The Influence of Switchgrass Establishment on Soil Organic Matter Pools in an Agricultural Landscape

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ABSTRACT

Agricultural activities have significant impacts on global biogeochemical cycles, particularly carbon and nitrogen. Conventional row-crop agriculture accelerates the decomposition of soil organic matter, contributing to atmospheric carbon and declining soil fertility. Planting perennial warm season grasses is a useful management alternative to row crop agriculture because these species have been shown to be effective at increasing soil carbon storage and retaining nitrogen. The objectives of this research were to examine how converting row crops to a native perennial warm season grass (*Panicum virgatum* L., common name switchgrass) influences the recovery of soil organic matter fractions and nitrogen retention within an agricultural watershed in the Shenandoah Valley of Virginia. Soil samples were analyzed for total carbon and nitrogen, three particulate organic matter fractions, root biomass, mineralizable carbon and nitrogen pools, and microbial biomass. Surprisingly, I observed significant declines in bulk soil organic matter and surface particulate organic matter pools following switchgrass establishment. There were no differences in mineralizable carbon and microbial biomass pools between row crop and switchgrass soils, but labile carbon pools and nitrogen immobilization increased as switchgrass stands matured. These results are potentially due to switchgrass litter inputs stimulating microbial communities and accelerating the decomposition of recalcitrant soil organic matter, leading to declines in soil organic carbon stocks. The results from this study will be used to understand the environmental and economic benefits of implementing switchgrass plantings in agricultural watershed as a means to mitigate agriculturally-induced effects on carbon storage and nitrogen retention in soils.

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my brother James, I hope to see you continue the tradition and become a member of the Virginia Tech class of 2020.

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INTRODUCTION

Agricultural activities have significant impacts on global biogeochemical cycles, particularly carbon and nitrogen (Vitousek et al., 1997; Lal., 2004b). Globally, soils are the largest pool of actively cycling carbon, accounting for at least 1500 Pg of carbon, with the primary source of this carbon coming from plant derived inputs (Lal, 2004a; Schlesinger, 2009). Intensive farming techniques and long-term cultivation of row crops critically impact soil fertility by removing much of the above-ground plant biomass that would contribute to litter fractions and by accelerating the decomposition of soil organic matter, which contributes to atmospheric carbon and decreases the ability of soils to retain nutrients (Mann, 1986; Davidson and Akerman, 1993; Paustian et al., 1997). Organic matter plays a crucial role in the soil environment by influencing a significant number of physical and chemical properties and processes including improving water holding capacity, soil stability, nutrient availability, and carbon sequestration (Tisdall and Oades, 1982; Lal, 2004b). Therefore, enhancing the formation of stable organic matter content in soils could potentially be an important strategy to offset anthropogenic CO_2 emissions while also improving nutrient retention and soil fertility.

Conventional agricultural practices such as tilling, albeit necessary for food production, often have high environmental costs that have raised concern for developing and implementing best management practices to increase carbon sequestration and promote soil fertility, while also offsetting nitrogen losses and greenhouse gas emissions. Farmers and land managers have turned to planting switchgrass (*Panicum virgatum* L.), a native perennial warm season grass, on marginal lands for bioenergy with the added benefit of stimulating soil organic matter accumulation and aiding soils in recovery from cultivation (McLaughlin et al., 2002; Lemus and Lal, 2005; Blanco-Canqui, 2010). Because of its extensive root system (Weaver and Darland,

1949; Ma et al., 2000b), switchgrass stimulates inputs of organic matter to the soil and microbial immobilization of nitrogen, effectively sequestering carbon and facilitating higher rates of nitrogen retention in soils (Bransby et al., 1998; Ma et al., 2000c; Zan et al., 2001; Frank et al., 2004; Leibig et al., 2005; Leibig et al., 2008; Anderson et al., 2009; Collins et al., 2010; Schmer et al., 2011). When compared to cultivated crop soils, switchgrass soils have been shown to have greater soil organic carbon content between 12 to 20%, with carbon accumulation rates from 40 to 1010 g C m^{-2} yr^{-1} reported over a variety of soil types and management histories (Ma et al., 2000c; Zan et al., 2001; Frank et al., 2004; Leibig et al., 2005; Leibig et al., 2008; Anderson et al., 2009; Collins et al., 2010; Schmer et al., 2011). Switchgrass also has an additional advantage over other conservation plantings of offering a commercially viable commodity to land-owners as the hay can be harvested and used for fodder or bioenergy production (McLaughlin et al., 2002; McLaughlin et al., 2005; Sanderson et al., 2006).

However, some studies have not observed increases in soil organic matter following switchgrass establishment, but rather decreases by up to 24% (Schmer et al., 2011; de Graaff et al., 2014; Strickland et al., 2015). These declines are likely caused by a stimulation of microbial decomposition of soil organic matter due to increases in root exudates, fine root litter, and detritus, which stimulates microbial activity (Kuzyakov et al., 2000; Fontaine et al., 2004; Phillips et al., 2012; de Graaff et al., 2014). The greater inputs of switchgrass root biomass in the surface soils provides a readily available energy source of carbon to microbes (Kuzyakov et al., 2000; Schimel et al. 2012), which may facilitate decomposition of more recalcitrant soil organic matter fractions (Fontaine and Barot, 2005).

Soil organic matter stability is influenced by the natural formation of aggregates (Adu and Oades, 1978; Nimmo, 2004). Conceptually, soil organic matter aggregates can be

differentiated by fractions with similar physical properties (e.g., particle size) and turnover rates (Parton et al., 1987; Cambardella and Elliott, 1992). Active fractions of the soil organic matter pool (*sensu* Parton et al. 1987) have short turnover times of days to months, and contain the most labile organic matter, predominantly that which is associated with microbial processes and byproducts. The slow organic matter pool has a turnover time of years to decades, and includes soil organic matter that is more physically protected and resistant to decomposition, such as fine roots and partially decomposed litter inputs (Parton et al., 1987). The passive soil organic matter pool has the longest turnover time of decades to centuries, and represents the humus material, or the very well decomposed and most recalcitrant organic matter, often associated with mineral surfaces and clay particles (Parton et al., 1987). Soil organic matter can cycle between these different pools based on the source of the organic matter as well as the physical environment of the soil (Parton et al., 1987; Cambardella and Elliott, 1992). Recently, this view of soil organic matter has been called into question for lacking insight to the complex microbial connections among plant exudates, litter decomposition, and stable soil organic matter formation (Schmidt et al. 2010; Cotrufo et al., 2013). New understanding of microbial dynamics is pointing to mechanisms of nutrient and soil organic matter dynamics that suggest these static views of organic matter fractions may underestimate the accessibility of "old" mineral associated fractions to microbial decomposition. However, the three pool conceptual model (*sensu* Parton et al. 1987) of organic matter dynamics is useful because it is easily compatible with physical fractions of organic matter that have distinct turnover dynamics and has been widely successful in describing soil recovery in agricultural systems (Garten and Wullshleger, 2000; Six et al., 2002).

The objectives of this research were to examine the soil organic matter dynamics in switchgrass plantings established within an agricultural watershed in the Shenandoah Valley of

Virginia. More specifically, I wanted to examine how switchgrass influences the recovery of different soil organic matter pools over time in order to identify mechanisms of soil organic matter formation. I used a variety of methods to measure carbon and nitrogen within the soil in order to quantify active, slow, and passive soil organic matter pools to understand stable soil organic matter formation, and to identify mechanisms for carbon sequestration and nitrogen retention within switchgrass soils. The results from this study will be used to understand the environmental and economic benefits of implementing switchgrass plantings within agricultural watersheds as a means to mitigate agriculturally-induced effects on soil carbon and nitrogen storage.

REVIEW OF LITERATURE

Switchgrass has been specified by the United States Department of Energy as an ideal bioenergy crop to be investigated not only for biofuel feedstock, but also for greater environmental benefits such as offsetting CO_2 emissions and improving soil quality (Mclaughlin et al., 2002). It has been well documented that switchgrass can sequester carbon and reduce nutrient losses in soils because of its extensive root system and high resource efficiency (Ma et al., 2000c; Zan et al., 2001; Frank et al,. 2004; Leibig et al., 2005; Leibig et al., 2008; Anderson et al., 2009; Collins et al., 2010; Schmer et al., 2011). However, the degree to which switchgrass influences these properties varies among different soils and land-use histories.

Switchgrass is well known for developing a very expansive and dense root system promptly after establishment, with coarse roots extending as deep as 3m (Weaver and Darland, 1949; Ma et al., 2000b). But, a majority of the root biomass, and thus soil organic carbon inputs, is allocated within the surface 50 cm of the soil (Weaver and Darland, 1949; Garten and Wullschleger, 1999; Ma et al, 2000b; Frank et al., 2004). Ma et al. (2000b) reported variability in root biomass production in different soil types, but on average over 50% of switchgrass roots were distributed within the top 30 cm of the soil in six year old switchgrass stands. Collins et al. (2010) sampled soils from five year old switchgrass plantings, and determined that 24% of carbon in the surface 15 cm was from new, root litter carbon inputs. These results were consistent with Garten and Wullschleger (2000), a study that used $\delta^{13}C$ analysis to determine that 19-31% of carbon in the uppermost 40 cm of five year old switchgrass soils was from new carbon inputs due to switchgrass root biomass additions. Because surface soils generally maintain an active microbial community, decomposition of these fine root litter inputs from

switchgrass production can be an important factor for soil organic carbon accumulation (Bransby et al., 1998; Garten and Wullschleger, 2000; Collins et al, 2010).

Switchgrass soils have been shown to have between 12-20% greater soil organic carbon concentrations when compared to cultivated crop soils, and soil organic carbon accumulation rates from 40 to 1010 g C m^{-2} yr^{-1} (Ma et al., 2000c; Zan et al., 2001; Frank et al., 2004; Leibig et al., 2005; Leibig et al., 2008; Anderson et al., 2009; Collins et al., 2010; Schmer et al., 2011). Leibig et al. (2005), assessed soil organic carbon in 42 switchgrass stands ranging from 2-18 years old with paired cultivated fields in Minnesota, North Dakota, and South Dakota, and found that on average switchgrass exhibited higher concentrations of soil organic carbon than cultivated cropland by 17.5% at 0-5 cm and 12.7% at 60-90 cm. In congruent studies, Leibig et al. (2008) and Schmer et al. (2011) determined soil organic carbon accrual rates of 50, 110, 240, and 209 g C m^{-2} yr^{-1} at depths of 0-20, 0-30, 0-90, and 0-120 cm, respectively, in superficial soils of five year old switchgrass stands in the same geographic region. This data is consistent with Zan et al. (2001), Frank et al. (2004), Anderson et al. (2009), and Collins et al. (2010), all of which observed increases in soil organic carbon stocks in younger switchgrass stands compared to corresponding row crop agricultural soils. In southwestern Quebec, Zan et al. (2001) reported 272 to 317 g C m^{-2} yr^{-1} organic carbon accumulation in four year old switchgrass soils from 30-60 cm. Frank et al. (2004) sampled a variety of switchgrass cultivars in North Dakota, and found an average soil organic carbon increase of 1010 g C m^{-2} yr^{-1} at a 90 cm profile three years after establishment. Collins et al. (2010) found 20% (~120 g C m^{-2}) soil organic carbon increases in superficial soils five years after switchgrass conversion in the state of Washington. Anderson et al. (2009) completed a review to assess soil organic carbon changes in switchgrass within the literature and reported a 1.8% (~ 40 g C m^{-2} yr^{-1}) annual increase in soil organic carbon in

published studies of switchgrass establishment with an average age of four years. The general increase in concentrations and rates of soil organic carbon is attributed to microbial decomposition of root biomass inputs from switchgrass establishment, but variation in quantities is due to differences in soil type, geographic location, site management history, and the maturity of the switchgrass.

