stability than a slow wetting method (capillary or vapor wetting) since the rapid water movement into the pore space inside an aggregate can cause high internal pressure from entrapped air (Kemper and Rosenau, 1986). The antecedent MC affects the rate of wetting and thus the size distribution of aggregates after wet-sieving can be affected by MC. Some studies to test aggregate stability in the field moist soils have shown that the MC was one of the most important determinants of aggregate stability (Perfect et al., 1990). The effect of antecedent MC at the time of sampling has been recognized since 1930s (Kolodny and Joffe, 1939). To minimize the confounding effect from different values of antecedent soil MC, aggregates are routinely air-dried to adjust MC to a constant level. Therefore, only the air-dried aggregates were used in this study also. However, higher MWD ratios and

TS of WSAs in July sampling indicate that soil MC at the time of sampling could still affect aggregate properties despite the standardization of MC through air-drying.

Field moist soils may be used for aggregate analysis to minimize the confounding effect from abiotic factors by air-drying aggregates and to account for the changes induced by biological factors. Evaporation of water brings mineral soil particles closer together, thereby, forming stronger bonds in aggregates (Attou and Bruand, 1998). Such stronger binding forces from abiotic factors may mask any effects of the weaker biological factors. Abiven et al. (2007) reported that polysaccharides content was associated with aggregate stability when a slow wetting method was used. Thus, probable changes in aggregate stability induced by plant exudates which are easily decomposed (Angers and Caron, 1998; Kuzyakov. and Domanski, 2000) may be detected by gentle pre-treatments in wet-sieving with field-moist soils.

The MWDpre was correlated with the MWDpost for 0-5 cm depth (r= 0.81, p <0.05) (Table 5.8A). The TS of WSAs was correlated with MWD ratios, but the other measurements of wet-sieving and dry-sieving were not correlated with the TS (Fig. 5.1). Since whole soils were used in the present study, the prior aggregate size distribution could affect the new aggregate size distribution after the wet-sieving. This trend also emphasizes the importance of the method of aggregate preparation. The breaking of aggregates manually may affect the aggregate size distribution prior to wet-sieving. A drop-shatter technique which breaks bulk soils by dropping from a certain height on to a hard surface can reduce the variability introduced in preparation of aggregates (Díaz-Zorita et al., 2002).

The TS increased with decrease in aggregates size, as was also reported by

Blanco-Canqui et al. (2005). The size of aggregate is one of the determinants of TS. Increase in aggregate size increases probabilities of including larger pores which are zone of weak strength (Dexter, 1988). Munkholm et al. (2002) reported that TS of aggregates could be used for predicting that of soil cores. Furthermore, TS of soil cores were negatively correlated with the macroporosity.

Despite the high variability in the TS, seasonal differences were observed even with the use of conservative statistical methods (non-parametric methods). The seasonal differences in TS of WSAs indicated that the MWDpost might not sufficiently represent aggregate stability by the wet-sieving method. However, MWD ratios were consistent with the data of the TS measurements, and could standardize any initial differences in aggregate size distribution.

The TS showed high variability among aggregates even after wet-sieving especially in the July sampling although 5 aggregates were used as experimental (measurement) or pseudo-replicates. Such a high variability could be attributed to distinct properties among individual aggregates. Further, high spatial variability of soil may be augmented because of differences between rhizospheric and bulk soils. The growth of roots can accentuate compressive and sheer stress decreasing porosity in close proximity of growing roots (Goss, 1991; Guidi et al., 1985, Bruand et al., 1996). Water uptake by plants results in localized drying and enmeshing of particles, and rhizodeposites can also enhance aggregation (Angers and Caron, 1998). All of these active processes of roots and microbes in the rhizosphere could develop distinctive properties as compared to bulk soils (Angers and Caron, 1998; Whalley et al., 2005; Gregory, 2006). Thus, a large number of aggregates must be analyzed to represent soil structural properties through the

methods of aggregates analyses.

Sainju (2006) compared SOC concentrations in aggregate size fractions using dry- and wet-sieving methods. The distribution of SOC concentration in aggregate fractions varied depending on the land use, cropping systems and soil properties. Under the Conservation Reserve Program (CRP), the SOC concentration was higher after wet-sieving than dry-sieving in the macroaggregates of both 4.75-2 and 2-0.25 mm size fractions. These trends imply that bigger sizes of aggregates preferentially protected SOC against washing by the wet-sieving procedure. The present study also show relatively higher C concentration in wet-sieved than in dry-sieved aggregates (4.75-8 mm). This observation is consistent across all cultivars, seasons, and depths. The disintegration of aggregates by wet-sieving might selectively remove weakly aggregated particles which probably consist of lower amount of organic binding materials. Therefore, the SOC concentration in aggregates could be higher after than before wet-sieving. This hypothesis may be corroborated by the fact that differences in SOC concentration between two sieving methods became more evident with increase in sampling depth.

The f_t and pore size distribution in aggregates differed neither among cultivars nor among seasons. Although there were no differences in pore size distribution among treatments, the high correlation between f_t and f_{trans} could represent the dynamic property of macropores and less variable property of micropores. The distribution of macropores could be easily affected by external factors such as land use changes, management practices, disturbances etc. (Kay, 1998). On the contrary, micropores are controlled more by soil texture than by structure (Brady and Weil, 1998).

The pore size distribution of intra-aggregates which is finer and more tortuous

(Horn et al., 1994) could make it harder to detect any probable changes in soil structure by pore size distribution among aggregates. No differences in pore size distribution of aggregates across soil depths could indicate that it might be difficult to observe treatments differences in intra-aggregates pore size distribution. Furthermore, there are several limitations of the aggregate analyses procedures. For example, each aggregate cannot show the characteristics of inter-aggregate pores (mostly bigger sizes of pores), pore continuity though the soil profile, etc. Accordingly, the intact core analysis or field measurements could complement aggregate analyses to adequately characterize soil structure.

5.6 Conclusion

No differences in aggregate properties were observed among four different switchgrass cultivars in this study. In contrast, seasonal differences due to moisture contents at the time of sampling strongly affected aggregate properties. When abiotic factors have great influences on soil structure, it might be difficult to determine the effects of treatments. Moreover, slight differences in aggregates among varieties might be difficult to detect from forceful pretreatments of aggregates. Finally, measurements in a bigger scale of soil structure would be able to complement the study from the smallest units in soil structure, aggregate.

