Title: Soil Carbon and Nitrogen Responses to Nitrogen Fertilizer and Harvesting Rates in Switchgrass Cropping Systems

Running Title Soil carbon storage in switchgrass cropping systems

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ABSTRACT

The environmental sustainability of bioenergy cropping systems depends upon multiple factors such as crop selection, agricultural practices, and the management of carbon (C), nitrogen (N), and water resources. Perennial grasses, such as switchgrass (*Panicum virgatum* L.), show potential as a bioenergy source due to high yields on marginal lands with low fertilizer inputs and an extensive root system that may increase sequestration of C and N in subsurface soil horizons. We quantified the C and N stocks in roots, free-particulate, and mineral-associated soil organic matter pools in a four year old switchgrass system following conversion from row-crop agriculture at the W.K. Kellogg Biological Station in southwest Michigan. Crops were fertilized with nitrogen at either 0, 84, or 196 kg N ha^{-1} and harvested either once or twice annually. Twice-annual harvesting caused a reduction of C and N stocks in the relatively labile roots and free-particulate organic matter pools. Nitrogen fertilizer significantly reduced total soil organic C and N stocks, particularly in the stable, mineral-associated C and N pools at depths greater than 15 cm. The largest ecosystem C stocks in combined switchgrass biomass and soil occurred in unfertilized plots with annual harvesting. These findings suggest that fertilization in switchgrass agriculture inhibits sequestration potential of the soil C pool.

INTRODUCTION

Managing the soil carbon cycle could help the bioenergy industry to deliver environmental benefits and mitigate the pace of climatic change. In addition to direct fossil fuel offsets, bioenergy cropping systems provide biogeochemical services such as the biological sequestration of atmospheric CO_2 in soil carbon reservoirs and biophysical services such as reduced latent heating from evapotranspiration (Paul et al., 2001; Torn et al., 1997; Trumbore, 2000). Carbon sequestration occurs when soil organic carbon (SOC) accumulates more rapidly than it is respired (as CO_2 or CH_4) by soil heterotrophs. Deeply-rooted perennial grasses offer high annual net primary productivity (NPP) and the potential to promote the accrual of SOC (Lal et al., 2004; Liebig et al., 2005).

Switchgrass is a perennial, warm-season C_4 bunchgrass that is native to North America, and is a promising bioenergy feedstock due to large aboveground yields and hardiness across climate zones, soil types, and landscapes (Bransby et al., 1998; Sanderson et al., 2006; Wright and Turhollow, 2010). Switchgrass is also suitable for marginal lands with low soil quality (Wright and Turhollow, 2010). The extensive rooting system of switchgrass and its C_4 photosystem efficiently use water and nutrients and reduce soil erosion (Vogel et al., 2002; Jung et al., 2011). Switchgrass rooting depths >1 meter may also promote the accrual of deep SOC pools in soils where SOC has been depleted by conventional row crop agriculture (Garten and Wullschleger, 2000; Frank et al., 2004).

The stability of SOC can be viewed as an ecosystem property with physical, chemical, and biological controls. For the purpose of estimating relative stability, SOC pools can be divided into protected and unprotected pools. Aggregate-protected and/or mineral-associated SOC can be isolated and quantified by size or density separation procedures (Baldock and Skjemstad, 2000; Kleber et al., 2005; von Lützow et al., 2007; Torn et al., 2013). The unprotected or free-particulate organic matter in the low-density light fraction (LF, < 1.8 g cm^{-3}) predominantly contains plant necromass (leaf and root litter) with typical turnover times < 10 years (Gregorich and Janzen, 1996; Six et al., 1998). The mineral-associated and aggregate-protected dense fraction (DF, > 1.8 g cm^{-3}) of SOC has mean residence times on the order of 10 to greater than 100 years (Baisden et al., 2002; Janzen et al., 1992; von Lützow et al., 2008).

Soil C storage in switchgrass plantations is a biogeochemical service that can be directly influenced through agricultural management practices. The responses of soil C and N pools to management practices are key indicators of the role that bioenergy landscapes can play in greenhouse gas abatement strategies (Robertson et al., 2011). Varied responses of SOC to switchgrass agriculture demonstrate the complexity in plant-soil interaction, and the need to study mechanisms of SOC accrual and stability (Table 1). Both fertilizer application rate and harvesting frequency can affect the accrual and long-term stability of SOC by modifying the extent to which organic matter enters protected and unprotected C pools (Stewart et al. 2014; Tiemann and Stuart Grandy 2014). In this study, we investigated soil C and N stocks in organic matter fractions of differing depth and stability (roots, LF, and DF) in response to two treatments: N fertilization rate and harvesting frequency, applied individually and in combination. We hypothesized that more frequent harvesting would reduce belowground C and N stocks due to preferential allocation of resources to aboveground biomass as opposed to roots, while applications of N-fertilizer to the soil surface would reduce the growth of roots deep into the mineral soil profile, and therefore attenuate the SOC and TN stocks in the unprotected and protected fractions (LF and DF).

MATERIALS AND METHODS

Field Site

The experiment was established at the W.K. Kellogg Biological Station (KBS) Long-term Ecological Research (LTER) site in southwest Michigan, USA (42° 249 N, 85° 249 W, elevation 288m), as part of the Great Lakes Bioenergy Research Center (GLRBC). Mean annual temperature at KBS is 10.1 °C; mean annual precipitation is 1027 mm (Robertson and Hamilton, 2015). The soil is the Kalamazoo soil series, a mixed, mesic-Typic Hapudalf developed on glacial outwash with a fine and coarse-loamy texture comprising 85% sand and silt (Crum, J.R. and Collins, 1995). Cropping history included corn-soybean and alfalfa rotations under conventional tillage prior to the planting of an upland switchgrass variety, "Cave-in-Rock", on July 11th 2008 at a seeding rate of 7.84 kg/ha.