Some research suggests that stand age has a considerable influence on switchgrass carbon sequestration. Ma et al. (2000c) sampled switchgrass stands in Alabama at two time-steps; two years and ten years following switchgrass establishment. There were no significant changes in soil organic carbon after two years, but 28-45% increases in the surface 30 cm of switchgrass soils after ten years (Ma et al., 2000c). Although many studies show increases in soil organic carbon under young switchgrass, this study implies the rate at which soil organic carbon is sequestered increases as switchgrass stands mature.

In addition to increasing bulk soil organic carbon stocks, changes in microbial biomass and mineralizable carbon pools under switchgrass soils have been observed (Ma et al., 2000a; Haney et al., 2010). The magnitude of the microbial biomass carbon pool and subsequent function to decompose organic matter is controlled by availability of soil carbon (Schimel et al., 2012: Haney et al., 2010), and additional root litter inputs from switchgrass establishment should increase microbial biomass and mineralizable carbon pools (Ma et al., 2000a; Haney et al. 2010).Ma et al. (2000a) observed considerable increases in carbon mineralization and microbial biomass carbon in two year old switchgrass stands in the southeastern United States. In the surface 15 cm of the soil, carbon mineralization increased by 112% and microbial biomass carbon increased by 168%. In addition, a 254% increase in carbon mineralization was observed at 15-30 cm (Ma et al., 2000a). Haney et al. (2010) compared long term cultivated fields with 10

year old switchgrass stands and also found significantly higher amounts of microbial biomass carbon and carbon mineralization rates in switchgrass soils. In this study, switchgrass exhibited carbon mineralization rates 37.5% higher than corn, and a 33% increase in microbial biomass carbon (Haney et al., 2010).

Although it is evident that switchgrass can increase soil organic carbon, some studies have observed decreases in soil organic carbon following switchgrass establishment (Schmer et al., 2011; de Graaff et al., 2014; Strickland et al., 2015). Strickland et al. (2015) reported a 24% decrease in overall soil organic carbon stocks within three year old switchgrass stands intercropped in loblolly pine plantations. When examining different carbon pools, a 29% and a 43% decrease was found in the mineralizable carbon and particulate organic matter carbon pools, respectively (Strickland et al., 2015). Schmer et al. (2011) observed significant soil organic carbon decreases of 290 and 560 g C m^{-2} at 2 of 10 sites sampled before and five years after switchgrass conversion in South Dakota. De Graaff et al. (2014) used a laboratory incubation to examine changes in microbial activity due to increased root-exudate inputs in switchgrass soils, and found that increasing root-exudates stimulated decomposition of soil organic matter and the loss of older soil organic carbon fractions. Because carbon is an energy source for soil microbes, the large inputs of root biomass from the switchgrass stands stimulates microbial mineralization of carbon (Schimel et al., 2012; de Graaff et al., 2014; Strickland et al., 2015). However, this stimulation of decomposition can prime microbes to not only decompose new litter inputs, but also native, more recalcitrant soil organic matter, leading to a decline in soil organic carbon concentrations (Dijkstra and Cheng., 2007; Phillips et al., 2012; de Graaff et al. 2014; Strickland et al. 2015). When considering soil carbon dynamics, it is necessary to consider soil nitrogen dynamics due to the intrinsic relationship between these two nutrient elements. When

microorganisms decompose soil organic matter to acquire and mineralize carbon, they are also facilitating a transformation of nitrogen (Schlesinger, 2009).

It is suggested that the increased soil organic carbon stocks from switchgrass establishment can change microbial nitrogen dynamics, specifically altering nitrogen availability for plant uptake (Tufekcioglu et al. 2003; Smith et al., 2013; Minnick et al., 2015). Tufekcioglu et al. (2003) reported that ten year old switchgrass stands in Iowa immobilized nitrogen into below ground biomass at a rate of 1.6 g N m^{-2} yr^{-1} in ten years (Tufekcioglu et al., 2003). Smith et al. (2013) reported strong decreases in nitrate leaching annually from the surface 50 cm soil profile of young switchgrass stands, declining from 2.5 g N m^{-2} yr^{-1} to < 0.5 g N m^{-2} yr^{-1} in 3 years. Minnick et al., (2015) found that nitrogen mineralization rates varied seasonally in switchgrass planted within the interbeds of loblolly pine plantations, observing lower rates during the growing season and higher rates during the non-growing season (Minnick et al. 2015). These results show that switchgrass does have the ability to alter nitrogen dynamics in soil, but more research is needed to understand the changes to specific mechanisms occurring during switchgrass establishment.

METHODS

Site Description: Merck, Sharp, & Dohme Corporation agricultural field site

The study site for this research project was located on property owned by the Merck, Sharp, & Dohme Corporation in Elkton, Virginia, USA. The site is located on the boundary of the Blue Ridge and the Ridge and Valley physiographic regions in the Shenandoah Valley. This region is one of the driest areas in Virginia, with a mean annual precipitation of 86 cm. The property is divided into two sections by the Shenandoah River, with the Stonewall pharmaceutical production plant located south of the river and an agricultural field site located north of the river (Fig. 1.). According to the United States Department of Agriculture, Natural Resources Conservation Service (USDA-NRCS), Soil Survey Division's official database of soil series descriptions (https://soilseries.sc.egov.usda.gov/osdname.asp), there are four distinct soil series present within the agricultural fields where this study was conducted (Table 1.). These soil series are commonly found in the Northern Appalachian region, specifically within river valleys, hills, or pasture landscapes. Soil texture varied by series, described as either fine sandy loams, loamy sands, or cobbly fine sandy loams (Table 1.).

The agricultural land at this site has undergone a variety of row crop cultivation since the mid-nineteenth century. In the last 30 years that the Merck, Sharp, & Dohme Corporation has owned the property, management has been primarily a rotation of corn and soybean row crop agriculture. Starting in 2011, replicate agricultural fields were converted to switchgrass stands to generate a chronosequence of switchgrass plantings ranging from one to four years of age (Fig. 1). Fields were chosen for switchgrass conversion based on the row crop rotation, following a pattern of establishing switchgrass on fields that had been on a soybean rotation the previous growing season in order to reduce nitrogen losses. The switchgrass stands were seeded using a

no-till drill, a piece of planting equipment that differs from traditional equipment by removing the need to till before the seeds are sown. Because the soils are not tilled, planting using a no-till drill minimizes soil disturbance and erosion, and thus is expected to increase yield. The switchgrass stands were managed to use the aboveground biomass for biofuel feedstock. The harvest of the switchgrass was delayed until after hard freezes in the winter in order to maximize nutrient translocation to root stocks.

In 2014, all fields at this site had been converted to switchgrass except for two fields that were maintained as row crop agriculture and one small plot of land from the United States Department of Agriculture Conservation Reserve Program (CRP) (Fig. 1.). The Conservation Reserve Program is a program for soil management that subsidizes farmers to remove land from row crop circulation in order to reduce soil erosion, recharge groundwater, improve soil fertility, and increase wildlife habitat. The CRP field at this agricultural field site had been abandoned and designated CRP land for 15 years.

Soil sampling description

In August 2014, soil samples were collected from switchgrass and row crop fields, as well as the CRP land. Switchgrass fields ranged from one to four years old, and were designated by their age (Fig 1.). Fields labeled "1st year switchgrass" refer to the youngest switchgrass, or those planted in 2014. Fields labeled "4th year" refer to the oldest switchgrass stands, or those planted in 2011. There was replication at the field level for all fields except for the 4th year switchgrass and CRP field. Within each field, transect points were created for sampling using a stratified random approach based on field size and topography. These transect points were intended to reduce in-field variation, and thus samples from these points were considered

representative of the entire field. At each transect point, a 100 m tape was pulled toward a randomly selected orientation. Samples were collected at five locations along the 100 m tape, with approximately 20 m between each location. Samples were collected at three depths: 0-20 cm, 20-40 cm, and 40-60 cm, resulting in 15 samples from each field (five samples at each depth increment).

Samples were collected using a common bucket auger method. Soil was removed from the ground and homogenized in a bucket before being placed in a plastic sample bag. Bulk soil samples were kept in coolers until they were returned to Virginia Tech (Blacksburg, VA, USA), and then stored at 4°C for further analysis. Each soil sample was passed through a 4 mm sieve before analysis (Sollins et al. 1999). Field water content and oven dry weight equivalent was determined by mass loss after 48 hours at 90° C in a standard convection oven. Soil pH was measured electrochemically.

A hammer core fitted with four, 5 cm diameter by 5 cm length metal sleeves was used to collect root biomass samples at the top 0-20 cm of the soil. Litter from the surface of the soil was removed and the hammer core was driven 20 cm into the soil (Elliott et al. 1999). Soil cores collected within the metal sleeves were removed, and coarse (>2 mm) and fine (<2 mm) roots were sieved from the soil cores. Roots were dried and weighed, and root biomass was estimated from summing the mass of the roots from each of the four metal sleeves.

Total soil organic carbon and total nitrogen

Bulk soil samples were analyzed to quantify the total amount of carbon and nitrogen within each sample. Total soil organic carbon and total nitrogen were estimated by dry combustion from a ~15 mg subsample of dried and ground soil using a FlashEA 1112 NC

Elemental Analyzer (CE Eltantech, Lakewood, NJ, USA) (Sollins et al. 1999). The results were expressed as a concentration of carbon (g C kg dry soil^{-1}) and nitrogen (g N kg dry soil^{-1}) by depth.

Particulate organic matter fractionation

I performed a particulate organic matter fraction technique (modified from Six et al., 2000) to isolate soil organic matter fractions and quantify the amount of carbon and nitrogen within each fraction. This procedure was completed on a subset of total soil samples collected from the row crop, switchgrass, and CRP fields. The subset included two replicate fields for each age of switchgrass and row crop fields; however, there were no replicate fields for the 4th year switchgrass and CRP fields. Soil samples were separated into particle size classes to operationally distinguish three different soil organic matter pools: macroaggregates (250-2000 µm), microaggregates (53-250 µm), and the mineral-associated soil organic matter fraction (<53 µm).