5.7 References

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		MC Mean SE			ρ _b ($(g \text{ cm}^{-3})$		RWI	$O(mg g^{-1})$	
		Mean	SE		Mean	SE		Mean	SE	
Depth 0-5 cm										
Cultivar	Alamo	0.23	(0.03)	c	1.29	(0.02)		14.71	(3.27)	
	GA-992	0.25	(0.03)	ab	1.28	(0.03)	ns	18.52	(5.89)	ns
	GA-993	0.24	(0.03)	bc	1.34	(0.01)		16.54	(1.55)	
	SL-93-2	0.25	(0.03)	a	1.28	(0.02)		12.26	(1.94)	
Month	Apr	0.28	(0.01)	b	1.32	(0.02)		18.25	(2.77)	ns
	Jul	0.14	(0.004)	c	1.30	(0.01)	ns			
	Oct	0.30	(0.01)	a	1.28	(0.02)		12.76	(1.90)	
Depth 5-10 cm	1									
Cultivar	Alamo	0.20	(0.01)		1.54	(0.01)		7.47	(1.43)	
	GA-992	0.20	(0.01)	ns	1.55	(0.01)	ns	6.13	(1.27)	ns
	GA-993	0.20	(0.02)		1.55	(0.01)		5.53	(1.13)	
	SL-93-2	0.20	(0.01)		1.54	(0.02)		4.84	(0.51)	
Month	Apr	0.23	(0.002)	a	1.51	(0.01)	b	6.87	(0.92)	ns
	Jul	0.14	(0.002)	с	1.57	(0.01)	а			
	Oct	0.22	(0.002)	b	1.55	(0.01)	а	5.12	(0.60)	
Depth 10-15 c	m									
Cultivar	Alamo	0.20	(0.02)		1.56	(0.02)		4.01	(0.62)	
	GA-992	0.19	(0.01)	ns	1.55	(0.01)	ns	3.93	(0.82)	ns
	GA-993	0.20	(0.01)		1.54	(0.02)		3.75	(0.81)	
	SL-93-2	0.20	(0.01)		1.60	(0.02)		3.80	(0.77)	
Month	Apr	0.22	(0.01)	а	1.57	(0.01)		4.95	(0.46)	a
	Jul	0.15	(0.004)	b	1.57	(0.02)	ns			
	Oct	0.22	(0.01)	а	1.56	(0.02)		2.79	(0.32)	b

Table 5. 1 Soil moisture content (MC), bulk density (ρ_b), and root weight density (RWD) among cultivars and sampling months (April and October)

Different lowercase letters in Month by each depth were significantly different (α =0.05) through the LSD test; ns: not significant

	С ((%)		N	(%)		C/N	ratio	
	Mean	SE		Mean	SE		Mean	SE	
Alamo	41.29	(0.64)		0.48	(0.07)		88.80	(12.20)	
GA-992	41.88	(0.64)	ns	0.42	(0.003)	ns	98.83	(2.16)	ns
GA-993	41.26	(0.58)		0.49	(0.03)		84.99	(5.48)	
SL-93-2	39.75	(1.31)		0.52	(0.06)		78.50	(12.00)	

Table 5. 2 C and N concentrations and C/N ratio in roots sampled in October 2007

ns: no significance

		MWDpre (mm)	MWDpost [#] (mm)	MWDratio	Macroaggregate (%)	Microaggregate (%)	Silt and clay size [#] (%)
Depth 0-5	cm	i i					i
Cultivar	Alamo	3.97 (0.21)	3.27 (0.22)	0.82 (0.03)	83.48 (1.49)	9.79 (0.71)	6.73 (1.06)
	GA-992	3.97 (0.29)	3.30 (0.18) ^{ns}	0.85~(0.04) ^{ns}	83.68 (1.44) ^{ns}	10.12 (1.13) ^{ns}	6.20 (0.55) ^{ns}
	GA-993	3.90 (0.26) ^{ns}	3.22 (0.20)	0.84 (0.04)	84.75 (1.90)	9.69 (1.49)	5.57 (0.62)
	SL-93-2	3.92 (0.32)	3.43 (0.28)	0.88 (0.03)	85.30 (1.73)	9.61 (1.43)	5.09 (0.44)
Month	Apr	4.86 (0.10) ^a	3.92 (0.17) ^a	0.81 (0.03) ^b	89.24 (1.05) ^a	6.45 (0.88) ^b	4.31 (0.36) ^b
	July	3.23 (0.14) ^c	3.00 (0.13) ^b	$0.93 (0.01)^{a}$	81.73 (0.92) ^b	11.90 (0.82) ^a	$6.37 (0.50)^{a}$
	Oct	3.73 (0.09) ^b	2.99 (0.10) ^b	0.80 (0.03) ^b	81.93 (0.90) ^b	11.06 (0.54) ^a	7.01 (0.68) ^a
Depth 5 -1	0 cm						
Cultivar	Alamo	4.61 (0.24)	2.72 (0.21)	0.60 (0.05)	81.32 (1.57)	12.07 (1.20)	6.62 (0.54)
	GA-992	4.58 (0.22) ^{ns}	2.56 (0.17) ^{ns}	0.57 (0.04) ^{ns}	79.25 (1.13) ^{ns}	13.48 (1.38) ^{ns}	7.27 (0.57) ^{ns}
	GA-993	4.50 (0.22)	2.52 (0.18)	0.57 (0.04)	80.55 (1.51)	12.87 (1.14)	6.58 (0.53)
	SL-93-2	4.27 (0.29)	2.61 (0.22)	0.62 (0.03)	79.25 (2.52)	13.56 (1.80)	7.19 (0.93)
Month	Apr	5.28 (0.05) ^a	2.90 (0.19)	0.55 (0.04)	84.04 (1.38) ^a	9.63 (1.07) ^b	6.33 (0.66)
	July	3.80 (0.15) ^c	2.42 (0.14) ^{ns}	0.64 (0.03) ^{ns}	78.23 (1.34) ^b	14.17 (1.02) ^a	7.60 (0.55) ^{ns}
	Oct	4.39 (0.09) ^b	2.49 (0.13)	0.57 (0.03)	78.02 (1.06) ^b	15.18 (0.80) ^a	6.80 (0.40)
Depth 10-	15 cm						
Cultivar	Alamo	4.55 (0.19)	1.25 (0.18)	0.27 (0.04)	65.91 (1.85)	22.75 (2.17)	11.34 (1.36)
	GA-992	4.76 (0.23) ^{ns}	1.13 (0.10) ^{ns}	0.24 (0.02) ^{ns}	68.56 (1.52) ^{ns}	22.13 (1.79) ^{ns}	9.31 (0.75) ^{ns}
	GA-993	4.69 (0.20)	1.20 (0.14)	0.26 (0.03)	68.85 (2.40)	21.09 (1.67)	10.06 (1.02)
	SL-93-2	4.63 (0.22)	1.14 (0.14)	0.25 (0.03)	65.45 (2.42)	23.80 (1.52)	10.75 (1.15)
Month	Apr	5.23 (0.06) ^a	1.12 (0.10)	0.17 (0.01)	68.59 (1.61)	20.65 (1.51)	10.76 (0.98)
	July	4.01 (0.14) ^c	1.14 (0.13) ^{ns}	0.15 (0.02) ^{ns}	65.81 (1.96) ^{ns}	23.67 (1.70) ^{ns}	10.52 (1.10) ^{ns}
	Oct	4.73 (0.07) ^b	1.29 (0.13)	0.15 (0.01)	67.18 (1.83)	23.00 (1.32)	9.82 (0.74)

Table 5. 3 The MWD before wet-sieving (MWDpre), MWD after wet-sieving (MWDpost), MWD ratio (MWDpost/MWDpre), and aggregate size fractions after wet-sieivng

[#] the data of MWDpost and silt-clay fractions were log-transformed for the analysis of variance (ANOVA); different lowercase letters in Month by each depth were significantly different (α =0.05) by the LSD test