The experimental design was a randomized split-plot arrangement: 4 replicate blocks each containing 8 plots measuring 4.6 m by 15.2 m. Each plot comprised one fertilization rate that was split into two harvest intensity treatments for a total of 64 plots, each with dimensions of 4.6 m by 7.6 m. Eight fertilization treatments were applied in 28 kg N/ha increments, from 0 to 196 kg N/ha once per year between 2009-2011. The recommended N application rates for warm season grass crops in this area is approximately 50-120 kg N/ha (Brejda 2000; Warnke, Dahl, and Jacobs 2009). Granular urea 46 % N (wt/wt) was broadcast on 17 June 2009, one year after plant establishment. In subsequent years, liquid urea ammonium nitrate (40% NH_4NO_3, 30% $CO(NH_2)_2$, 30% H_2O) was applied as a foliar spray at a concentration of 28 % N (wt/wt) in May 2010 and 2011. The plots sampled for this study were those fertilized once annually at rates of 0, 84, and 196 kg N/ha. Harvest intensity treatments were once per year (in November, after a killing frost) or twice per year (July and November) (http://lter.kbs.msu.edu/datatables/375).

Sample Collection and Analysis

Soil samples for this study were collected in July and November of 2011, immediately following the biomass harvest. In 2011 the mean annual temperature and total annual precipitation were 9.6 °C and 1125 mm (http://lter.kbs.msu.edu/datatables/7). Two soil cores from each plot were collected by first removing the litter layer and then pushing a 5cm steel tube (5 cm diameter with plastic liner) to a soil depth of 60 cm using a hydraulic GeoProbe ™. A total of 8 cores per treatment (2 cores per each of 4 replicate blocks) were extracted and capped in the field. The liners were split on-site, sectioned into four depth intervals (0 - 5, 5 - 15, 15 -30, 30 - 60 cm), and sealed in separate plastic bags before being packed with ice in coolers and shipped to Baylor University where they were stored at -20°C until processed. Each soil sample bag was allowed to warm to room temperature and then weighed as an initial step before handling. Each depth interval for all bulk soil cores were individually homogenized before being processed and analyzed separately. An initial sub sample (50 - 100g) was oven dried at 50 °C for at least 24 hours (to constant mass) to determine soil dry weight for bulk density calculations. A subset of the soils were also oven dried at 105ºC to quantify any potential bias in soil masses obtained at 50 °C (Table S6). Soil bulk density was calculated by dividing the oven-dried weight by the soil core volume for each depth interval after correcting for the mass of the gravel fraction (>2 mm) (http://lter.kbs.msu.edu/datatables/308).

The remaining soil used to calculate SOC and TN stocks was air dried, picked for roots, and sieved to 2 mm. Roots were hand-picked with tweezers, lightly brushed of any adhered soil and placed in an aluminum tray for drying. Roots and a subsample of the sieved soil was placed in the drying oven at 50 °C for at least 24 hours, weighed, and stored for further analysis. Approximately 20 g of the soil subsample was placed in a 50mL centrifuge tube with approximately 30 mL of sodium iodide (NaI) solution (density =1.8 g/cm^3). After shaking for 30 seconds by hand, the tubes were centrifuged at 82 × g for 20 minutes. The solution was then allowed to settle before the floating LF was decanted onto glass fiber filters (Whatman, GFF) under vacuum. The LF was rinsed with deionized water to remove residual NaI, then dried in the oven at 50°C for 24 hours before being transferred to a glass vial for storage until C and N elemental analysis. The DF (> 1.8 g cm^{-3}) remaining in the centrifuge tube was drained and rinsed of residual NaI solution, dried, and stored for future analysis.

The remaining subsample of root-free, oven-dried soil (< 2 mm) was homogenized in a planetary ball-mill before determining weight percent C and N. The roots were pulverized and homogenized using dry ice and a Scienceware™ Micro-mill grinder. An initial group of soils treated with 10% hydrochloric acid (HCl) to remove inorganic C produced no detectable carbonate at any sampled depth interval. Therefore, HCl pretreatment was deemed unnecessary for the remaining samples. The soil, root, and LF samples were weighed into tin capsules and combusted in a Thermo Scientific Flash EA 1112 Series NC Soil Analyzer to obtain total organic C and total N concentrations. SOC and TN stocks (kg m^{-2}) were calculated from the elemental concentration, soil layer bulk density, and soil layer depth (Stock = concentration (g/g) x soil density (g/cm^3) x depth interval (cm)). The C and N stocks in the mineral-associated, dense fraction (C_{DF} and N_{DF}, respectively) were calculated as the difference between whole soil and the free light fraction (C_{LF} and N_{LF}, respectively) stocks: C_{DF} = (SOC –C_{LF}); N_{DF} = (total N – N_{LF}).

The aboveground switchgrass C and N stocks were estimated as the product of biomass yield and C and N concentrations obtained from KBS LTER datatables (KBS LTER Datatables: Costech Elemental Combustion System CHNS-O, 2004; Total Soil Carbon and Nitrogen, 2009; Plant Carbon and Nitrogen, 2012). Total ecosystem carbon stocks were calculated from the sum of above and below ground stocks as: Total ecosystem C stock = (total aboveground biomass C + standing root biomass C + soil C_{LF} + soil C_{DF}). For plots harvested twice annually, the total aboveground biomass C was estimated from the sum of the July and November biomass C yields.