I placed a 2000 µm sieve in a basin with water approximately 1 cm above the sieve mesh. I gently sprinkled a ~50 g subsample of bulk soil at field water content evenly onto the sieve mesh and allowed the sample to settle in the water for five minutes. I sieved the soil by moving the sieve up and down at an angle within the water for two minutes. I removed the sieve from the water and rinsed the outside with water to ensure that no soil particles were lost during sieving. I poured the water and soil particles that passed through the 2000 µm sieve onto a 250 µm mesh sieve in a second basin with water approximately 1 cm above the sieve mesh. I repeated the sieving procedure for two minutes. I removed the sieve from the water and rinsed the outside of the sieve with water into the basin. I backwashed the soil particles that remained on the 250 µm

sieve into pre-weighed drying pans. This collected the macroaggregate soil organic matter fraction (250- 2000 µm). This process was repeated once more with a 53 µm sieve. The particles that remained on the 53 µm sieve were backwashed into pre-weighed drying pans. This collected the microaggregate soil organic matter fraction (53- 250 µm). The water and soil particles that passed through the 53 µm sieve were then poured into pre-weighed drying pans. This collected the mineral associated soil organic matter fraction (<53 µm). I placed all the pre-weighed drying pans containing soil fractions and water in a convection oven and dried the fractions at 60° C for 48 hours (Six et al., 2000).

Each separated fraction of soil was analyzed to quantify the amount of carbon and nitrogen within each fraction. Soil organic carbon and nitrogen were estimated by dry combustion from a ~15 mg subsample of dried and ground soil using a FlashEA 1112 NC Elemental Analyzer (CE Eltantech, Lakewood, NJ, USA) (Sollins et al. 1999). The results were expressed as a concentration of carbon (g C kg dry soil^{-1}) or nitrogen (g N kg dry soil^{-1}) of the fraction within the total soil sample by depth.

Mineralizable carbon and nitrogen

I conducted a short term, aerobic incubation experiment (modified from Strickland et al., 2010) to estimate the amount of mineralizable carbon and nitrogen within the soil samples. This experiment was conducted on the top 0- 20 cm soil samples from subset selected for the particulate organic matter fractionation procedure.

I distributed ~10 g of field moisture soil into 50 mL centrifuge tube. I placed air-tight caps with a septum onto the centrifuge tubes and flushed the soil with CO_2 compressed free air at 2 bar for two minutes. I placed the centrifuge tubes in a 20 °C incubator for approximately 24

hours. After 24 hours, I measured the CO_2 headspace concentration from each centrifuge tube using an infrared gas analyzer (IRGA; Model LI-7000, Li-Cor Biosciences, Lincoln, Nebraska, USA) (Paul et al, 2001). Following this initial incubation, each soil sample was adjusted to reach a 65% water holding capacity. Samples were assayed following the described procedure on days 3, 4, 6, 9, 23, and 33, with the experiment ending on day 34. During the duration of the experiment, soils were adjusted with water to maintain a 65% water holding capacity, and stored uncapped in the incubator in between incubation periods. The total mineralizable carbon was calculated by integrating the amount of CO_2 produced over time, and the results were expressed as a concentration of mineralizable carbon (g C kg dry soil^{-1}) (Strickland et al.,2010).

I took ~10 g subsamples of each soil samples on day 0 and day 34 day of the experiment to analyze for potential nitrogen mineralization (Roberston et al. 1999). Inorganic nitrogen was extracted from each subsample by adding 50 ml of 2 M KCl and shaking the sample at 250 RPM for 30 minutes on an orbital shaker. I collected the extracts by gravity filtering the soil solution through Whatman #42 filter paper, and NH_4^+ and NO_3^- concentrations were estimated using a Lachat QuikChem 8500 Flow Injection Analyzer (Lachat Instruments, Loveland, CO, USA). Total inorganic nitrogen was calculated as the sum of NH_4+ and NO_3- concentrations, and the results were expressed as a concentration of mineralizable nitrogen (g N kg dry soil^{-1}) (Robertson et al. 1999; Paul et al. 2001).

Microbial biomass

I used a simultaneous chloroform fumigation-extraction (sCFE) method to quantify the microbial biomass in the soil. The sCFE method determines the amount of microbial biomass in the soil from the amount of carbon extracted after the soil is treated with chloroform (Fierer and

Schimel, 2003; Strickland et al., 2010). I completed this procedure for the top 0-20 cm soil samples from the subset selected for the particulate organic matter fractionation procedure.

I weighed out two ~10 g subsamples of each sample into 70 mL glass tubes identified as a chloroform exposed sample and a control sample. I dispensed 1 mL of EtOH-free chloroform to the chloroform exposed samples, and sealed both the chloroform exposed and the control samples with chloroform resistant caps. I then placed all glass tubes onto a side arm shaker for four hours at 150 rev min^{-1}. After four hours, I gravity filtered the soil extracts through Whatman #42 filter paper and collected the filtrate in plastic vials. The filtrates were then bubbled with compressed air for 60 minutes to remove the remaining chloroform. The extracts were measured for labile carbon using a using a Model 1010 Total Organic Carbon Analyzer (OI Analytical, College Station, TX, USA), and the results were expressed as a concentration of microbial biomass carbon (g C kg dry $soil^{-1}$) (Fierer and Schimel, 2003; Strickland et al., 2010).

Bulk Density

An individual set of soil samples were collected for bulk density at all fields previously sampled in April of 2015. At each site, five samples were collected along a 100 m transect, with approximately 20 m between each sampling location. Transect points had been previously designated in each field using a stratified random allocation based on field size and year of switchgrass establishment. Samples were collected at three depths: 0-20 cm, 20-40 cm, and 40-60 cm, resulting in 15 samples from each field.

A hammer core with 5 cm diameter and 5 cm length metal sleeves was used to collect the bulk density samples. I cleared the litter from the surface of the soil and drove the hammer core 20 cm into the soil (Elliott et al. 1999). I removed the metal sleeve containing the soil core and

used a knife to level the ends of the sample with the top and bottom of the metal sleeve. I placed rubber caps on the top and bottom of the sleeve to keep the core intact for transportation and analysis. I used an auger to remove soil in the hole to reach the next depth profile. I repeated this hammer core method to obtain soil cores from the 20-40 cm and 40-60 cm depth profiles. Samples were transported to the Blacksburg, VA campus of Virginia Tech for analysis.

Samples were placed in a standard convection oven at 105 °C for ~24 hours then weighed to obtain the dry mass. Bulk density (g cm^{-3}) was calculated as the amount of dry weight of the soil (g) within the volume of the core (cm^3) (Elliott et al. 1999).

Statistical analyses

The mean and standard error of each treatment type was calculated by depth for each response variable: bulk density, soil pH, total organic carbon and nitrogen, root biomass carbon, macroaggregate soil organic matter carbon and nitrogen (250-2000 µm), microaggregate soil organic matter carbon and nitrogen (53-250 µm), mineral-associated carbon and nitrogen (<53 µm), mineralizable carbon and nitrogen, and microbial biomass carbon. The distribution of each response variable followed a normal distribution, so a one-way analysis of variance (ANOVA) was used to test for significant differences in soils between the age of switchgrass establishment, row crop agriculture, and CRP land. Since the 4^{th} year switchgrass and CRP treatments did not include replication at the field level, supplementary ANOVAs were performed including only treatments with replicate fields to determine if additional statistical power yielded differences in the insights about the role of switchgrass on soil organic matter dynamics. The two sets of analyses yielded very similar results (Table 2.), and the ANOVAs including all treatment types are reported. If the F-test from the ANOVA was statistically significant ($\alpha \leq 0.05$), Tukey's

honest significant difference test was used to compare treatment means. All analyses were performed using R software version 3.2.0 (R Development Core Team, 2015).

RESULTS

Bulk Density and pH

Soil bulk density ranged from 1.23 ± 0.05 to 1.59 ± 0.06 g cm^{-3} within the surface 60 cm across treatments, generally increasing with depth (Table 3.). Overall differences between treatments were small, but mean values were significantly different in the 0-20 cm depth (Table 3.; $P \leq 0.05$). At 0-20 cm, bulk density was highest in 2nd year switchgrass (1.50 ± 0.04 g cm^{-3}) and lowest in CRP soils (1.23 ± 0.05 g cm^{-3}). There were no significant differences in bulk density among row crop, 1st year, 3rd year, or 4th year switchgrass treatments. Bulk density varied slightly across treatments between 20-40 and 40-60 cm, but means were not statistically significant (Table 3.)

Soil pH ranged from 5.7 ± 0.1 to7.0 ± 0.1 within the surface 60 cm (Table 3.), and there were significant differences in mean pH across treatments at all three depth profiles. At 0-20 cm, pH was highest in the older switchgrass treatments (6.3 ± 0.1 to 6.5 ± 0.20, and lowest in 1st year switchgrass soils (Table 3.; $P \leq 0.01$). The lowest pH at the 20-40 cm depth increment was also 1st year switchgrass, and pH was highest in 2nd year switchgrass soils (Table 3.; $P \leq 0.05$). At 40-60 cm, pH was also highest in 2nd year switchgrass treatments, but lowest in 3rd year switchgrass treatments (Table 3.; $P \leq 0.05$). There was no variation in pH of CRP and row crop soils at any depth increment (Table 3.).

Total organic carbon and nitrogen

There were significant differences in total organic carbon across treatments, but the most pronounced pattern observed at all depth profiles was the substantial decrease in mean soil organic carbon concentrations between 1st year and all subsequent switchgrass treatments (Fig.

2a-c). At 0-20 cm, total organic carbon ranged from 8.94 ± 0.48 to 19.77 ±1.53 g C kg dry soil^{-1}, with the lowest concentrations observed in 4th year switchgrass soils and the highest concentrations observed in CRP soils (Fig. 2a.; $P \leq 0.0001$). Surprisingly, the concentration of soil organic carbon was 53% lower in 2nd year switchgrass (9.0 ± 0.52 g C kg dry soil^{-1}) soils compared to 1st year switchgrass (19.05 ± 2.85 g C kg dry soil^{-1}) soils. Soil organic carbon concentrations in 3rd year and 4th year switchgrass soils remained low at 8.94 ± 0.48 and 9.60 ± 0.61 g C kg dry soil^{-1}, respectively, and row crop concentrations, 13.22 ± 1.7 g C kg dry soil^{-1}, were not statistically different than any other treatment.

Mean total soil organic carbon concentrations decreased with depth, but the overall pattern across treatments was the same. At 20-40 cm, total soil organic carbon ranged from 3.78 ± 0.28 to 8.21 ± 1.15 g C kg dry soil^{-1}, however the lowest and highest concentrations were observed in the 3rd year and 1st year switchgrass stands, respectively (Fig. 2b.; $P \leq 0.001$). From 1st year switchgrass (8.21 ± 1.15 g C kg dry soil^{-1}) to 2nd year switchgrass (4.12 ± 0.43 g C kg dry soil^{-1}) treatments, total soil organic carbon also decreased by 50%, and concentrations remained low in 3rd year switchgrass (3.78 ± 0.28 g C kg dry soil^{-1}) and 4th year switchgrass (5.58 ± 0.35 g C kg dry soil^{-1}) soils. The mean total organic carbon concentration in row crop soils, 6.49 ± 0.99 g C kg dry soil^{-1}, was also not statistically different than any other treatment. Total soil organic carbon ranged from 2.33± 0.14 to 5.12 ± 1.31 g C kg dry soil^{-1} at 40-60 cm depth, and 2nd year switchgrass soils (2.33 ± 0.14 g C kg dry soil^{-1}) showed total soil organic carbon concentrations 52% lower than the 1st year switchgrass soils (4.83 ± 0.65 g C kg dry soil^{-1}) (Fig. 2c.; $P \leq 0.01$). Total soil organic carbon concentrations in 3rd year switchgrass and 4th year switchgrass were similar to 2nd year switchgrass soils at 2.65 ± 0.25 and 2.45 ± 0.3 g C kg

dry soil^{-1}, respectively, and the mean row crop total carbon concentration, 3.93 ± 0.61 g C kg dry soil^{-1}, did not differ statistically from any other treatment.