We	t-sieved		1-2 mm				2-4.75 mm	ı		4.75-8 mm	ı	
aggre	egate size	Mean	SE	Med [#]	_	Mean	SE	Med [#]	Mean	SE	Med [#]	_
Depth 0-5	cm											
Cultivar	Alamo	433.1	(48.7)	434.8		192.6	(11.8)	195.1	90.4	(11.6)	72.5	
	GA-992	348.0	(48.0)	308.6		165.9	(16.2)	182.7	77.8	(11.3)	66.2	
	GA-993	519.0	(217.0)	311.0	ns	238.3	(48.6)	216.3 ^{ns}	92.9	(19.9)	72.3	ns
	SL-93-2	542.0	(191.0)	334.0		226.6	(48.0)	183.2	84.8	(10.4)	71.6	
Month	Apr	316.8	(37.6)	290.8		154.7	(13.6)	145.3 ^b	58.3	(3.2)	60.69	c
	July	765.0	(185.0)	496.0		282.7	(41.1)	218.7 ^a	118.7	(12.7)	111.4	а
	Oct	299.0	(34.5)	268.2		180.2	(16.8)	179.2 ^{ab}	82.4	(9.4)	74.93	b
Depth 5-10) cm											
Cultivar	Alamo	410.0	(64.2)	354.1		123.3	(13.1)	121.8	58.4	(9.1)	53.15	
	GA-992	337.1	(56.9)	261.6		125.6	(14.7)	109.9	50.3	(5.9)	47.58	
	GA-993	473.0	(197.0)	292.0	ns	166.9	(37.7)	124.2 ^{ns}	46.5	(7.6)	42.05	ns
	SL-93-2	481.0	(197.0)	267.0		160.1	(42.5)	101.8	62.4	(8.6)	55.55	
Month	Apr	341.5	(40.5)	300.5	ab	107.9	(7.2)	108.1 ^b	48.0	(2.6)	46.72	
	July	685.0	(190.0)	401.0	а	202.6	(36.7)	146.7 ^a	56.5	(7.9)	45.8	ns
	Oct	249.3	(11.4)	249.7	b	121.4	(14.1)	108.4 ^b	58.6	(8.4)	59.66	
Depth 10-1	5 cm											
Cultivar	Alamo	400.0	(57.7)	402.4		115.8	(14.2)	98.1	51.1	(12.2)	38.3	
	GA-992	383.8	(51.0)	345.5		115.2	(14.2)	91.0	52.1	(7.4)	47.87	
	GA-993	627.0	(218.0)	386.0	ns	157.0	(43.0)	108.0 ^{ns}	75.1	(14.8)	48.5	ns
	SL-93-2	382.1	(30.8)	391.1		98.6	(13.9)	85.0	84.0	(38.5)	48.7	
Month	Apr	401.9	(30.0)	394.2		98.6	(9.6)	88.0	46.8	(5.2)	41.22	b
	July	610.0	(162.0)	458.0	ns	153.9	(31.9)	118.9 ^{ns}	106.6	(27.9)	72.7	а
	Oct	333.2	(40.5)	300.3		112.4	(14.1)	93.9	43.3	(7.5)	39.63	b

Table 5. 4 Treatment effects on tensile strength (kPa) of three different size fractions of wet-sieved aggregates

[#]Med: Median value; Different lowercase letters in Month by each depth were significantly different (α =0.05) by the Mann-Whitney Test; ns: not significant

		C in dry-sieved	aggregates (%)	C in wet-sieved	aggregate (%)
		Mean	SE	Mean	SE
Depth 0-5 cm	n				
Cultivar	Alamo	1.20	(0.07)	1.24	(0.04)
	GA-992	1.14	$(0.02)_{\rm ns}$	1.34	$(0.06)_{\rm ns}$
	GA-993	1.21	(0.05)	1.31	(0.03)
	SL-93-2	1.22	(0.04)	1.27	(0.05)
Month	Apr	1.22	(0.04)	1.37	$(0.04)^{a}$
	July	1.17	$(0.03)^{ns}$	1.25	$(0.02)^{b}$
	Oct	1.18	(0.05)	1.25	$(0.04)^{b}$
Depth 5-10 c	m				
Cultivar	Alamo	0.89	(0.03)	0.99	(0.03)
	GA-992	0.88	$(0.02)_{\rm ns}$	0.98	$(0.03)_{ns}$
	GA-993	0.90	(0.03)	1.00	(0.02)
	SL-93-2	0.88	(0.04)	0.98	(0.03)
Month	Apr	0.91	(0.03)	0.99	(0.03)
	July	0.89	$(0.02)^{ns}$	1.01	$(0.02)^{\rm ns}$
	Oct	0.86	(0.02)	0.96	(0.02)
Depth 10-15	cm				
Cultivar	Alamo	0.61	(0.02)	0.82	(0.04)
	GA-992	0.62	$(0.03)_{\rm ns}$	0.87	$(0.03)_{ns}$
	GA-993	0.62	(0.04)	0.86	(0.05)
	SL-93-2	0.60	(0.02)	0.90	(0.06)
Month	Apr	0.62	(0.02)	0.83	(0.03)
	July	0.62	$(0.02)^{\rm ns}$	0.92	$(0.04)^{\rm ns}$
	Oct	0.60	(0.03)	0.85	(0.04)

Table 5. 5 C concentration in dry-sieved and wet-sieved aggregates (4.75-8 mm)

Different lowercase letters in Month by each depth were significantly different (α =0.05) by the LSD test; ns: not significant

		ft Moon SE Mod [#]				f _{trans}			fstorage			fresidual	
		Mean	SE	Med [#]	Mean	SE	Med [#]	Mean	SE	Med [#]	Mean	SE	Med [#]
Depth 0-5	5 cm												
Cultivar	Alamo	0.44	(0.01)	0.42	0.06	(0.02)	0.05	0.22	(0.02)	0.21	0.15	(0.02)	0.18
	GA-992	0.44	(0.00)	0.44	0.06	(0.01)	0.05	0.23	(0.02)	0.23	0.14	(0.02)	0.16
	GA-993	0.44	(0.01)	0.43	0.07	(0.01)	0.06	0.23	(0.02)	0.21	0.13	(0.02)	0.16
	SL-93-2	0.44	(0.00)	0.44	0.06	(0.01)	0.06	0.23	(0.02)	0.22	0.14	(0.02)	0.16
Month	Apr	0 44	(0.01)	0 44	0.07	(0.02)	0.07	0.22	(0.02)	0.21	0.15	(0.02)	0.17
	July	0.43	(0,00)	0.43	0.05	(0.01)	0.05	0.24	(0.02)	0.22	0.14	(0.01)	0.16
	Oct	0.44	(0.00)	0.44	0.07	(0.01)	0.06	0.23	(0.01)	0.22	0.14	(0.01)	0.15
Denth 5-1	10 cm												
Cultivar	Alamo	0.43	(0, 01)	0.42	0.06	(0, 01)	0.05	0.21	(0, 02)	0.18	0.15	(0, 02)	0.18
Cultival	GA-992	0.43	(0.01)	0.42	0.00	(0.01)	0.05	0.21	(0.02)	0.10	0.13	(0.02)	0.16
	GA-993	0.43	(0.01)	0.43	0.06	(0.01)	0.08	0.20	(0.02)	0.19	0.14	(0.01)	0.15
	SI 03 2	0.43	(0.01)	0.42	0.00	(0.01)	0.06	0.23	(0.02)	0.21	0.14	(0.01)	0.15
	51-75-2	0.45	(0.00)	0.42	0.00	(0.01)	0.00	0.25	(0.02)	0.24	0.14	(0.01)	0.15
Month	Apr	0.44	(0.01)	0.44	0.07	(0.01)	0.07	0.22	(0.02)	0.22	0.15	(0.01)	0.16
	July	0.43	(0.00)	0.42	0.06	(0.01)	0.06	0.22	(0.02)	0.19	0.14	(0.01)	0.16
	Oct	0.43	(0.01)	0.44	0.07	(0.01)	0.07	0.22	(0.02)	0.20	0.14	(0.01)	0.16
Depth 10	-15 cm												
Cultivar	Alamo	0.44	(0.01)	0.44	0.09	(0.02)	0.08	0.18	(0.02)	0.16	0.17	(0.01)	0.18
	GA-992	0.42	(0.01)	0.42	0.07	(0.01)	0.07	0.20	(0.02)	0.18	0.15	(0.02)	0.18
	GA-993	0.42	(0.01)	0.42	0.07	(0.01)	0.07	0.20	(0.02)	0.18	0.15	(0.02)	0.19
	SL-93-2	0.42	(0.01)	0.42	0.07	(0.01)	0.06	0.21	(0.02)	0.20	0.14	(0.02)	0.15
Months	Apr	0.43	(0.01)	0.42	0.07	(0.01)	0.07	0.19	(0.01)	0.18	0.17	(0.01)	0.19
	July	0.42	(0.01)	0.42	0.07	(0.01)	0.07	0.20	(0.02)	0.19	0.15	(0.02)	0.16
	Oct	0.44	(0.01)	0.43	0.09	(0.01)	0.08	0.20	(0.02)	0.18	0.15	(0.01)	0.16