Deep soil core samples were collected immediately prior to switchgrass establishment in June 2008 by KBS staff, and sectioned at depth intervals of: 0 - 10cm, 10 – 25 cm, 25 – 50 cm, and 50 – 100 cm. These samples were passed through a 2mm sieve, oven dried at 60 °C, and stored in air-tight glass jars at room temperature. Subsamples were sent to Baylor University in 2016 for C and N elemental analysis. Soil C and N stocks were calculated, as described above, using elemental concentration values measured at Baylor and KBS bulk soil density values from the GLBRC Sustainability Data Catalog (KBS LTER Datatables: Soil Bulk Density, 2013). The initial (pre-switchgrass) soil C and N stocks provide a meaningful baseline against which to evaluate the switchgrass treatment effects. However, differences in sampling depth intervals preclude direct quantitative comparisons of initial soil C and N stocks to those for switchgrass treatments using statistical analysis methods.

Statistical Analyses

To test for treatment effects on C and N stocks, we used a 3-way analysis of variance (ANOVA) General Linear Model Univariate. The fixed factors in this analysis were fertilization rate, harvest frequency, and depth intervals. Homoscedasticity of data was checked by the Levene's test prior to ANOVA. The p-value < 0.05 was chosen as the significance level in testing for differences between experimental treatments. The 84 kg N/ha fertilization rate was omitted from the ANOVA due to a lack of data for the November sampling of the twice-annual harvest treatment. Analyses were performed with IBM SPSS statistics 21.0 software (SPSS Inc., Chicago, IL).

RESULTS

Ecosystem Carbon and Nitrogen Stocks were Highest in Unfertilized Switchgrass Treatments

The combination of twice-annual harvesting and high rates of N fertilization generated the largest aboveground biomass C and N stocks, however the root C stock in the annually harvested treatments were significantly larger than twice-annually harvested plots ($p = 0.018$) (Figure 1, Table S1). The SOC and TN stocks were highest in unfertilized plots (Figure 2). The SOC stocks were 13% higher in unfertilized plots than in plots fertilized at a rate of 196 kg N ha^{-1} ($p = 0.004$, Figure 2a). The soil TN stocks were also higher in unfertilized plots both in annually-harvested ($p = 0.006$, Figure 2b) and twice-annually harvested treatments ($p = 0.055$).

In Figure 3, the C_{DF} was the largest contributor to the total ecosystem C stock, and the total ecosystem C stock was significantly affected by fertilizer practices in annually harvested plots. Most notably, high N fertilization rates attenuated the total ecosystem C stocks (Figure 3) due to smaller soil C_{DF} stocks.

Treatment Effects on Soil C and N Pools

Fertilization Reduced SOC and TN in the Dense Fraction. The addition of N-fertilizer reduced C_{DF} ($p = 0.003$) and N_{DF} ($p = 0.005$) stocks by 14% relative to unfertilized controls across the 60 cm soil profile (Figure 4). The fertilizer treatments did not significantly affect C_{LF} and N_{LF} stocks ($p = 0.725$ and $p = 0.261$, respectively) or the root C and N stocks ($p = 0.253$ and $p = 0.225$, respectively).

Twice-annual Harvesting Increased C and N in the Dense Fraction. Soil N_{DF} stocks were 12 % larger in the twice-annually harvested plots ($p = 0.037$). The C_{LF} stocks were 32 % larger and N_{LF} stocks were 18 % larger through 60cm in twice-annually harvested plots ($p = 0.049$ and $p = 0.073$, respectively), compared to annually-harvested plots (Figure 5a, b). No major differences were observed between harvest treatments for overall LF mass. The C_{LF} and N_{LF} stocks declined significantly with depth in all treatments ($p \leq 0.01$) and on average 70% of these stocks were located in the upper 15cm (Figure 5a, b). The root C and N stocks were considerably more variable than other C and N pools. Nevertheless, twice-annual harvesting significantly reduced standing root biomass and root C stocks ($p = 0.026$, $p = 0.018$, respectively; Table S1; Figure 5c).

Soil C and N pools Changed Seasonally

The SOC and TN stocks declined by 9 % from July to November, and SOC stocks were also significantly smaller with N fertilization for both seasons ($p=0.025$, Table S3). The late season decline in SOC and TN were driven by a reduction in C_{DF} and N_{DF} stocks, which occurred between the July and November harvests (Table S4). The LF mass was 28 % larger with N-fertilizer application ($p = 0.043$, Table S3), however the C_{LF} and N_{LF} stocks showed no significant seasonal changes between July and November harvest dates. Root N stocks increased from July to November ($p = 0.008$, Table S4), but no other significant changes were apparent between harvest dates and among fertilization treatments for root biomass, root C stocks, and root N stocks.

DISCUSSION

A review of recent publications on switchgrass agriculture shows substantial variability in the response of SOC stocks to N fertilizer applications (Table 1). The complex interplay of substrate quality (plant residue chemistry), nutrient availability, soil redox gradients, and microbial enzyme capacity/activity and community structure, soil mineralogy and available surface area may contribute to disparate responses of SOC and the effects of N-fertilization across switchgrass field trials.

We found several important changes in soil C and N with harvesting and fertilizer treatments. The SOC and TN stocks were significantly larger in unfertilized switchgrass stands. Approximately half of the SOC and TN stocks are found at depths >15 cm (Figure 2), and predominantly in the mineral–associated dense fraction (Figure 3, Figure 4). Twice-annual harvesting caused a reduction in the root C and free-particulate C_{LF} stocks.