Total nitrogen results mirrored those of total soil organic carbon at the 0-20 cm depth profile (Fig. 2d.). There were significant differences in total nitrogen across treatments, and a pronounced decrease between 1st and 2nd year switchgrass treatments. Total nitrogen ranged from 0.81 ± 0.03 to 1.93 ± 0.14 g N kg dry soil^{-1}, and the lowest and highest concentrations were also observed in the 4th year switchgrass and CRP soils (Fig. 2d; $P \leq 0.001$). Total nitrogen was 47% lower in 2nd year switchgrass (0.92 ± 0.03 g N kg dry soil^{-1}) soils than 1st year switchgrass (1.74 ± 0.26 g N kg dry soil^{-1}). The mean total nitrogen concentration 3rd year switchgrass (0.92 ± 0.03 g N kg dry soil^{-1}) was similar to that of 2nd year switchgrass and 4th year switchgrass, and concentrations in row crop soils were not statistically different than any other treatment.

At deeper depth increments, total nitrogen was similar across all treatments, except for 4th year switchgrass, where nitrogen content was significantly greater (Fig. 2e, f.). At 20-40 cm, total nitrogen ranged from 0.48 ± 0.03 to 1.65 ± 0.65 g N kg dry soil^{-1} across treatments (Fig. 2e; $P \leq 0.0001$). Total nitrogen concentrations were similar in row crop (0.76 ± 0.08 g N kg dry soil^{-1}), 1st year switchgrass (0.84 ± 0.08 g N kg dry soil^{-1}), 2nd year switchgrass (0.49 ± 0.04 g N kg dry soil^{-1}), and 3rd year switchgrass treatments (0.48 ± 0.03 g N kg dry soil^{-1}), but 4th year soils exhibited concentrations 50-70% greater compared to the other treatments. The CRP total nitrogen concentration (0.92 ± 0.04 g N kg dry soil^{-1}) was not statistically different than any other treatment. Similar patterns were observed at 40-60 cm, with total nitrogen concentrations ranging from 0.37 ± 0.02 to 1.80 ± 0.53 g N kg dry soil^{-1} (Fig. 2f; $P \leq 0.0001$). Mean total nitrogen concentrations were similar in CRP (0.7 ± 0.07 g N kg dry soil^{-1}), row crop (0.57 ± 0.05 g N kg dry soil^{-1}), 1st year switchgrass (0.58 ± 0.05 g N kg dry soil^{-1}), 2nd year switchgrass (0.37

± 0.02 g N kg dry soil^{-1}), and 3rd year switchgrass (0.39 ± 0.03 g N kg dry soil^{-1}). 4th year switchgrass total nitrogen concentrations were significantly greater than all other treatments at 1.80 ± 0.53 g N kg dry soil^{-1}.

Particulate organic matter carbon and nitrogen

At 0-20 cm depth, particulate organic matter fractions of carbon and nitrogen showed patterns similar to that of the total organic carbon and total nitrogen pools; there were significant differences in mean concentrations across treatments, but the greatest difference was between the 1st year switchgrass and the older switchgrass establishments (Table 4.). Both carbon and nitrogen concentrations were greatest in the macroaggregate fraction and lowest in the mineral-associated fraction for all treatments except 3rd year switchgrass, where the lowest concentrations were seen in the microaggregate fraction. Mean concentrations of carbon within macgroaggregates ranged from 3.58 ± 0.38 to 11.45 ± 1.5 g C kg dry soil^{-1}, and concentrations were lowest in 2nd year switchgrass soils and highest in CRP soils (Table 4.; $P \leq 0.0001$). The concentration of carbon in the 2nd year switchgrass was 50% lower than 1st year switchgrass (7.26 ± 0.6 g C kg dry soil^{-1}), and 3rd year and 4th year switchgrass treatments were also low at 4.01 ± 0.44 and 4.63 ± 0.56 g C kg dry soil^{-1}, respectively. The mean row crop carbon concentration was higher than all switchgrass treatments (8.39 ± 1.82 g C kg dry soil^{-1}), but not statistically different from CRP and 1st year switchgrass concentrations. These same patterns were seen within the nitrogen analysis; concentrations ranged from 0.34 ± 0.03 to 1.1 ± 0.15 g N kg dry soil^{-1}, with the lowest and highest concentrations seen within 2nd year switchgrass and CRP soils, respectively (Table 4.; $P \leq 0.001$). The 1st year switchgrass treatment showed nitrogen concentrations 55% greater than 2nd year soils, and concentrations in the 3rd year switchgrass

(0.39 ± 0.05 g N kg dry soil^{-1}) and 4th year switchgrass (0.42 ± 0.04 g N kg dry soil^{-1}) soils were similarly low like the 2nd year treatment. The mean row crop nitrogen concentration (0.79 ± 0.19 g N kg dry soil^{-1}) was not statistically different from CRP, 1st year switchgrass, 3rd year switchgrass, or 4th year switchgrass treatments

The microaggregate fraction showed carbon and nitrogen results similar to that of the macroaggregate fraction at 0-20 cm, however, concentrations were lowest in the row crop soils and highest in the 3rd year switchgrass soils (Table 4.). Carbon concentrations ranged from 1.87 ± 0.12 to 4.8 ± 0.32 g C kg dry soil^{-1} (Table 4.; $P \leq 0.0001$), and there was a 42% difference in concentrations between 2nd year switchgrass (2.74 ± 0.2 g C kg dry soil^{-1}) and 1st year switchgrass (4.75 ± 0.33 g C kg dry soil^{-1}) treatments. 3rd year switchgrass (1.87 ± 0.12g C kg dry soil^{-1}) and 4th year switchgrass (2.93 ± 0.32 kg dry soil^{-1}) concentrations were similar to that of 2nd year switchgrass concentrations, and the row crop carbon concentration (4.8 ± 0.32 g C kg dry soil^{-1}) was greater than all switchgrass treatments, and similar to the CRP (4.46 ± 0.61 kg dry soil^{-1}) and 1st year switchgrass treatments. Nitrogen concentrations ranged from 0.19 ± 0.01 to 0.44 ± 0.04 g N kg dry soil^{-1}, and the difference between 1st year switchgrass (0.41 ± 0.03g N kg dry soil^{-1}) and 2nd year switchgrass (0.28 ± 0.02 g N kg dry soil^{-1}) treatments was 32% (Table 4.; $P \leq 0.0001$). The 4th year switchgrass mean nitrogen concentration (0.27 ± 0.02 g N kg dry soil^{-1}) was higher than the younger switchgrass treatments, but statistically different from CRP (0.42 ± 0.04 g N kg dry soil^{-1}) and 1st year switchgrass treatments.

Within the mineral-associated fraction, mean carbon and nitrogen concentrations were lowest in 2nd year switchgrass treatments and highest in row crop treatments (Table 4.). Carbon ranged from 1.62 ± 0.16 to 3.43 ± 0.52 g C kg dry soil^{-1}, and there was a 45% difference between 2nd year switchgrass and 1st year switchgrass treatments (2.96 ± 0.25 g C kg dry soil^{-1}) (Table 4.;

$P \leq 0.05$). Carbon concentrations in 3rd year switchgrass and 4th year switchgrass treatments were also lower than the row crop treatment at 2.43 ± 0.63 and 2.09 ± 0.22 g C kg dry soil^{-1}, respectively. Nitrogen concentrations ranged from 0.16 ± 0.02 to 0.34 ± 0.06 g N kg dry soil^{-1} (Table 4.; $P \leq 0.05$). The mean nitrogen concentration within 2nd year switchgrass treatments (0.16 ± 0.02 g N kg dry soil^{-1}) was 41% lower than the 1st year switchgrass treatments (0.27 ± 0.02 g N kg dry soil^{-1}), but the values were not statistically different. There were no differences in nitrogen concentrations between 1st year switchgrass, 3rd year switchgrass (0.22 ± 0.05 g N kg dry soil^{-1}), 4th year switchgrass (0.18 ± 0.02 g N kg dry soil^{-1}), and CRP (0.22 ± 0.02 g N kg dry soil^{-1}) treatments, and all were lower than the mean row crop concentration (0.34 ± 0.06 g N kg dry soil^{-1}).

At the 20-40 and 40-60 cm depth profiles, there were also significant differences seen in mean carbon and nitrogen concentrations across treatments within some particulate organic matter fractions, but the patterns observed were more variable (Table 4.). At 20-40 cm, mean carbon concentrations were significantly different across treatments within the macroaggregate (Table 4.; $P \leq 0.001$), microaggregates (Table 4.; $P \leq 0.01$), and mineral-associated (Table 4.; $P \leq 0.05$) fractions. Nitrogen concentrations were significantly different across treatments for the macroaggregate fraction (Table 4.; $P \leq 0.05$), but there were no significant differences across treatments within the microaggregate and mineral-associated fractions (Table 4.). From 40-60 cm, there were significant differences in carbon concentrations across treatments for the macroaggregate (Table 4.; $P \leq 0.05$), microaggregate (Table 4.; $P \leq 0.01$), and mineral-associated fractions (Table 4.; $P \leq 0.05$). Additionally, nitrogen concentrations were significantly different across treatments for the macroaggregate (Table 4.; $P \leq 0.05$), microaggregate (Table 4.; $P \leq 0.01$), and mineral-associated fractions (Table 4.; $P \leq 0.01$).

Root biomass carbon

The concentration of carbon from root biomass in switchgrass soils initially declined substantially following conversion from row crop; however, mean carbon concentrations consistently increased over time (Fig. 3.). Carbon from root biomass was 85% lower in 1st year switchgrass treatments (0.42 ± 0.15 g C kg dry soil^{-1}) when compared to row crop soils (2.74 ± 0.74 g C kg dry soil^{-1}). Soils within the 2nd year, 3rd year, and 4th year switchgrass treatments showed annual increases, with mean values of 0.5 ± 0.14, 1.43 ± 0.44, 1.92 ± 0.3 g C kg dry soil^{-1}, respectively. Although there was a visible increase in carbon across the chronosequence, the pattern observed was not statistically significant.

Mineralizable carbon and nitrogen, and microbial biomass

There was little variation in mineralizable and microbial biomass carbon across treatments, but switchgrass greatly influenced mineralizable nitrogen concentrations (Fig. 4.). Mineralizable carbon ranged from 0.063 ± 0.005 to 0.076 ± 0.005 g C kg dry soil^{-1}, and there were no statistically significant differences in means across treatments (Fig. 4a.). Microbial biomass carbon showed similar patterns (Fig. 4b.). Mean values ranged from 0.029 ± 0.003 to 0.056 ± 0.004 g C kg dry soil^{-1}, and were not statistically significant across treatments.