Table 5. 6 Total porosity (f_t) and pore size distribution: transmission pore (f_{trans}), storage pore ($f_{storage}$), and residual and bonding pore ($f_{residual}$)

Depth		()-5 cm				5	-10 cm			10-15 cm					
Principle components	PC1	PC2	PC3	PC4	PC5	PC1	PC2	PC3	PC4	PC5	PC1	PC2	PC3	PC4	PC5	
Eigenvalue	5.74	2.82	2.12	2.03	1.11	4.62	3.30	2.94	1.84	1.23	3.98	3.58	2.51	1.77	1.41	
Proportion	0.34	0.17	0.13	0.12	0.07	0.27	0.19	0.17	0.11	0.07	0.23	0.21	0.15	0.10	0.08	
Cumulative ratio	0.34	0.50	0.63	0.75	0.81	0.27	0.47	0.64	0.75	0.82	0.23	0.44	0.59	0.70	0.78	
Eigenvectors																
MĊ	0.28	-0.20	0.19	0.06	-0.10	-0.32	0.04	-0.06	-0.36	-0.34	-0.30	-0.16	-0.27	0.03	0.38	
BD	0.04	0.21	0.04	0.27	0.77	0.20	-0.17	0.07	-0.02	0.62	0.17	0.07	-0.01	0.23	-0.35	
MWDpre	0.38	0.05	-0.12	0.13	-0.04	-0.38	0.13	-0.07	-0.17	-0.10	-0.11	-0.30	-0.19	0.33	0.30	
MWDpost	0.30	0.26	-0.26	0.14	-0.13	-0.13	0.45	-0.23	-0.05	0.20	0.33	-0.33	-0.01	0.06	-0.13	
MWDratio	-0.17	0.33	-0.25	-0.03	-0.11	0.19	0.36	-0.20	0.11	0.24	0.37	-0.22	0.06	-0.05	-0.22	
Macro	0.35	0.22	-0.22	0.02	-0.05	-0.35	0.29	0.01	0.24	0.07	0.34	-0.29	-0.13	0.14	0.21	
Micro	-0.34	-0.07	0.22	-0.05	0.21	0.32	-0.25	0.06	-0.3 0	0.08	-0.28	0.30	-0.06	-0.12	-0.37	
SC	-0.22	-0.39	0.13	0.05	-0.23	0.26	-0.25	-0.16	0.00	-0.36	-0.19	0.06	0.33	-0.07	0.20	
TS1	-0.26	0.16	-0.33	-0.22	0.14	0.27	0.19	-0.01	0.33	-0.17	0.11	0.21	0.42	0.03	0.36	
TS2	-0.29	0.14	-0.29	-0.24	0.09	0.32	0.22	0.00	0.33	-0.12	0.32	0.18	0.25	-0.06	0.36	
TS5	-0.30	0.10	-0.23	-0.03	-0.29	0.25	0.21	0.04	0.05	-0.45	0.33	0.25	0.09	-0.20	0.14	
C-dry	0.15	0.19	0.10	-0.40	-0.14	-0.24	-0.04	0.23	0.42	0.05	0.35	0.06	-0.30	-0.04	-0.05	
C-wet	0.22	-0.10	-0.12	-0.38	-0.06	-0.12	-0.23	0.35	0.38	-0.10	0.14	0.17	-0.11	-0.40	-0.04	
\mathbf{f}_{t}	0.17	-0.17	-0.12	-0.47	0.27	-0.19	-0.30	-0.22	0.20	0.03	-0.12	-0.32	0.01	-0.53	0.08	
f _{trans}	0.15	-0.38	-0.14	-0.35	0.24	-0.17	-0.34	-0.27	0.27	-0.02	-0.01	-0.33	-0.01	-0.54	0.00	
f _{storage}	-0.01	0.41	0.43	-0.20	-0.05	0.01	0.14	0.52	-0.15	0.01	0.05	0.31	-0.44	-0.02	0.20	
f _{residual}	-0.03	-0.29	-0.46	0.30	0.01	0.04	-0.04	-0.54	0.07	0.01	-0.09	-0.25	0.46	0.13	-0.18	

Table 5. 7 Principal component analysis conducted with the 15 parameters measured during three sampling times

BD: bulk density; MC: moisture content at the sampling time; MWDpre: Mean weight diameter (MWD) before wet-sieving; MWDpost: MWD after wet-sieving; MWDratio: MWDpost/MWDpre; Macro: macroaggregate; Micro: microaggregate ; SC: silt and clay; TS1: tensile strength of wet-sieved aggregates 2-4.75 mm size; TS5: tensile strength of wet-sieved aggregates 4.75-8 mm size; C-dry: C concentration in dry-sieved aggregates (4.75-8 mm); C-wet: C concentration in wet-sieved aggregates (4.75-8 mm); f_i: total porosity; f_{trans}: porosity of transmission pores; f_{storage}: porosity of storage pores; f_{residual}: porosity of residual and bonding pores. *Notes:* Italic and bold numbers showed the parameters which mainly explained the properties of the principal component

	MC	BD	Mpre	Mpost	Mratio	Macro	Micro	SC	TS1	TS2	TS5	C-dry	C-wet	\mathbf{f}_{t}	$\mathbf{f}_{\text{trans}}$	$\mathbf{f}_{\text{storage}}$	$\mathbf{f}_{residual}$
BD	-0.11																
Mpre	0.59	0.14															
Mpost	0.26	0.19	0.82														
Mratio	-0.65	0.06	-0.40	0.19													
Macro	0.36	0.17	0.85	0.86	-0.06												
Micro	-0.40	0.00	-0.80	-0.79	0.12	-0.91											
SC	-0.14	-0.38	-0.58	-0.63	-0.06	-0.74	0.39										
TS1	-0.56	-0.03	-0.46	-0.28	0.40	-0.27	0.40	-0.06									
TS2	-0.55	-0.10	-0.58	-0.36	0.45	-0.37	0.49	0.02	0.91								
TS5	-0.57	-0.26	-0.54	-0.32	0.43	-0.37	0.34	0.26	0.63	0.66							
C-dry	0.14	-0.15	0.22	0.25	0.02	0.32	-0.21	-0.36	-0.08	-0.04	-0.29						
C-wet	0.26	-0.25	0.40	0.23	-0.22	0.38	-0.37	-0.25	-0.11	-0.16	-0.34	0.29					
\mathbf{f}_{t}	0.21	-0.09	0.22	0.12	-0.18	0.24	-0.29	-0.05	-0.04	-0.07	-0.30	0.36	0.44				
$\mathbf{f}_{\text{trans}}$	0.28	-0.17	0.17	-0.07	-0.36	0.13	-0.25	0.12	-0.17	-0.17	-0.28	0.05	0.53	0.78			
$\mathbf{f}_{\text{storage}}$	-0.08	0.12	-0.10	0.03	0.17	0.06	0.11	-0.30	0.00	-0.01	-0.01	0.38	-0.08	-0.08	-0.44		
f _{residual}	-0.02	-0.05	0.09	0.07	-0.02	-0.06	-0.07	0.25	0.11	0.11	0.09	-0.35	-0.11	-0.05	0.13	-0.92	
RWD	0.17	0.02	0.41	0.22	-0.14	0.25	-0.29	-0.10	-0.15	-0.05	-0.02	-0.11	0.47	0.32	0.34	-0.24	0.17