Changes in Soil Carbon and Nitrogen Stocks

The unfertilized SOC stocks measured 0.78 kg C m^{-2} larger than the fertilized treatment over the course of the study to 60 cm depth (3.7 years), corresponding to steady-state change of 0.21 kg C m^{-2} yr^{-1}. An annualized rate of 0.21 kg C m^{-2} y^{-1} to 60 cm depth is similar to those reviewed by Anderson-Teixeira (2009), where the average SOC accrual was 0.1 kg C m^{-2} y^{-1} to 30 cm for fertilized sites. None of the perennial grass sites they reviewed were unfertilized. Follett et al. (2012) also observed an accrual rate of 0.2 kg C m^{-2} y^{-1} to 150 cm, where half of the SOC accumulated at depths below 30 cm. These relative rates of SOC change are relatively modest, and we note that Ruan et al. (2016) significant SOC accrual at the KBS GLBRC site, but took fewer samples and did not fractionate nor include root biomass. Nevertheless, modest SOC accrual rates can lead to significant C sequestration if the accrual occurs within protected soil pools with potential for long-term stability. The N fertilizer treatment may attenuate long-term sequestration potential by affecting both the accrual depth and mineral association of C and N stocks (Liebig et al. 2005; Schrumpf et al. 2013).

Nitrogen Fertilizer Reduced Soil C_{DF} and N_{DF} Stocks

The N fertilizer treatment had significantly lower C_{DF} and N_{DF} stocks compared to the unfertilized control, mainly at depths > 15cm (Figures 2 and 3). This result is important because deeper soil C pools have longer mean residence times, which can be attributed to lower O_2 availability and slower rates of decomposition and mineralization (Trumbore, 2000; Gill and Burke, 2002; Rumpel and Kögel-Knabner, 2010). The residence time (radiocarbon age), and the thermodynamic stability of SOM typically increases with soil depth (Wang et al., 1996; LaRowe and Van Cappellen 2011; Keiluweit et al. 2016). Radiocarbon dating and laboratory incubation studies indicate that SOM associated with soil minerals (both mineral-bound and aggregate-occluded) has greater stability against biodegradation than free-particulate SOM (Torn et al., 1997; Trumbore, 2000; Paul et al., 2001).

The causal mechanism for the C_{DF} response to N fertilizer remains unclear, but we consider two mechanisms likely. First, molecular level studies of grassland SOM suggest that roots and microbial biomass are the predominant sources of organic matter in the dense fraction (or humin fractions) (Otto et al., 2005; Rasse et al., 2005; Simpson et al., 2007). Our measurements at KBS indicate that root biomass C is ~30% lower in the fertilized plots (196 kg N ha^{-1}) than the unfertilized plots, though the effect was not statistically significant in 2011 samples ($p = 0.25$, Table S1). Nevertheless, a reduction in root C may have contributed to a reduction in C_{DF} and N_{DF} over the 3.7 year duration of the study.

Second, N fertilization may also reduce SOM accrual in the dense fraction by indirect effects on SOM decomposition rates, caused by changes to SOM chemical composition and/or microbial activity. For instance, high rates of N fertilization can increase root decomposability through the reduction of root C:N ratios (Garten Jr. et al., 2011). Furthermore, soil nutrient availability can affect microbial community structure and activity and promote or retard the decomposition of SOM (Chen et al., 2014; Nottingham et al., 2015). Chen et al. (2014) demonstrate that N fertilizer added to soil in combination with fresh plant residues tends to accelerate the mineralization of organic matter. Acceleration of the decomposition rate may reduce the accrual of SOC and TN.

Twice-Annual Harvesting Reduced LF and Root C and N Stocks

Mechanisms for the reduction in C_{LF} and N_{LF} pools with twice-annual harvesting (Figure 5a, 5b) could be due to a more efficient removal of aboveground biomass and therefore less incorporation into the soil C and N pools, or the increased exposure at the soil surface favoring increased erosion (physical transport) and aerobic (biotic) or photic (chemical) decomposition of surface residues and associated LF organic matter. In the present study, root C stocks below 15 cm represented 30-45% of total root C to 60 cm for all samples collected in November. The smaller root C and N stocks observed in the twice-annually harvested treatment (Figure 5c, 5d; Table S1) may be from the mid-season harvesting disturbance which could modify resource allocation to aboveground biomass. The 12% reduction in root

C stocks with fertilization at the deepest depth (30-60cm) may be a function of nutrient availability at the surface. The reduced root C and N inputs may also have contributed to the lower C_{LF} and N_{LF} pools in the twice-annual harvesting treatments, as root biomass can be transformed into LF SOM (Ma et al., 2000).

Soil Dense Fraction C and N Declined Rapidly Between Summer and Fall Harvests

The rapid decline of the C_{DF} and N_{DF} pools over the intervening months between July and November harvests is surprising, given the presumed stability of this fraction (Table S3, S4). There are several mechanisms that might explain such a rapid reduction of C_{DF} and N_{DF} stocks between harvests. (1) Seasonal soil aggregate stability could diminish between seasons as a function of increased annual precipitation and cooler temperatures (Dimoyiannis 2009; Bach and Hofmockel 2016). (2) The priming of microorganisms by surface residues from mid-season harvesting and the soil disturbance associated with that harvest could accelerate the mineralization of the more stable DF SOM (Kuzyakov et al., 2000). (3) Alternatively (or additionally), mid-season harvesting could cause a reallocation of photosynthate from root growth to shoot growth, leading to a decline in the substrates supporting mineral-associated microbial biomass, thus diminishing the C_{DF} and N_{DF} between harvests (De Vries et al., 2015). The reduction in C_{DF} was larger in the unfertilized treatments between harvests, however the unfertilized plots had significantly larger C_{DF} and N_{DF} stocks at both harvest dates. This implies that high rates of N fertilization and harvesting, which reduce the production of root and LF C and N stocks, may affect microbial physiology and SOC cycling associated with the reduction of C_{DF} and N_{DF} stocks (Kallenbach et al., 2015).