Unlike mineralizable and microbial carbon, mineralizable nitrogen concentrations varied across treatments (Fig. 4c). The maximum concentration of mineralizable nitrogen was observed in CRP soils, 0.010 ± 0.0034 g N kg dry soil^{-1}, and there was an observed decline in concentrations over time. Mineralizable nitrogen concentrations in row crop, 1st year, 2nd year, and 3rd year treatments were 0.0077 ± 0.0044, 0.0062 ± 0.0014, 0.0015 ± 0.0005, and 0.0015 ± 0.007 g N kg dry soil^{-1}, respectively. 4th year soils not only continued this pattern of decline, but

actually exhibited an uptake of -0.00026 ± 0.005 g N kg dry soil^{-1}. There was a strong pattern of decreasing nitrogen mineralization across treatments, however, the pattern was not statistically significant (Fig. 4c; P= 0.07).

The proportion of actively cycling carbon within the bulk soil organic carbon pool increased across switchgrass treatments; when mineralizable carbon is presented as a proportion of the bulk soil organic carbon pool there were significant differences among treatments (Fig. 5.; $P \leq 0.0001$). The proportions of labile carbon from CRP, row crop, and 1st year switchgrass soils were similar, with labile pool proportions of 0.36%, 0.45%, and 0.46% of bulk carbon pools, respectively. The 2nd and 3rd year switchgrass treatments showed greater proportions of labile carbon pools of 0.76% and 0.82%, respectively. There was a slight decrease in the proportion of labile carbon within the 4th year switchgrass soils, but it was not significantly different from 2nd and 3rd year switchgrass treatments.

DISCUSSION

The need to implement more efficient agricultural management practices to improve soil fertility and promote carbon sequestration is evident, from the necessity of promoting long-term agricultural sustainability as well as the need to offset anthropogenic CO_2 emissions (Vitousek et al., 1997; Lal, 2004a). Management practices targeted at enhancing soil organic matter storage is an effective way to achieve both of these goals (Tisdall and Oades, 1982; Lal and Kimble, 1997; Lal, 2004b). Previous studies have demonstrated that converting agricultural lands to switchgrass pastures typically increase soil organic matter content, contributing to the recovery of soil structure, nutrient retention capacity, and resulting in improved soil fertility (Schimel, 1986; Bransby et al., 1998; Ma et al., 2000c; Zan et al., 2001; Frank et al., 2004; Leibig et al., 2005; Leibig et al., 2008; Anderson et al., 2009; Collins et al., 2010; Schmer et al., 2011). However, my results exhibit different patterns: notably significant decreases in soil organic matter pools (carbon, nitrogen, and POM fractions) following the conversion from row-crop agriculture to switchgrass plantings.

Using a field experiment to examine how converting long-term, row crop fields to perennial switchgrass pastures influences the recovery of soil organic matter over one to four years, my results show that soils under switchgrass plants lost bulk soil organic matter and surface particulate organic matter pools following switchgrass establishment. Mean volumetric quantities of total organic carbon in the top 0-20 cm of soil profiles, estimated by the addition of particulate organic matter fractions, and root biomass, as well as bulk organic carbon concentrations declined following switchgrass plantings (Fig. 6.). The 1st year switchgrass treatment did not differ significantly from the row crop treatment, there were significant declines as switchgrass aged from one to two years old, and pools did not recover up to four years after

establishment (Fig. 6.). Similar patterns were observed in the total organic carbon and the particulate organic matter fractions (Fig. 6.). The 2nd year switchgrass treatment showed total soil organic carbon concentrations 53% lower than the 1st year treatment, and 50%, 42%, and 45% lower within the macroaggregate, microaggregate, and mineral-associated particulate organic matter fractions, respectively. The concentration of carbon in these pools within 3rd year and 4th year switchgrass treatments were similar to those of the 2nd year treatment, indicating there was no recovery of soil organic matter within 4 years of switchgrass planting in these fields.

These results are in stark contrast to other studies that typically show increases in soil organic matter after planting switchgrass generally within a few years. Other studies have reported switchgrass plantings having up to 20% more soil organic carbon than comparable row crops, and carbon accumulation rates anywhere from 40 to 1010 g C m^{-2} yr^{-1} (Ma et al., 2000c; Zan et al., 2001; Frank et al., 2004; Leibig et al., 2005; Leibig et al., 2008; Anderson et al., 2009; Collins et al., 2010; Schmer et al., 2011). Switchgrass has also been shown to increase particulate organic matter content rather rapidly as stands mature (Dou et al, 2013), which is why it is significant and surprising that my results did not show recovery of particulate organic matter pools, particularly within the macroaggregate fraction of switchgrass soils. The macroaggregate fraction is representative of the active soil organic matter pool (sensu Parton et al. 1987), and their formation is dependent on their protection from disturbances (Elliott, 1986).). Cultivation can decrease macroaggregate stabilization, breaking them down into smaller microaggregate and mineral-associated particles (Adu and Oades, 1978; Elliott, 1986). Without tilling or after tilling has ceased, macroaggregate formation and resistance should increase (Elliott, 1986). In contrast, my results showed a decline in the macroaggregate fraction as switchgrass stands matured.

Although these patterns were significant and consistent across organic matter fractions, the root biomass fraction exhibited a different trend. Root biomass initially decreased significantly one year after switchgrass conversion, and steadily increased in switchgrass stands over time (Fig. 6.). Root biomass was 85% lower in 1st year switchgrass than row crop treatments, probably reflecting low plant inputs to soils. Following second year plants, root mass consistently increased across the 2nd year, 3rd year, and 4th year treatments. Although these increases in root biomass do not accompany increases in soil organic carbon, other studies have shown that switchgrass enhances soil organic matter primarily through building root stocks (Garten and Wullschleger, 2000; Collins et al, 2010). Collins et al (2010) reported soil organic carbon increases five years after switchgrass establishment, and determined that 24% was from new, root litter inputs from switchgrass. Consistently, Garten and Wullschleger (2000) showed that 19-31% of soil organic carbon under switchgrass stands was derived from switchgrass root carbon inputs. These studies were conducted in the surface soils (0-50 cm), which is where the majority of switchgrass root biomass is found in the soil. It has been shown that over 50% of switchgrass roots exist as fine roots within the top 50 cm of the soil (Weaver and Darland, 1949; Garten and Wullschleger, 1999; Ma et al, 2000b). Microbial activity is generally high in surface soils, so decomposition of switchgrass fine root litter inputs can contribute to soil organic carbon accumulation (Bransby et al., 1998; Garten and Wullschleger, 2000; Collins et al, 2010).

Nitrogen within surface bulk soil organic matter and particulate organic matter pools exhibited very similar patterns to carbon (Fig. 2d,). There were no significant differences between 1st year switchgrass and row crop soils, but nitrogen concentrations declined over time and pools did not recover up to four years after establishment. These results are understandable

because alterations to soil organic carbon pools are usually consistent to those of soil nitrogen pools (Mclauchlan, 2006).

The patterns of declines in soil organic carbon and increases in root biomass observed indicate that the influence of switchgrass on soil organic matter pools occurs two years after planting, or when the stands become established. Ma et al, (2000c) showed that switchgrass did not increase soil organic carbon two years after planting, but after ten years, it increased to 45%. This suggests that time, and stand development, needs to be considered when panting switchgrass to promote soil organic matter accumulation. During the first growing season of switchgrass stands at our field site, fields did not produce any substantial aboveground biomass. The bulk soil organic matter and particulate organic matter results reflect this as there were no changes within these pools in 1st year switchgrass soils. However by the second growing season, stands were producing an average of 1120 g m-2 above ground biomass, and it was this treatment where we saw soil organic matter stocks declined significantly (Fig. 6.). This, along with the substantial increases in root biomass in 2nd year stands, implies that stands had become established, and effected soil organic matter pools, two years following conversion.

Labile pools of soil organic matter

Previous studies have shown that microbial and active pools of carbon recover most rapidly in soils following abandonment from agriculture (Burke et al., 1995). In switchgrass systems, microbial biomass and mineralizable carbon pools have been shown to increase due to increased inputs of soil organic matter from root litter inputs (Ma et al., 2000a; Haney et al. 2010). My results showed no significant variation in concentrations of mineralizable carbon (Fig. 4a.) and microbial biomass (Fig. 4b.) pools across treatments, but the similarities in their patterns

and magnitudes are important, especially with respect to the proportion of total carbon that they represent. Carbon within these pools is fluid; labile carbon from plant inputs (e.g. root litter and/or exudates) is consumed by microbes and incorporated into microbial biomass, and microbial biomass contributes to the labile carbon pool. Furthermore, the amount of carbon in the mineralizable pool as represented by potential soil respiration showed increases in labile carbon pools (Fig. 5.) and nitrogen immobilization (Fig. 4c.) as switchgrass stands matured. As a proportion of the bulk soil organic carbon pool, labile pools were statistically similar in CRP, row crop, and 1st year switchgrass soils, patterns consistent with the bulk soil organic and particulate organic carbon pools. However, labile pools increased by 39% between 1st year and 2nd year switchgrass soils, and 7% between 2nd year and 3rd year switchgrass soils (Fig. 5.). Nitrogen mineralization pools showed contrasting trends, with nitrogen concentrations steadily decreasing over time, even displaying a net immobilization of nitrogen in 4th year switchgrass soils (Fig. 4c.).

The contrasting patterns in carbon and nitrogen mineralization suggest that soils under switchgrass tend toward a state of increasing nitrogen limitation, which promotes nitrogen immobilization. Previous studies in switchgrass and in grasslands in general have shown that nitrogen immobilization is influenced by the C:N ratio plant litter inputs and soil organic matter (Barrett and Burke, 2000; Barrett and Burke, 2002; Tufekcioglu et al., 2003). At the field scale, such changes in soil stoichiometry are predicted to result in enhanced nitrogen retention and decreased run-off (Tufekcioglu et al., 2003). The changes in the relative cycling of carbon and nitrogen observed in my microcosm study suggest changes in microbial dynamics in soils under switchgrass plants. The decrease in potential nitrogen mineralization suggests that microbes are increasingly nitrogen limited in soils under switchgrass plantings, perhaps associated with

changes in soils and nutrient availability following the conversion from row-crops to switchgrass and the cessation of fertilizer inputs. Recent work has demonstrated that nitrogen-limited microbes may selectively decompose older, more recalcitrant organic matter pools in order to acquire nitrogen; thus nitrogen limitation can contribute to enhanced decomposition (Kuzyakov et al., 2000; Minnick et al., 2015).