Table 5. 8A. Correlation coefficient between two parameters measured in April and October for 0-5 cm depth

MWDpre: Mean weight diameter (MWD) before wet-sieving; MWDpost: MWD after wet-sieving; MWDratio: MWDpost/MWDpre; Macro: macroaggregate size fraction (> 0.25 mm); Micro: microaggregate size fraction (0.053-0.25 mm); SC: silt and clay size fraction (< 0.053 mm); TS1: tensile strength of wet-sieved aggregates 1-2 mm size; TS2: tensile strength of wet-sieved aggregates 2-4.75 mm size; TS5: tensile strength of wet-sieved aggregates 4.75-8 mm size; C-dry: C concentration in dry-sieved aggregates (4.75-8 mm); C-wet: C concentration in wet-sieved aggregates (4.75-8 mm); f_t : total porosity; f_{trans} : porosity of transmission pores; $f_{storage}$: porosity of storage pores; $f_{residual}$: porosity of residual and bonding pores; MC: moisture content; BD: bulk density

Notes: Italic and bold numbers showed the significant correlation coefficient (α =0.05)

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	MC	BD	Mpre	Mpost	Mratio	Macro	Micro	SC	TS1	TS2	TS5	C-dry	C-wet	\mathbf{f}_{t}	$\mathbf{f}_{\text{trans}}$	f _{storage}	$\mathbf{f}_{residual}$
BD	-0.49																
Mpre	0.72	-0.36															
Mpost	0.24	-0.27	0.43														
Mratio	-0.35	0.00	-0.36	0.68													
Macro	0.34	-0.40	0.66	0.59	0.06												
Micro	-0.28	0.47	-0.61	-0.53	-0.07	-0.94											
SC	-0.31	0.08	-0.46	-0.45	-0.03	-0.68	0.38										
TS1	-0.49	0.06	-0.32	0.02	0.34	-0.12	0.08	0.15									
TS2	-0.53	0.15	-0.45	0.07	0.48	-0.15	0.10	0.19	0.85								
TS5	-0.13	-0.08	-0.34	0.06	0.39	-0.22	0.16	0.25	0.45	0.59							
C-dry	0.06	-0.14	0.15	-0.05	-0.20	0.48	-0.45	-0.33	-0.19	-0.20	-0.21						
C-wet	-0.12	-0.05	-0.08	-0.55	-0.50	0.14	-0.12	-0.11	-0.13	-0.13	-0.16	0.68					
\mathbf{f}_{t}	0.17	0.00	0.22	-0.15	-0.34	0.08	-0.13	0.06	-0.21	-0.29	-0.40	0.16	0.16				
$\mathbf{f}_{\text{trans}}$	0.12	-0.02	0.10	-0.23	-0.32	0.05	-0.14	0.16	-0.26	-0.33	-0.35	0.24	0.23	0.81			
$\mathbf{f}_{\text{storage}}$	0.01	0.05	0.00	-0.12	-0.14	0.08	0.06	-0.33	0.03	0.04	0.13	0.16	0.27	-0.36	-0.63		
$\mathbf{f}_{residual}$	-0.03	-0.04	0.02	0.25	0.27	-0.10	-0.02	0.31	0.07	0.07	-0.07	-0.31	-0.44	0.28	0.40	-0.94	
RWD	0.20	-0.23	0.18	-0.04	-0.08	0.01	-0.16	0.32	0.25	0.17	0.30	0.11	0.31	-0.06	-0.20	0.19	-0.11

 Table 5.8B. Correlation coefficient between two parameters measured in April and October for 5-10 cm depth

	MC	BD	Mpre	Mpost	Mratio	Macro	Micro	SC	TS1	TS2	TS5	C-dry	C-wet	\mathbf{f}_{t}	$\mathbf{f}_{\text{trans}}$	$\mathbf{f}_{\text{storage}}$	f _{residual}
BD	-0.34																
Mpre	0.64	0.06															
Mpost	-0.15	0.19	0.23														
Mratio	-0.39	0.13	-0.14	0.93													
Macro	0.03	0.03	0.42	0.71	0.56												
Micro	-0.04	0.01	-0.46	-0.63	-0.48	-0.85											
SC	0.01	-0.07	-0.07	-0.32	-0.30	-0.52	0.00										
TS1	-0.27	-0.10	-0.28	-0.12	0.01	-0.03	-0.10	0.21									
TS2	-0.38	0.19	-0.26	0.19	0.28	0.23	-0.32	0.07	0.71								
TS5	-0.52	0.16	-0.51	-0.16	0.01	-0.21	0.20	0.07	0.13	0.30							
C-dry	-0.18	0.23	0.01	0.37	0.37	0.51	-0.36	-0.39	-0.17	0.20	0.04						
C-wet	-0.16	0.15	-0.36	-0.15	-0.04	-0.02	0.01	0.01	-0.04	0.21	0.37	0.44					
\mathbf{f}_{t}	0.35	-0.30	0.19	0.15	0.07	0.09	-0.14	0.06	-0.24	-0.21	-0.20	-0.21	0.05				
f _{trans}	0.20	-0.20	0.15	0.34	0.27	0.19	-0.20	-0.03	-0.24	-0.14	-0.13	-0.04	0.08	0.91			
$\mathbf{f}_{\text{storage}}$	0.11	0.00	-0.13	-0.24	-0.20	-0.10	0.26	-0.22	-0.10	0.12	0.16	0.34	0.22	-0.31	-0.33		
f _{residual}	-0.10	0.00	0.12	0.11	0.07	0.03	-0.20	0.27	0.15	-0.13	-0.16	-0.41	-0.26	0.18	0.11	-0.96	
RWD	-0.31	0.29	0.60	0.25	0.12	0.42	-0.46	-0.05	0.15	0.34	0.24	0.32	0.05	-0.05	-0.06	-0.06	0.10

 Table 5.8C. Correlation coefficient between two parameters measured in April and October for 10-15 cm depth



Fig. 5. 1 Loading plots for each depth with the first two principal components

BD: bulk density; MC: moisture content at the sampling time; MWDpre: Mean weight diameter (MWD) before wet-sieving; MWDpost: MWD after wetsieving; MWDratio: MWDpost/MWDpre; Macro: macroaggregate size fraction (> 0.25 mm); Micro: microaggregate size fraction (0.053-0.25 mm); SC: silt and clay size fraction (< 0.053 mm); TS1: tensile strength of wet-sieved aggregates 1-2 mm size; TS2: tensile strength of wet-sieved aggregates 2-4.75 mm size; TS5: tensile strength of wet-sieved aggregates 4.75-8 mm size; C-dry: C concentration in dry-sieved aggregates (4.75-8 mm); C-wet: C concentration in wet-sieved aggregates (4.75-8 mm); f_t : total porosity; f_{trans} : porosity of transmission pores; $f_{storage}$: porosity of storage pores; $f_{residual}$:

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CHAPTER 6

SYNTHESIS AND FUTURE RESEARCH PLANS

A paradigm shift from maximum to sustainable agricultural production also applies to cultivation of bioenergy crops. If biomass production is not sustainable, the environmental debt would outweigh the benefits of producing biofuel feedstock. Soil organic matter (SOM) is a key determinant of soil quality (Tate, 1992), and thus can be a good indicator to determine sustainable bioenergy crop cultivation.