SUMMARY

Although a primary objective in bioenergy production is maximizing aboveground biomass for use as biofuel feedstock, energy conservation and soil C storage are also valuable biogeochemical services (Robertson et al., 2008) that can further reduce the carbon intensity of bioenergy systems. Our results show that the largest total ecosystem C stocks (above + below ground) were achieved with the least energy-intensive agricultural practices: no N fertilizer and a single postseason harvest. Harvest intensity and N-fertilizer rates affected the magnitude of soil C and N storage, as well as the depth and relative stability of the C and N pools. The changes in SOC occurred primarily at depths greater than 15 cm and in the dense fraction of the SOC pool where organo-mineral associations could provide a mechanism for long-term soil C storage. The N-fertilizer treatments caused a reduction in soil C stocks, particularly in the mineral-associated fraction, while the combination of annual harvesting and N-fertilization reduced soil N stocks in the mineral-associated fraction. The twice-annual harvest treatment reduced LF and root C pools. Unfertilized switchgrass plots contained 15% more SOC, on average, 4 years after planting than did plots under high fertilization rates. Ruan et al. (2016) recently demonstrated the high carbon cost of fertilizing biomass crops such as switchgrass. Our findings demonstrate that management practices that minimized carbon emissions from N fertilization and mechanical harvesting also enhanced the magnitude and longevity of soil carbon storage.

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Supporting Information Available:

1 File: Microsoft Word document containing tabular and graphical data for C and N stocks in root biomass and soil organic matter fractions, testing for effects of N fertilizer, harvesting frequency, and seasonality.

Figure 1

Figure 1

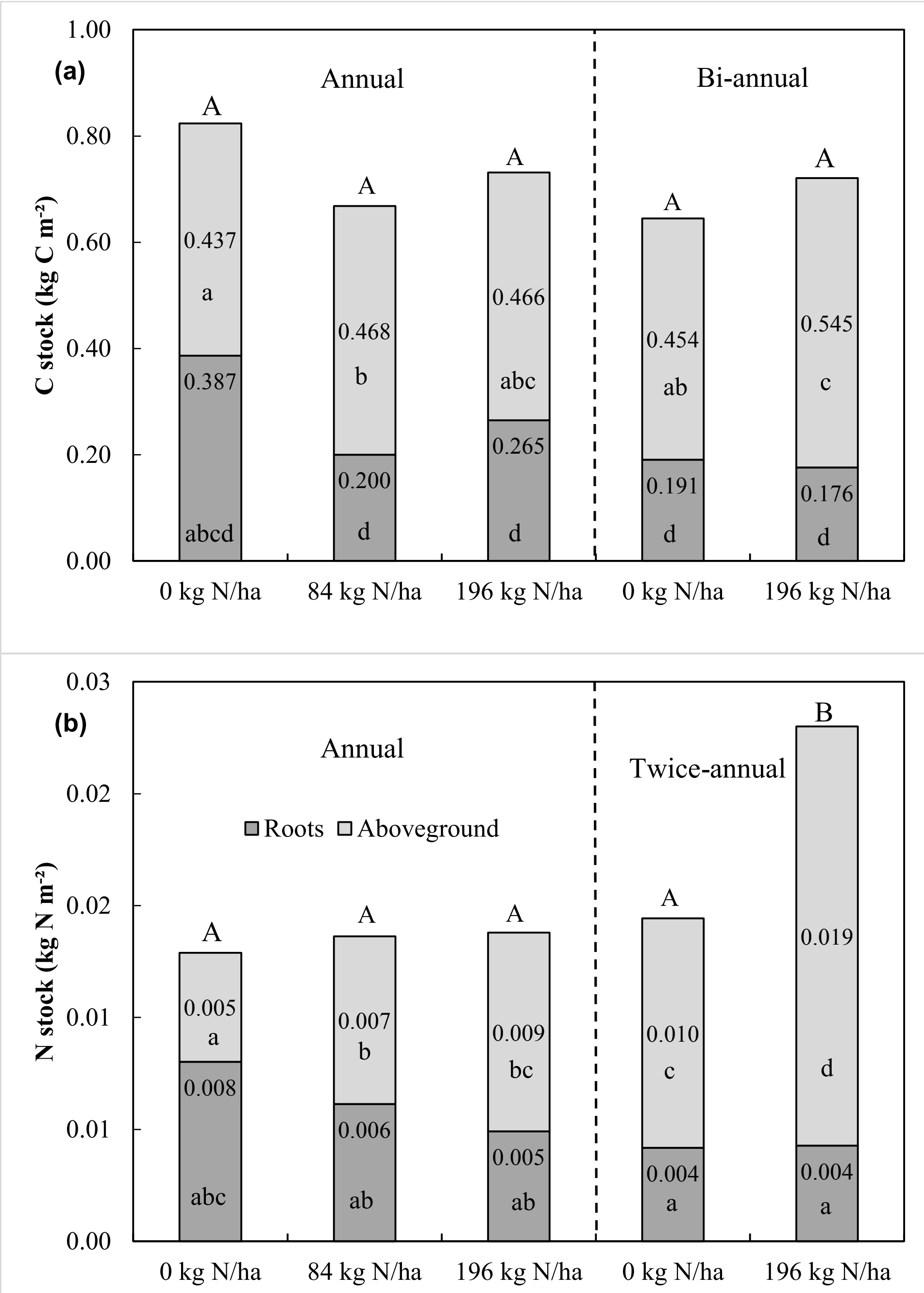

Figure 1. Carbon (a) and Nitrogen (b) stocks in total annual aboveground (sum of 2011 July and November harvests) and belowground (root) biomass after 3 full growing seasons under the harvesting and fertilizer treatments. Standing root biomass C and N were measured in November. Lower case letters within each panel represent significant differences in biomass stocks. Upper case letters signify significant differences in total biomass stocks (above + belowground) (Tukey letter ($P > 0.10$)).