Concepts such as microbial "mining" and "priming" describe conditions when increased soil organic matter inputs, such as root litter, to the soil environment stimulate microbial activity (Kuzyakov et al., 2000; Fontaine et al., 2003; Chen et al., 2014; Strickland et al., 2015). Microbial priming is operationally defined as circumstances where microbial activity is stimulated by substrate additions, which result in rates of carbon mineralization in excess of the actual substrate addition (Kuzyakov et al., 2000). Microbial mining describes a similar phenomena in which microbial demand for nitrogen facilitates the decomposition of organic matter to mineralize nitrogen (Cotrufo et al., 2013). For example, in recovering agricultural soils where the cessation of fertilizer and a sudden increase in above and below-ground litter inputs stimulate microbial activity, increased demand for nitrogen may alter microbial dynamics and stimulate the decomposition of recalcitrant organic matter pools to mobilize nitrogen (Cotrufo et al., 2013). Thus, additions of fresh organic matter to the soil can provide the nitrogen-limited microbial community with resources to stimulate their activity to breakdown organic matter (Kuzyakov et al., 2000; Fontaine et al., 2004; Phillips et al., 2012; de Graaff et al., 2014). Roots release an abundance of extracellular compounds into the soil that are a source of readily available carbon for soil microbes, which promotes microbial growth and activity to mine for additional nitrogen by decomposing soil organic matter (Kuzyakov and Xu, 2013). Although plants and microbes are in competition for nitrogen, microbes exceed plants in nitrogen uptake

when it is mineralized from soil organic matter (Kuzyakov and Xu, 2013). This immobilizes nitrogen within microbial biomass and *primes* soil microbes to decompose not only the new litter inputs, but also native, more recalcitrant soil organic matter, leading to a decline in bulk soil organic carbon and promotes importantly a mobilization of nitrogen from organic matter (Kuzyakov et al., 2000; Fontaine et al., 2004).

Microbial dynamics such as priming and mining are complex, and not yet fully understood. A priming effect can be driven by many different factors, including the amount and type of soil organic matter substrate, as well as soil organic matter accessibility to microbial communities and nitrogen limitation (Fierer et al. 2003; Kuzyakov et al., 2000). It has also been proposed that the capacity to which switchgrass systems can promote carbon sequestration is dependent on the original soil organic carbon stocks at the site prior to switchgrass establishment (Garten and Wullschleger et al, 2000; Strickland et al., 2015). Agricultural sites generally present reduced carbon stocks because of cultivation, and so planting switchgrass appears to increase soil organic carbon from these originally low quantities (Six et al., 2002). In forested or no-till systems, where organic carbon stocks may be closer to saturation levels, priming effects have been observed when labile carbon inputs are increased in the soil (Dijkstra and Cheng, 2007; Phillips et al., 2012; Strickland et al., 2015). This is inconsistent with our results, which suggest that microbial mobilization of older organic matter fractions occurs on agricultural lands that have a history of long-term agriculture and low soil organic carbon stocks relative to other soils in the region.

The transformation of fresh litter inputs into soil organic matter is a primary process for soil carbon sequestration. The results from this study and other studies that propose priming effects indicate the importance of investigating the microbial processes that connect litter

decomposition with stable soil organic matter formation. New insights into microbial dynamics has unveiled mechanisms of nutrient cycling and soil organic matter properties that can better explain decomposition than previously used static soil organic matter models.

Conclusions

Most previous studies have shown that the general outcome of establishing switchgrass in agricultural land is an increase in the accumulation of soil organic carbon. For example, in other studies of four year old switchgrass plantings, there have been reports of soil organic carbon accumulation rates up to 300 g C m^{-2} yr^{-1} (Zan et al., 2001; Anderson et al., 2009). In contrast, my results show a negative effect of switchgrass plantings on soil organic matter storage. The declines in bulk and surface particulate organic matter pools, as well as increases in labile soil organic matter pools and nitrogen immobilization are consistent with a proposed priming effect occurring in the soil following switchgrass establishment.

Here, we present a case where converting agricultural lands to switchgrass plantings does not result in increased soil organic matter content over a short time scale (1-4 years). These results suggest that soil organic matter dynamics in switchgrass plantings are dependent upon a complex interplay of plant production, microbial dynamics and soil formation. Investigating how switchgrass influences the microbial community composition following conversion and as switchgrass stands develop could be useful to better understand our results. Soil microbial community composition can be responsive to changes in plant cover or substrate inputs (Wieland et al., 2001; Lauber et al., 2008), and examining extra-cellular enzyme profiles and fungal/bacterial ratios in these switchgrass soils could better identify mechanisms causing the priming effect and subsequent declines in soil organic matter pools.

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Table 1. Classification of soils within row crops, switchgrass and conservation reserve program (CRP) fields at the Merck Sharp & Dohme facility in on the Shenandoah River in Elkton, VA.

Treatment	Field I.D.	Soil Series	Texture	Geomorphic position	Soil order	Family classification
CRP	CRP	Millrock	loamy sand	flood plains	Entisols	Mixed, mesic Alfic Udipsamments
Row crop	01A	Monongahela	cobbly fine sandy loam	stream terraces	Ultisols	Fine-loamy, mixed, semiactive, mesic Typic Fragiudults
	04A, 07A	Chavies	fine sandy loam	stream terraces/ toe slope	Alfisols	Coarse-loamy, mixed, active, mesic Ultic Hapludalfs
1st year switchgrass	01	Monongahela	fine sandy loam	stream terraces	Ultisols	Fine-loamy, mixed, semiactive, mesic Typic Fragiudults
	06	Allegheny	fine sandy loam	stream terraces/ toe slope		
2nd year switchgrass	02	Monongahela	fine sandy loam	stream terraces	Ultisols	Fine-loamy, mixed, semiactive, mesic Typic Fragiudults
	07	Allegheny	fine sandy loam	stream terraces/ toe slope		
3rd year switchgrass	03, 05	Allegheny	fine sandy loam	stream terraces/ toe slope	Ultisols	Fine-loamy, mixed, semiactive, mesic Typic Hapludults
	04	Chavies	fine sandy loam	stream terraces/ toe slope	Alfisols	Coarse-loamy, mixed, active, mesic Ultic Hapludalfs
4th year switchgrass	03A	Allegheny	fine sandy loam	stream terraces/ toe slope	Ultisols	Fine-loamy, mixed, semiactive, mesic Typic Hapludults

Table 2. Results for one-way analysis of variances (ANOVA) including all treatments (row crop, 1st year switchgrass, 2nd year switchgrass, 3rd year switchgrass, 4th year switchgrass, CRP) and only treatments with replicate fields (row crop, 1st year switchgrass, 2nd year switchgrass, 3rd year switchgrass). DF denotes degrees of freedom, and values that are significant ($\alpha \leq 0.05$) are in bold.

Variable	Depth (cm)	All Treatments			Only Treatments with Replicate Fields		
		DF	F ratio	p-value	DF	F ratio	p-value
bulk density	0-20	5,52	3.254	**≤ 0.05**	3,44	1.417	0.25
	20-40	5,51	1.564	0.187	3,43	1.916	0.141
	40-60	5,50	0.56	0.73	3,42	0.73	0.54
pH	0-20	5,54	5.663	**≤ 0.001**	3,46	9.114	**≤ 0.0001**
	20-40	5,53	2.419	**≤ 0.05**	3,46	3.599	**≤ 0.05**
	40-60	5,47	2.642	**≤ 0.05**	3,40	3.607	**≤ 0.05**
total organic carbon	0-20	5,54	7.736	**< 0.0001**	3,46	8.074	**≤ 0.001**
	20-40	5,53	4.909	**≤ 0.001**	3,46	6.463	**≤ 0.001**
	40-60	5,47	4.464	**≤ 0.01**	3,40	6.046	**≤ 0.01**
total nitrogen	0-20	5,54	5.427	**≤ 0.001**	3,46	5.25	**≤ 0.01**
	20-40	5,53	7.648	**≤ 0.0001**	3,46	8.472	**≤ 0.0001**
	40-60	5,47	15.89	**≤ 0.0001**	3,40	7.677	**≤ 0.001**
macroaggregate carbon	0-20	5,34	6.962	**≤ 0.0001**	3,28	5.668	**≤ 0.01**
	20-40	5,33	6.61	**≤ 0.001**	3,28	7.609	**≤ 0.001**
	40-60	5,27	3.393	**≤ 0.05**	3,22	2.053	0.136
microaggregate carbon	0-20	5,34	18.8	**≤ 0.0001**	3,28	32.59	**≤ 0.0001**
	20-40	5,33	4.85	**≤ 0.01**	3,28	7.181	**≤ 0.001**
	40-60	5,27	5.885	**≤ 0.01**	3,22	1.833	0.1358
mineral-associated	0-20	5,34	2.448	**≤ 0.05**	3,28	3.172	**≤ 0.05**

carbon	20-40	5,33	3.253	**≤ 0.05**	3,28	4.642	**≤ 0.01**
	40-60	527	5.458	**≤ 0.05**	3,22	8.988	**≤ 0.001**
macroaggregate nitrogen	0-20	5,34	5.762	**≤ 0.001**	3,28	4.089	**≤ 0.05**
	20-40	5,33	3.545	**≤ 0.05**	3,28	3.031	**≤ 0.05**
	40-60	5,27	3.241	**≤ 0.05**	3,22	1.801	0.176
microaggregate nitrogen	0-20	5,34	12.07	**≤ 0.0001**	3,28	18.49	**≤ 0.0001**
	20-40	5,33	0.922	0.479	3,28	1.149	0.347
	40-60	5,27	5.256	**≤ 0.01**	3,22	2.854	0.0605
mineral- associated nitrogen	0-20	5,34	2.614	**≤ 0.05**	3,28	3.306	**≤ 0.05**
	20-40	5,33	2.047	0.975	3,28	2.602	0.0714
	40-60	5,27	4.5237	**≤ 0.01**	3,22	5.1391	**≤ 0.01**
mineralizable carbon	0-20	5,34	0.85	0.524	3,28	0.948	0.431
mineralizable nitrogen	0-20	5,34	3.3685	0.07	3.28	1.86	0.159
microbial Biomass	0-20	5,34	1.926	0.116	3,28	0.328	0.805
proportion of labile carbon	0-20	5,34	8.912	**≤ 0.0001**	3,28	10.7	**≤ 0.0001**

Table 3. Soil bulk density and pH for row crops, switchgrass, and conservation reserve program (CRP) fields. Values represent treatment means ± standard error (n= 5).

Treatment	Depth (cm)	Bulk Density (g cm^{-3})	pH
CRP	0-20	1.23 ± 0.05	6.1 ± 0.2
	20-40	1.45 ± 0.03	6.6 ± 0.2
	40-60	1.55 ± 0.05	6.8 ± 0.2
Row crop	0-20	1.42 ± 0.05	6.1 ± 0.1
	20-40	1.44 ± 0.04	6.5 ± 0.1
	40-60	1.51 ± 0.04	6.5 ± 0.1
1^{st} year switchgrass	0-20	1.41 ± 0.04	5.7 ± 0.1
	20-40	1.55 ± 0.07	6.2 ± 0.1
	40-60	1.59 ± 0.06	6.5 ± 0.1
2^{nd} year switchgrass	0-20	1.50 ± 0.04	6.5 ± 0.1
	20-40	1.55 ± 0.03	6.8 ± 0.1
	40-60	1.57 ± 0.05	7.0 ± 0.1
3^{rd} year switchgrass	0-20	1.40 ± 0.03	6.3 ± 0.1
	20-40	1.53 ± 0.02	6.5 ± 0.1
	40-60	1.52 ± 0.03	6.4 ± 0.2
4^{th} year switchgrass	0-20	1.40 ± 0.02	6.5 ± 0.2
	20-40	1.56 ± 0.06	6.6 ± 0.1
	40-60	1.50 ± 0.02	6.7 ± 0.1

Table 4. Mean carbon and nitrogen concentrations within three particulate organic matter fractions of row crops, switchgrass and conservation reserve program (CRP) fields at three depth increments. Values represent fraction means ± standard error across treatments at depth. Tukey's honest significant difference test results are displayed if the F-test from the one-way ANOVA was statistically significant ($\alpha \leq 0.05$). Letters that are the same indicate means that are not significantly different.