Cycles of carbon (C) and nitrogen (N) are strongly coupled from photosynthesis to decomposition because of elemental constituents for organisms and SOM (McGill and Cole, 1981; Asner et al., 1997). However, there is no consensus about the effects of N on SOM pools and its dynamics (Johnson & Curtis, 2001; Christopher and Lal, 2007; Khan et al., 2007; Reay et al., 2008). Therefore, a series of field and laboratory studies were conducted to evaluate the effects of N fertilization needed to optimize biomass yields, on SOC under switchgrass, a promising bioenergy crop. This study evaluated changes in SOC concentration and pool and biomass production following N fertilization, examined possible mechanisms underpinning the effects of N on organic matter (OM) decomposition, and measured the changes in soil structural properties. Field studies were conducted in Ohio and Tennessee. Studies conducted in Ohio showed that the

aboveground biomass responded positively to N fertilization, but the belowground biomass did not. Even though the aboveground biomass was harvested and removed from the plots and no significant increase in belowground biomass was observed with N fertilization, there was a positive relationship between N rates and SOC stocks. Similarly, a significant increase in SOC concentrations with N application was also observed in Tennessee. Since no other pathways of C inputs were measured, the probable mechanisms for the accrual of the SOC pools are not confidently identified. However, these results emphasize the need for studying other pathways of C input such as litter production during a growing season, root turnover, or rhizodepostion (Rasse et al., 2005; Gregory, 2006; Johnson et al., 2006). The measurements of root biomass at a specific phonological stage might not precisely represent the total input of C associated with root production and turnover. Thus, more diverse routes of the C inputs need to be carefully examined.

The observed reduction in decomposition of OM by the addition of N may be responsible for the increase in SOC pools associated with N fertilization. In general, application of N had a positive effect on SOC through increase in the amount of residues returns. Yet, the possibility of increase in SOC pools because of the decrease in SOC decomposition with N fertilization has not been widely evaluated in a field scale study. The data presented in Chapter 3 based on a laboratory study conducted under the controlled environment indicated a negative effect of N application on OM decomposition. Several studies in forest ecosystems (Bowden et al., 2004; Compton et al., 2004) have also documented decrease in soil respiration with N deposition. Moreover, the effects of N on OM decomposition may vary depending on the SOC fractions (Neff et al.,

2002; Hagedorn et al., 2003; Bradford et al., 2008). Additionally, interactions with other elements may play important roles in moderating SOC pool and dynamics, which are important to understanding the impact of changing environment.

The data in Chapter 3 support the priming effect of root addition on SOC. However, the amount of C lost from the priming effect was offset by the formation of new C from added root-C substrates. In several previous studies on SOC dynamic, the priming effect was not considered to be important. The additional CO₂ production after adding C substrates was thought to be either a short-term effect or a misconception that microbial turnover or pool substitutions contributed to the increased CO₂, rather than the real CO₂ derived from the decomposition of SOC pools. However, several studies have shown the occurrence of a real priming effect as well as an apparent priming effect (Kuzyakov et al., 2000). Plant growth could induce a rhizosphere priming effect (Kuzyakov, 2002; Cheng and Kuzyakov, 2005). Steinbeiss et al. (2008) showed that the priming effect could also be caused by easily decomposable compounds transported to deeply down the soil profile. These studies have emphasized the importance of the priming effect in SOC dynamics.

The data represented in Chapter 4 support the conclusion that soil structural properties are associated more with changes in root growth than those in SOC concentrations after N fertilization. Campbell et al. (1993) showed higher aggregate stability with higher rates of N fertilization because of an increase in residues returns and the SOC concentration. The latter is a key determinant of soil quality, however, the present study highlight some discrepancies between structural stability and SOC concentration following N fertilization. In the Tennessee experiments, SOC concentration
per se did not affect soil structure, since aggregate stability was lower in plots receiving higher rates of N fertilizer despite higher levels of SOC concentration. The results of this experiment confirmed the important role of roots in stabilizing soil structure (Tisdall and Oades, 1982; Jastrow et al., 1998). It is well known that root growth decreases under a sufficient nutrient regime (Thornley, 1972), and that roots play important roles in formation and stabilization of aggregates (Tisdall and Oades, 1982). The impact of nutrient input on root growth and that of root growth on aggregation have not been collectively assessed in the available literature. The present study, with a focus on switchgrass, showed that higher rates of N application decreased root biomass or length, and thus reduced aggregate stability. Although the changes in the microbial community with N fertilization were not investigated in the present study, the role of fugal hyphae in aggregate formation and stabilization should be considered importantly in the future study. Several studies have shown that N application causes a shift of microbial community from fungal-dominated to bacterial-dominated community (Bradley et al, 2006). Such a shift in microbial community may affect aggregate stability. Thus, additional research is needed to monitor changes in the microbial community as a result of N fertilization.

The data in Chapter 5 showed the importance of abiotic environments at the time of soil sampling (i.e., antecedent soil moisture content), and sample preparation to determine aggregate stability and soil structure.

In conclusion, this study showed that;

· aboveground biomass of switchgrass increased with increasing rates of N application,

- the response of switchgrass root biomass to the addition of N might not be obvious, but root length could be decreased by continuous N application for a long period,
- SOC concentration/pool generally increased with the increase in N application and could lead to the retardation of SOC decomposition following N addition,
- although the increase in SOC was associated with the addition of N, the adversely affected root proliferation under the excessive rate of N fertilizers could result in attendant decrease in soil structural properties.

Therefore, future research needs to focus on

- the avenue of C input to soil such as root exudates, root turnover, or litter production through growing seasons,
- · mechanisms and magnitude of the priming effects,
- · mechanisms for the decrease in OM decomposition associated with the addition of N,
- life cycle analysis and evaluation of soil quality and environmental effects under switchgrass following N application for sustainable cultivation, and
- \cdot a shift in microbial community with N addition and its influences on soil structure.

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APPENDICES

APPENDIX A: Data not presented in Chapter 3

	CO_2	Cum	MC	NO ₃ -	$\mathrm{NH_4}^+$	IN	pН	Beta	Phe	Pero
Cum	-0.61									
MC	-0.02	0.01								
NO ₃ -	-0.74	0.67	0.05							
$\mathrm{NH_4}^+$	0.01	0.05	0.19	0.12						
IN	-0.73	0.67	0.06	1.00	0.18					
pН	0.68	-0.77	-0.10	-0.88	0.01	-0.87				
Beta	0.26	-0.01	-0.27	-0.18	-0.17	-0.19	0.20			
Phe	-0.35	0.39	-0.11	0.31	-0.08	0.31	-0.34	-0.18		
Pero	0.57	-0.54	0.45	-0.49	0.08	-0.49	0.52	0.24	-0.62	
Oxi	0.39	-0.32	0.47	-0.34	0.03	-0.34	0.34	0.14	0.14	0.69

Appendix A1. Correlation matrix of parameters measured during the incubation study

*CO₂: CO2 flux rate; CumCO₂: Cumulative CO₂; MC: moisture content; IN: inorganic N concentration; Beta: β -glucosidase activity; Phenol: phenol oxidase activity; Pero: peroxidase activity; Oxi: oxidative enzyme activity

*Data from five sampling dates (0, 10, 45, 100, and 200) were used for CO₂, CumCO₂, MC, NO3-, NH4+, IN, that from four sampling dates (10, 45, 100, and 200) were used for , and that from three sampling dates (10, 45, and 200) were used for Phenol, Pero, and Oxi in correlation coefficients calculation.