Figure 2

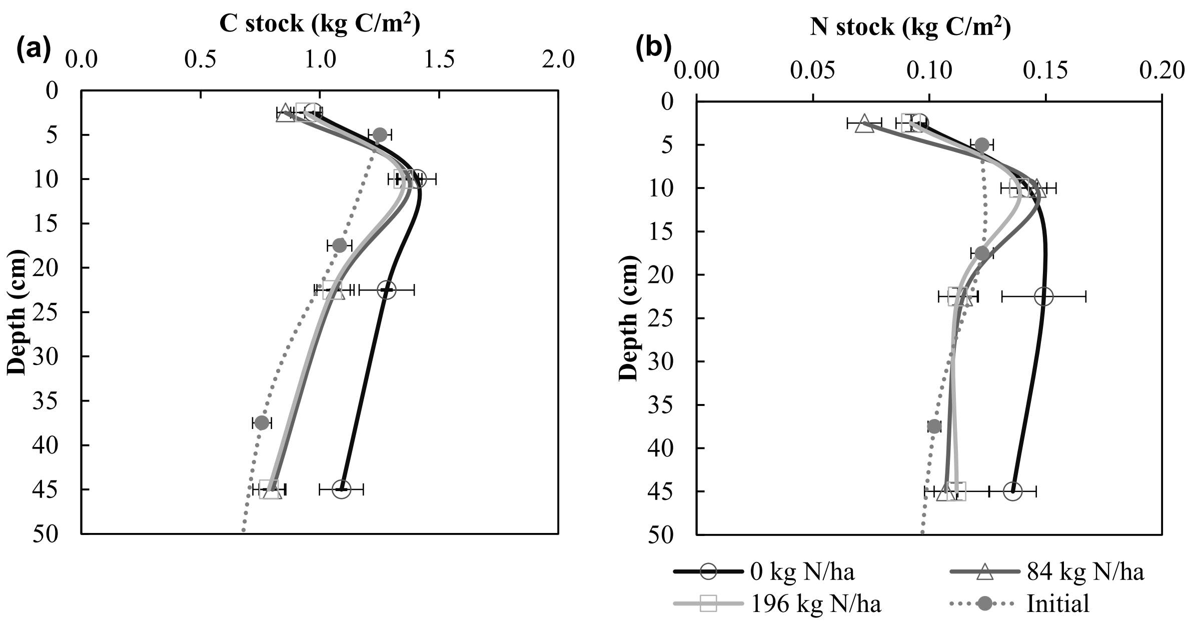

Figure 2: Soil C (a) and TN (b) stocks (roots, LF, DF) at different fertilization rates (open symbols) in Fall 2011. Initial soil C and TN stocks (closed symbols, n = 4) were sampled adjacent to the experimental plots at time of switchgrass establishment. Plotted values are averages across harvest treatments for 0 and 196 kg N/ha (n = 8) and the single annual harvest data for the 84 kg N/ha (n = 4) fertilization rate at each soil depth interval. Horizontal bars are standard error for replicated field plots.

Figure 3

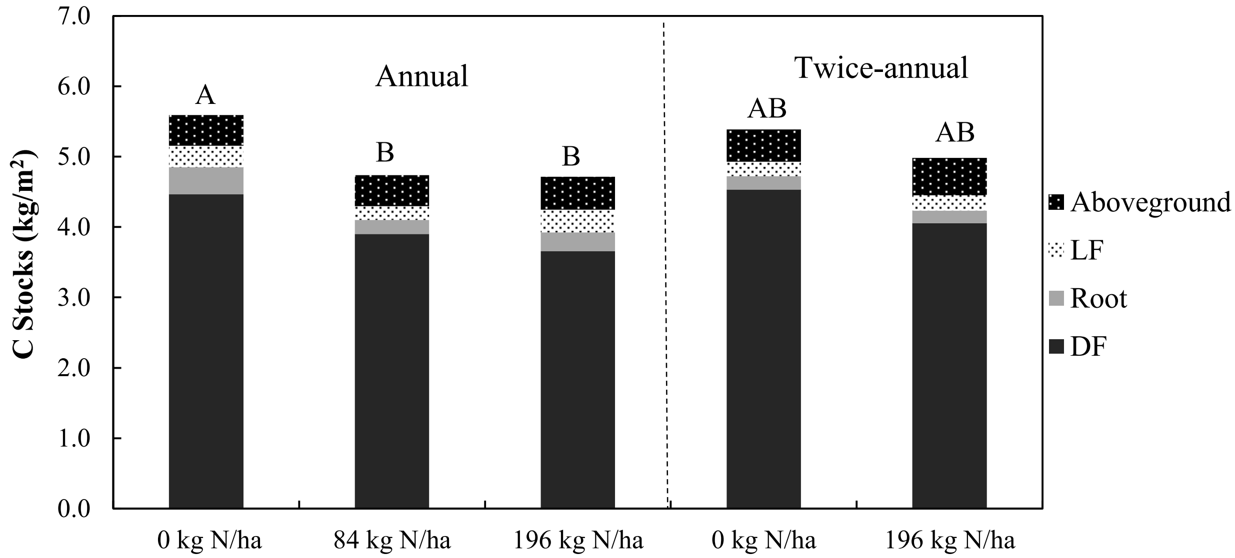

Figure 3: Total ecosystem C stocks for switchgrass cropping systems after the 3rd full growing season under fertilizer and harvest intensity treatments. Total Ecosystem C stock = (total aboveground biomass + root C stock + soil C stock (light + dense fraction)). Upper case letters represent significant differences ($P < 0.10$) between Total Ecosystem C stocks.