Variable	Depth (cm)	Fraction	Treatment					
			CRP	Row crop	1st year switchgrass	2nd year switchgrass	3rd year switchgrass	4th year switchgrass
g C kg dry soil^{-1}	0-20	250- 2000 µm	11.45±1.50^{A}	8.39±1.82^{AB}	7.26±0.60^{ABC}	3.58±0.38^{C}	4.01±0.44^{C}	4.63±0.56^{BC}
		53- 250 µm	4.46±0.61^{AB}	4.80±0.32^{A}	4.75±0.33^{A}	2.74±0.20^{C}	1.87±0.12^{C}	2.93±0.32^{BC}
		<53 µm	2.24±0.24^{AB}	3.43±0.52^{A}	2.96±0.25^{AB}	1.62±0.16^{B}	2.43±0.63^{AB}	2.09±0.22^{AB}
	20-40	250- 2000 µm	4.01±0.59^{A}	3.46±0.55^{A}	2.42±0.41^{AB}	1.18±0.12^{B}	1.78±0.20^{B}	2.15±0.27^{AB}
		53- 250 µm	1.97±0.30^{ABC}	2.71±0.36^{A}	2.49±0.44^{AB}	1.22±0.26^{BC}	1.01±0.16^{C}	1.63±0.22^{ABC}
		<53 µm	1.90±0.22^{AB}	2.79±0.35^{A}	2.05±0.38^{AB}	1.46±0.19^{B}	1.53±0.13^{B}	1.63±0.19^{AB}
	40-60	250- 2000 µm	5.77±3.02^{A}	1.61±0.23^{AB}	1.48±0.34^{B}	0.93±0.08^{B}	1.31±0.19^{B}	1.14±0.20^{AB}
		53- 250 µm	2.13±0.58^{A}	1.82±0.42^{AB}	1.29±0.18^{ABC}	0.72 ± 0.11^{C}	0.62±0.10^{C}	0.59±0.09^{BC}
		<53 µm	1.64±0.48^{AB}	2.13±0.47^{A}	1.55 ± 0.25^{AB}	0.79 ± 0.07^{B}	0.67±0.06^{B}	0.74±0.14^{B}
g N kg dry soil^{-1}	0-20	250- 2000 µm	1.10±0.15^{A}	0.79±0.19^{AB}	0.62±0.05^{ABC}	0.34±0.03^{C}	0.39±0.05^{BC}	0.42±0.04^{BC}
		53- 250 µm	0.42±0.06^{AB}	0.44±0.04^{A}	0.41±0.03^{AB}	0.28±0.02^{C}	0.19±0.01^{C}	0.27±0.02^{BC}
		<53 µm	0.22±0.02^{AB}	0.34±0.06^{A}	0.27±0.02^{AB}	0.16±0.02^{B}	0.22±0.05^{AB}	0.18±0.02^{AB}
	20-40	250- 2000 µm	0.41±0.04^{A}	0.31±0.06^{A}	0.23±0.03^{AB}	0.12±0.01^{B}	0.26±0.06^{AB}	0.19±0.04^{AB}
		53- 250 µm	0.24±0.04	0.25±0.04	0.25±0.04	0.13±0.03	0.20±0.09	0.15±0.02
		<53 µm	0.23±0.01	0.29±0.05	0.22±0.03	0.19±0.02	0.18±0.01	0.17±0.02
	40-60	250- 2000 µm	0.56±0.27^{A}	0.18±0.02^{AB}	0.16±0.03^{B}	0.12±0.01^{B}	0.19±0.03^{AB}	0.14±0.02^{AB}
		53- 250 µm	0.26±0.04^{A}	0.2±0.05^{AB}	0.14±0.02^{AB}	0.10±0.02^{B}	0.09±0.03^{B}	0.07±0.01^{B}
		<53 µm	0.21±0.02^{AB}	0.24±0.07^{A}	0.19±0.02^{AB}	0.12±0.02^{AB}	0.09±0.01^{B}	0.10±0.01^{AB}

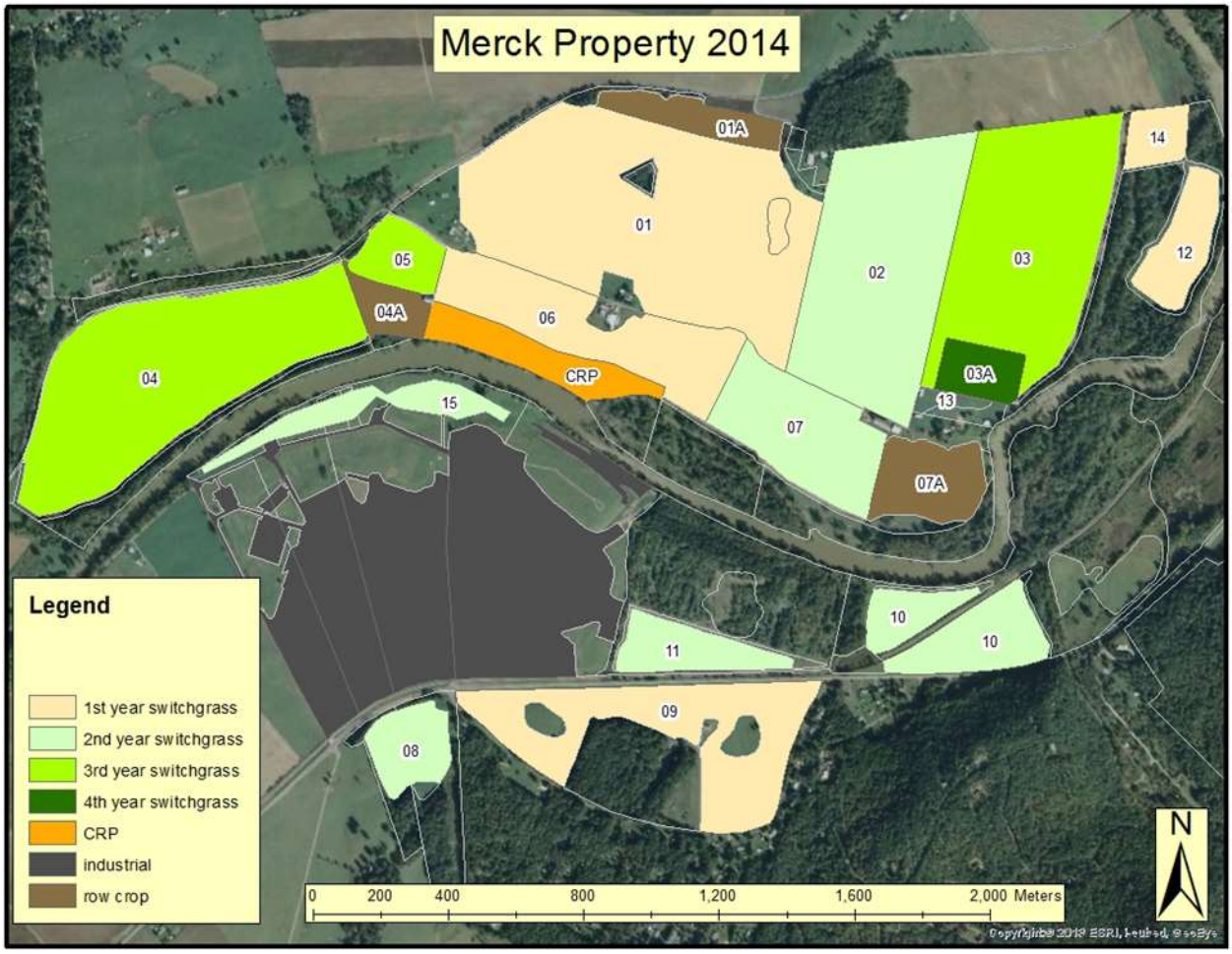


Fig. 1. Map of agricultural fields at the Merck Sharp & Dohme facility on the Shenandoah River in Elkton, VA. (Used with permission from the Virginia Tech Conservation Management Institute).

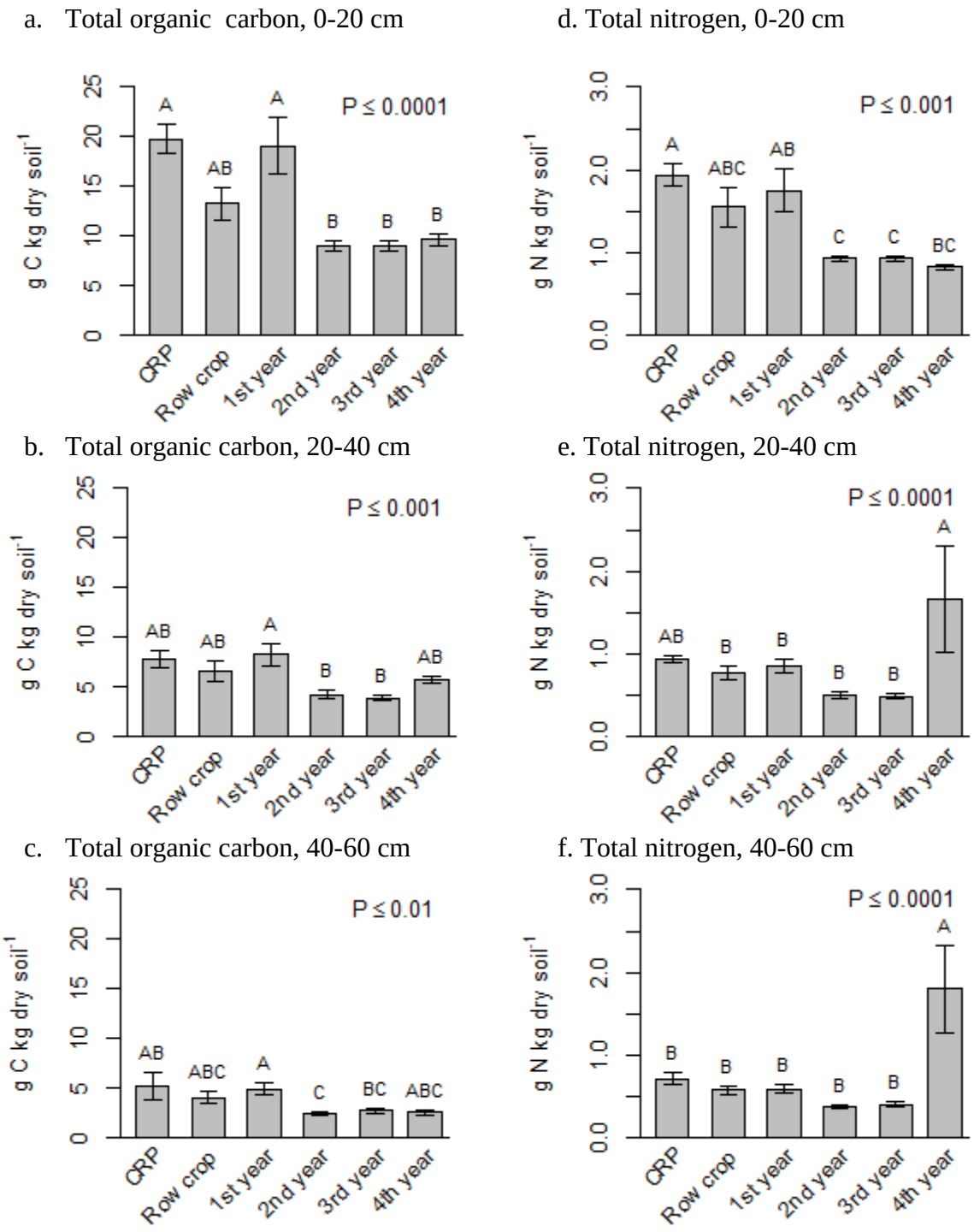


Fig. 2. Total soil organic carbon (a-c) and nitrogen (d-f) in row crops, switchgrass and conservation reserve program (CRP) fields. Values represent treatment means and error bars represent standard error (n= 15 for row crop, 3rd year; n= 10 for 1st year, 2nd year; n= 5 for CRP, 4th year). Means with the same letter are not significantly different ($\alpha \leq 0.05$).