C treatment	N treatment	k_l	k _r	Cl
C0	N0	0.0112	0.0006	2.22
C0	NL	0.0076	-0.0045	3.96
C0	NH	0.0068	-0.0076	5.54
CL	N0	0.0076	-0.0094	7.62
CL	NL	0.0126	0.0009	3.16
CL	NH	0.0074	-0.0065	5.27
СН	N0	0.0133	0.0028	3.76
СН	NL	0.0130	0.0021	3.76
СН	NH	0.0103	-0.0016	4.46

Appendix A2. Estimates from an exponential plus linear model

Exponential plus linear model

$$C_{\min} = C_l[1 - \exp(k_l t)] + k_r t$$

Cmin: mineralized carbon; k_l : decomposition constant for the labile C pool; k_r : decomposition constant for the recalcitrant C pool; C_l : labile C pool; t: time

The correlation coefficient between k_l and k_r was 0.928.

Appendix A3. Testing for coincidence of three regression lines (response variable: CO₂-C efflux rates, predictor variable: soil pH) built by three different rates of N treatments (N0, NL, NH). The test was performed in each C level separately.

		AN	OVA		F-test statistics for coincidence							
	Source	df	SS	MS	F-value p-value							
C0	рН	1	$1.87*10^{-3}$	1.87*10 ⁻³								
	N_trt	2	7.40*10 ⁻⁷	3.70*10 ⁻⁷	$E = (7.4*10^{-7} + 1.22*10^{-5})/4 = 0.177 = 0.040$							
	pH:N_trt	2	1.22*10 ⁻⁵	6.10*10 ⁻⁶	$\Gamma = \frac{1.83*10^{-5}}{1.83*10^{-5}} = 0.177 = 0.949$							
	residuals	39	7.12*10 ⁻⁴	1.83*10 ⁻⁵								
CL	pH N_trt pH:N_trt residuals	1 2 2 39	6.71*10 ⁻³ 9.40*10 ⁻⁶ 2.40*10 ⁻⁶ 4.39*10 ⁻³	$\begin{array}{c} 6.71^{*}10^{-3} \\ 4.70^{*}10^{-6} \\ 1.20^{*}10^{-6} \\ 1.13^{*}10^{-4} \end{array}$	$F = \frac{(9.4*10^{-6} + 2.4*10^{-6})/4}{1.13*10^{-4}} = 0.026 \qquad 0.999$							
СН	pH N_trt pH:N_trt residuals	1 2 2 39	9.34*10 ⁻³ 7.88*10 ⁻⁵ 6.90*10 ⁻⁶ 8.17*10 ⁻³	9.34*10 ⁻³ 3.94*10 ⁻⁵ 3.40*10 ⁻⁶ 2.09*10 ⁻⁴	$F = \frac{(7.88 \times 10^{-5} + 6.9 \times 10^{-6})/4}{2.09 \text{E-04}} = 0.102 \qquad 1.000$							

A linear regression model is

CO_2 -C efflux rates = pH + N_trt + pH * N_trt

where N_trt is an indicator variable (N0, NL, NH).

Appendix A4. Individual values (closed circle) and mean values (cross circle) for δ^{13} C from the initial soil and SOC at the end of incubation (200 days).



N0: no addition of N = 0; NL: low level of N = 0.021 mg N/g soil; NH: high level of N = 0.083 mg N/g soil; C0: no addition of C = 0; CL: low level of C = 5 mg root/g soil; CH: high level of C = 10 mg root/g soil

APPENDIX B: Data not presented in Chapter 4

	BD	RWD	RLD	SOC	TN	P0.2	5 I	P100	P1500	Macro	Micro	SC	N	/WD _{pre}	MWD _{post}	MWD _{ratio}	TS5	ГS2	Sand	Silt
RWD	-0.22																			
RLD	-0.30	0.53	3																	
SOC	-0.12	0.1	1 0.0	8																
TN	0.03	0.18	8 -0.0	6 0.9	5															
P0.25	0.25	0.32	2 -0.1	5 0.3	3 (.38														
P100	0.55	0.3	1 0.0	3 0.4	0 (0.57	0.68													
P1500	0.21	0.60	6 0.0	3 0.2	4 (0.38	0.55	0.72												
Macro	-0.12	0.62	2 0.6	5 0.0	4 (0.12	0.14	0.35	0.59	9										
Micro	-0.16	-0.51	1 -0.7	1 0.0	4 -(.04 -	0.25	-0.51	-0.64	4 -0.82	1									
SC	0.43	-0.37	7 -0.2	0 -0.1	3 -(0.14	0.10	0.10	-0.12	2 -0.6	1 0.0)3								
MWD _{pre}	0.56	-0.04	4 -0.2	0 0.5	6 (.71	0.37	0.77	0.3	7 -0.0	1 -0.1	8	0.26							
MWD _{pos}	t 0.28	0.55	5 0.4	1 0.3	3 ().51	0.35	0.77	0.7	1 0.72	2 -0.2	- 27	0.26	0.62						
MWD _{rati}	o -0.43	0.54	4 0.8	5 -0.3	6 -(.40 -	0.15	-0.23	0.17	7 0.68	8 -0.4	18 -	0.52	-0.65	0.18	8				
TS5	0.02	0.50	6 0.2	9 -0.0	7 (0.04	0.16	0.27	0.59	9 0.40	6 -0.4	16 -	0.16	-0.01	0.40	0.38	3			
TS2	0.19	0.29	9 0.1	4 -0.3	1 -(0.13	0.16	0.15	0.3	1 0.24	4 -0.2	- 29	0.01	0.08	0.3	0.21	0.48			
Sand	-0.20	0.05	5 0.4	9 0.4	6 (.32 -	0.24	0.22	0.12	2 0.40	0 -0.3	- 39	0.19	0.14	0.3	1 0.30	0.24	-0.65		
Silt	-0.61	-0.36	5 -0.0	7 -0.5	3 -(.60 -	0.13	-0.65	-0.80	6 -0.52	2 0.3	38	0.42	-0.76	-0.75	5 0.03	-0.74	-0.02	-0.30)
Clay	0.73	0.33	3 -0.2	5 0.2	2 (0.38	0.28	0.48	0.73	3 0.25	5 -0.1	- 3	0.29	0.66	0.53	-0.23	0.57	0.45	-0.3	5 -0.78

Appendix B1. Correlation matrix of parameters for 0-5 cm depth

BD: bulk density; RWD: root weight density; RLD: root length density; SOC: soil organic carbon; TN: total soil nitrogen; P0.25: volumetric moisture content at suction 0.25 kPa; P100: volumetric moisture content at suction 100 kPa; P1500: volumetric moisture content at suction 1500 kPa; Macro: percentage of macroaggregate size fractions (> 0.25 mm); Micro: percentage of microaggregate size fractions (0.053-0.25 mm); SC: percentage of silt and clay size fractions; MWDpre: mean weight diameter (MWD) before wet-sieving; MWDpost: MWD after wet-sieving; MWDpratio: MWDpost to MWDpre; TS5: tensile strength of water stable aggregates in size 4.75-8 mm; TS2: tensile strength of water stable aggregates in size 2-4.75 mm; Sand: percentage of sand in soil textural analysis; Silt: : percentage of silt in soil textural analysis