Figure 4

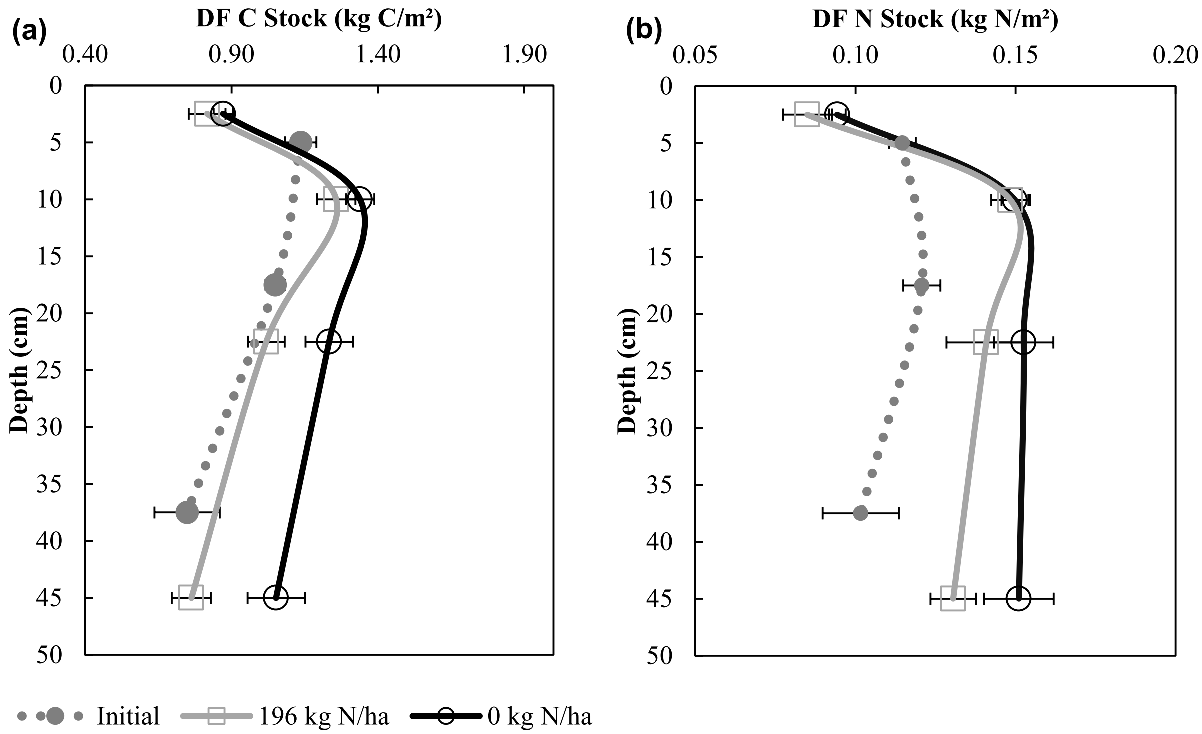

Figure 4: Averaged DF C (a) and N (b) stocks by depth in 0 and 196 kg N/ha (open symbols) treatments sampled in November 2011 with harvest intensities of annual and bi-annual pooled by depth interval (n=8). Initial stocks (closed symbols, n=4) were sampled adjacent to the experimental plots at time of switchgrass establishment at different depth intervals. Horizontal bars are standard errors for replicated field plots.