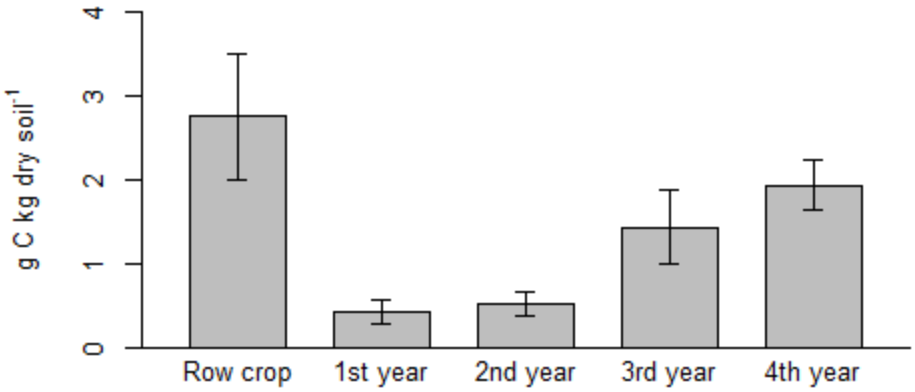
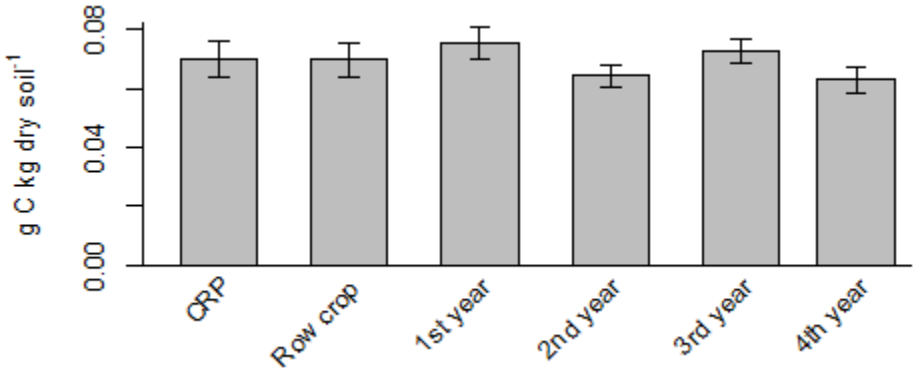
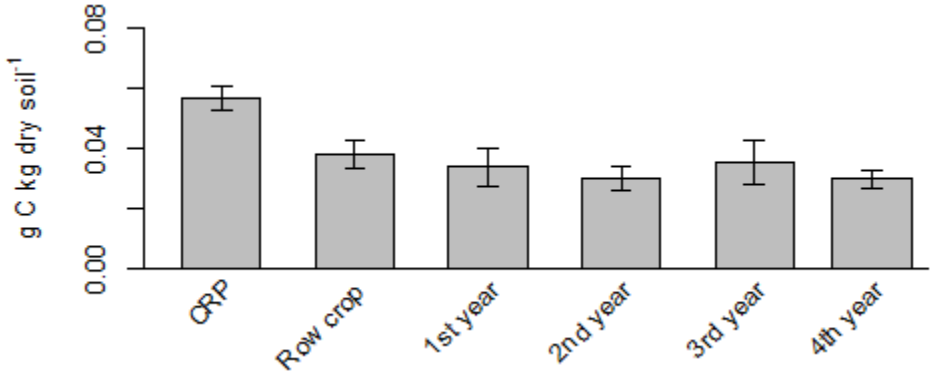


Fig. 3. Root biomass carbon at 0-20 cm in row crops, switchgrass and conservation reserve program (CRP) fields. Values represent treatment means and error bars represent standard error (n= 5).

a. Mineralizable carbon



b. Microbial biomass carbon



c. Mineralizable nitrogen

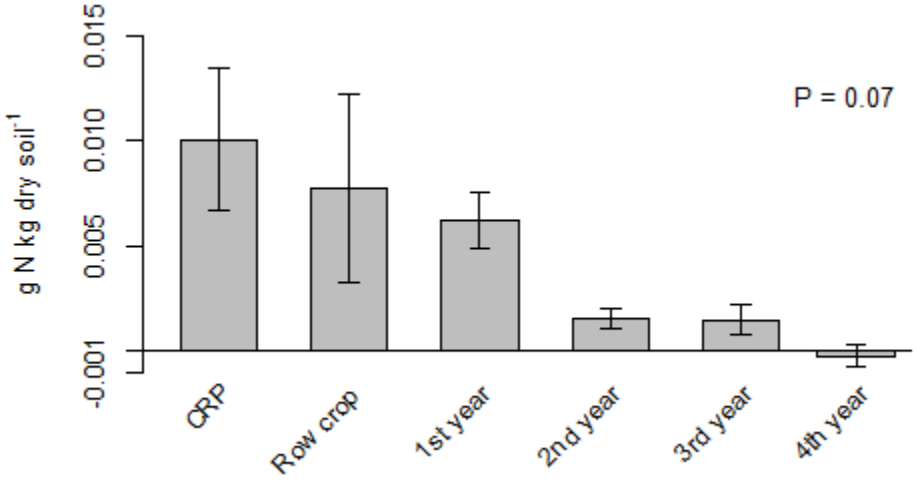


Fig. 4. Mineralizable carbon (a) and nitrogen (b), and microbial biomass (c) in soils collected at 0-20 cm from row crops, switchgrass and conservation reserve program (CRP) fields. Values represent treatment means and error bars represent standard error (n= 8 for row crop, 1st year, 2nd year, 3rd year; n= 4 for CRP, 4th year).

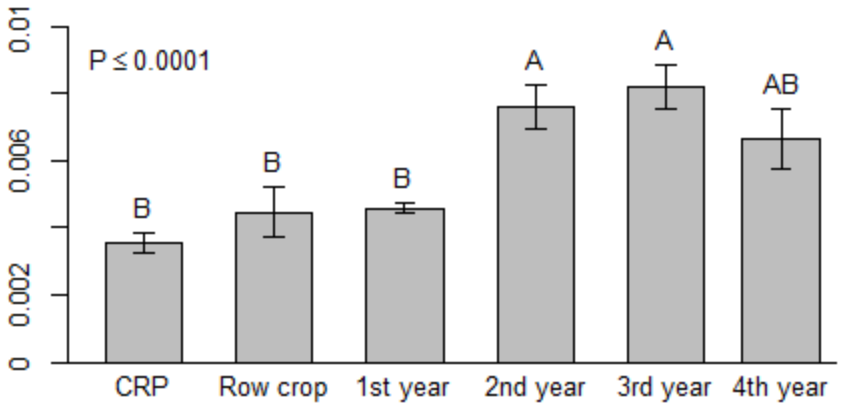


Fig. 5. Proportion of labile carbon of the bulk carbon pool in soils at 0-20 cm collected from row crops, switchgrass and conservation reserve program (CRP) fields. Values represent treatment means and error bars represent standard error (n= 8 for row crop, 1st year, 2nd year, 3rd year; n= 4 for CRP, 4th year). Means with the same letter are not significantly different ($\alpha \leq 0.05$).

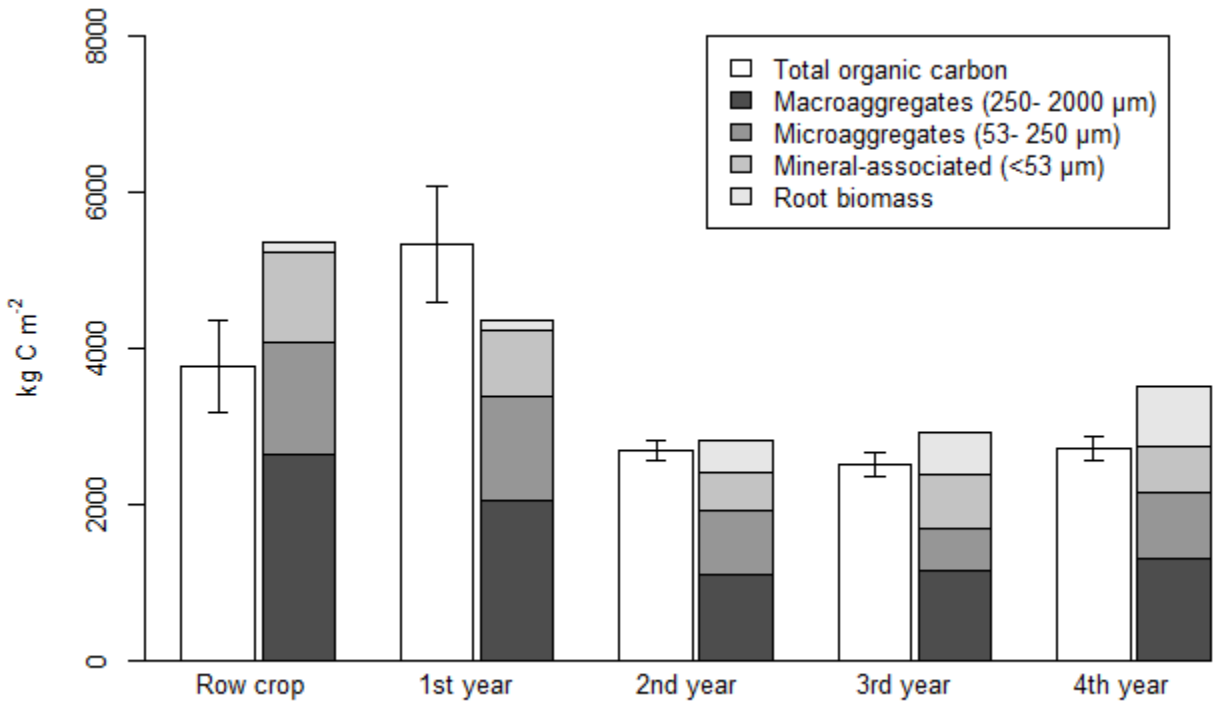


Fig. 6. Soil carbon inventory in particulate organic matter and root fractions at 0-20 cm in row crops, switchgrass and conservation reserve program (CRP) fields. Values represent treatment means and error bars represent standard error.