	BD	RWD	RLD	SOC	ΓN	P0.25	P100	P1500	Macro	Micro	SC	Ν	4WD _{pre} N	MWD _{post} N	MWD _{ratio} 7	S5 1	rsa s	Sand	Silt
RWD	-0.14	Ļ																	
RLD	0.09	0.4	1																
SOC	0.15	0.00	0.03	3															
TN	-0.07	-0.02	2 0.15	5 0.70															
P0.25	0.29	-0.09	9 -0.03	0.25	-0.09														
P100	0.55	-0.43	3 -0.16	6 0.20	0.25	0.53													
P1500	0.50	-0.1	5 -0.17	0.56	0.33	0.65	0.64	4											
Macro	0.33	0.52	2 0.54	0.26	0.20	0.19	0.05	5 0.3	6										
Micro	-0.25	-0.42	7 -0.54	-0.18	-0.16	-0.26	0.04	4 -0.3	3 -0.9	1									
SC	-0.25	-0.23	3 -0.18	-0.23	-0.15	0.11	-0.2	-0.1	5 -0.4	0.0-0.0)1								
MWD _{pre}	-0.04	-0.1	7 -0.41	-0.16	0.46	-0.38	0.24	4 -0.0	8 -0.24	4 0.2	22	0.10							
MWD _{post}	0.15	0.44	4 0.28	8 0.11	0.45	0.00	0.18	3 0.2	4 0.7	8 -0.6	64 -	-0.45	0.32						
MWD _{ratio}	0.23	0.50	0 0.49	0.22	0.11	0.22	0.00	0.2	4 0.9	1 -0.7	76 ·	-0.52	-0.39	0.74					
TS5	0.08	-0.10	6 0.20	0.05	0.55	-0.26	0.30	0.0	3 0.1	8 -0.0)9 .	-0.22	0.59	0.48	0.06				
TS2	0.10	-0.20	6 -0.41	0.21	0.50	-0.37	0.26	-0.0	6 -0.1	5 0.2	28 -	-0.28	0.42	0.16	-0.15	0.62			
Sand	0.02	-0.07	7 0.46	6 0.11	0.02	0.36	0.18	3 0.0	9 0.32	2 -0.3	33 .	-0.09	-0.24	0.21	0.33	0.05	-0.60		
Silt	-0.33	0.17	7 0.51	-0.72	-0.62	-0.30	-0.57	7 -0.7	3 0.0	6 -0.3	37	0.46	-0.59	-0.19	0.08	0.05	-0.48	0.14	
Clay	0.31	-0.1	5 -0.58	3 0.67	0.59	0.22	0.51	1 0.6	8 -0.12	2 0.4	42 ·	-0.42	0.61	0.14	-0.14	-0.06	0.57	-0.32	-0.98

Appendix B2. Correlation matrix of parameters for 5-10 cm depth

BD: bulk density; RWD: root weight density; RLD: root length density; SOC: soil organic carbon; TN: total soil nitrogen; P0.25: volumetric moisture content at suction 0.25 kPa; P100: volumetric moisture content at suction 100 kPa; P1500: volumetric moisture content at suction 1500 kPa; Macro: percentage of macroaggregate size fractions (> 0.25 mm); Micro: percentage of microaggregate size fractions (0.053-0.25 mm); SC: percentage of silt and clay size fractions; MWDpre: mean weight diameter (MWD) before wet-sieving; MWDpost: MWD after wet-sieving; MWDratio: MWDpost to MWDpre; TS5: tensile strength of water stable aggregates in size 4.75-8 mm; TS2: tensile strength of water stable aggregates in size 2-4.75 mm; Sand: percentage of sand in soil textural analysis; Silt: : percentage of silt in soil textural analysis
	BD 1	RWD	RLD	SOC	TN	P0.25	P100	P1500	Macro	Micro	SC	Ν	1WD _{pre} 1	MWD _{post} N	AWD _{ratio}	rss 1	ГS2	Sand	Silt
RWD	0.03																		
RLD	-0.06	0.78	}																
SOC	-0.06	0.08	0.47	7															
TN	0.23	-0.01	0.44	0.40															
P0.25	-0.20	0.26	6 0.16	6 0.03	-0.50														
P100	0.19	0.17	0.03	0.28	0.13	0.39													
P1500	0.26	0.17	-0.02	2 0.42	0.12	0.40	0.9 3	3											
Macro	-0.05	0.27	0.35	5 0.55	-0.03	0.41	0.31	l 0.5	0										
Micro	0.28	-0.01	0.43	-0.13	0.51	-0.67	-0.33	-0.40	0 -0.4	5									
SC	-0.19	-0.27	-0.47	-0.44	-0.41	0.16	-0.03	- 0.1′	7 -0.6	3 -0.4	12								
MWD _{pre}	0.35	-0.14	0.21	-0.21	0.53	-0.45	0.07	7 -0.09	9 -0.3	2 0.5	57 -	0.17							
MWD _{post}	0.11	0.53	8 0.72	2 0.47	0.29	0.16	0.19	0.32	2 0.7	7 -0.0)1 -	0.78	-0.06						
MWD _{ratio}	-0.10	0.48	8 0.75	5 0.53	-0.01	0.33	0.15	5 0.32	2 0.8	<i>o</i> -0.2	- 25	0.60	-0.50	0.88					
TS5	0.34	0.19	0.12	2 -0.18	0.50	-0.37	0.14	4 0.03	8 -0.3	9 0 .5	54 -	0.18	0.57	-0.09	-0.40				
TS2	0.33	0.00	0.18	-0.05	0.74	-0.43	0.05	5 0.02	2 -0.1	6 0.6	63 -	0.39	0.62	0.22	-0.12	0.75			
Sand	-0.50	0.21	0.2	-0.26	-0.35	-0.20	-0.30	-0.3	6 -0.4	3 -0.1	4	0.40	-0.52	-0.04	0.07	-0.63	-0.64	4	
Silt	-0.57	0.20	0.48	3 0.09	-0.09	-0.27	-0.44	4 -0.4	7 -0.1	9 0.2	24	0.05	-0.53	0.01	0.17	-0.10	-0.07	7 0.4	0
Clav	0.61	-0.22	2 -0.42	7 -0.04	0.14	0.28	0.4ϵ	5 0.49	9 0.2	5 -0.2	- 20	0.11	0.57	0.00	-0.17	0.19	0.12	7 -0.5	54 -0.99

Appendix B3. Correlation matrix of parameters for 10-15 cm depth

BD: bulk density; RWD: root weight density; RLD: root length density; SOC: soil organic carbon; TN: total soil nitrogen; P0.25: volumetric moisture content at suction 0.25 kPa; P100: volumetric moisture content at suction 100 kPa; P1500: volumetric moisture content at suction 1500 kPa; Macro: percentage of macroaggregate size fractions (> 0.25 mm); Micro: percentage of microaggregate size fractions (0.053-0.25 mm); SC: percentage of silt and clay size fractions; MWDpre: mean weight diameter (MWD) before wet-sieving; MWDpost: MWD after wet-sieving; MWDratio: MWDpost to MWDpre; TS5: tensile strength of water stable aggregates in size 4.75-8 mm; TS2: tensile strength of water stable aggregates in size 2-4.75 mm; Sand: percentage of sand in soil textural analysis; Silt: : percentage of silt in soil textural analysis