Figure 5

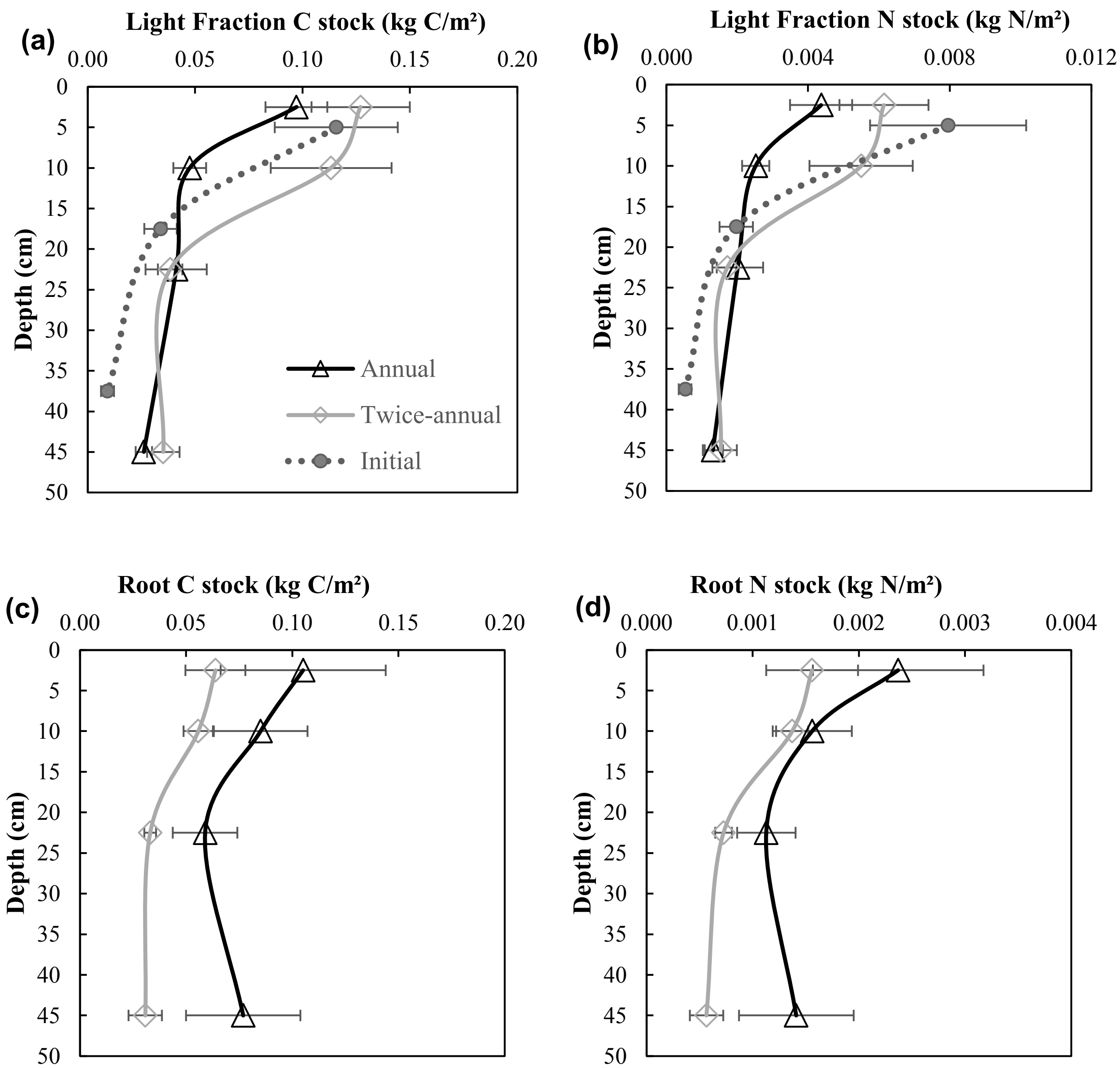

Figure 5: End of season distribution of LF (a, b) and Root (c, d) C and N stocks for annual (open triangles) and twice-annual (open diamonds) harvest frequencies with initial LF stocks shown where measured (closed circles). Horizontal bars are standard error for replicated field plots (n=8, annual and twice-annual harvest; n=4, time zero).

Table 1. Summary of literature on soil C pool responses to N fertilizer in switchgrass plantations

	Location	**Stand Age**	**Soil Depth interval (cm)**	**N Fertilization Rate (kg N ha^{-1})**	**Soil C Response to Fertilization**
(Liebig et al., 2008)	Ten sites in NE, ND, SD	5 years	0 - 30	31 to 104	Linear increase (P=0.03) *
			0 - 120	31 to 104	Linear increase (P=0.07)
(Jung and Lal, 2011)	Three sites in OH	6 years	10 - 20	0, 50, 100, 200	Increase in SOC (P = 0.05) *
			0 - 30	0, 50, 100, 200	No change in SOC
(Stewart et al., 2014)	NE	9 years	0 - 5	60	Increase in SOC (P=0.05) *
			0 - 30	60, 120	Increase in SOC (P<0.01) *
(Follett et al., 2012)	NE	9 years	0 - 30	60	Increase in SOC (P=0.10)
			0 - 15	60	Increase in SOC (P=0.06)
(Heggenstaller et al., 2009)	IA	3 years	0 - 100	65	Increase in roots
			0 - 100	140	Increase in roots
			0 - 100	220	No change in roots
(Lee et al., 2007)	SD	4 years	0 - 60	112, 224	Increase in SOC
(Ruan et al., 2016)	KBS, MI	3 years	0- 100	0 to 196	No change in SOC
(Ma et al., 2000)	AL	4 years	0 - 225	112	No change in SOC
			0 - 225	224	No change in SOC

* P values for significant treatment effects

Supporting Information

Contents

Table S1. Carbon and Nitrogen Stocks (kg m^{-2}) for Soil and Roots. Values are averages for 4 replicated plots. ANOVA results for harvesting and fertilizer treatment effects.

Harvesting frequency		Annual	Annual	Biannual	Biannual	3 way ANOVA		
Fertilization rate:		0kg N/ha	196 kg N/ha	0kg N/ha	196 kg N/ha		F Value	P Value
	Soil Depth (cm)	(kg/m^2)	(kg/m^2)	(kg/m^2)	(kg/m^2)			
Soil C stock	0-5	0.968	0.859	0.978	1.020	Harvest	0.416	0.522
	5-15	1.440	1.305	1.375	1.396	Fertilizer	**9.409**	**0.004**
	15-30	1.336	1.011	1.225	1.093	Depth	**16.958**	**0.000**
	30-60	1.022	0.808	1.160	0.765	H x F	0.618	0.436
	0-60	4.766	3.983	4.737	4.274	H x F x D	0.739	0.534
Soil N Stocks	0-5	0.093	0.081	0.098	0.104	Harvest	3.879	0.055
	5-15	0.140	0.134	0.145	0.143	Fertilizer	**8.213**	**0.006**
	15-30	0.139	0.109	0.159	0.115	Depth	**11.227**	**0.000**
	30-60	0.128	0.106	0.144	0.118	H x F	0.011	0.917
	0-60	0.501	0.430	0.546	0.480	H x F x D	0.339	0.797
Root Biomass	0-5	1.552	1.549	0.914	0.885	Harvest	**5.243**	**0.026**
	5-15	1.385	0.956	1.134	0.981	Fertilizer	2.075	0.156
	15-30	0.955	0.911	0.523	0.609	Depth	0.991	0.405
	30-60	2.039	0.747	0.899	0.411	H x F	0.525	0.472
	0-60	5.930	4.163	3.470	2.886	H x F x D	0.195	0.899
Root C stock	0-5	0.110	0.100	0.061	0.067	Harvest	**5.96**	**0.018**
	5-15	0.099	0.071	0.057	0.055	Fertilizer	1.340	0.253
	15-30	0.067	0.051	0.030	0.036	Depth	1.393	0.256
	30-60	0.111	0.043	0.044	0.018	H x F	0.827	0.368
	0-60	0.387	0.265	0.191	0.176	H x F x D	0.035	0.991
Root N Stock	0-5	0.0027	0.0020	0.0015	0.0016	Harvest	3.347	0.074
	5-15	0.0018	0.0013	0.0014	0.0013	Fertilizer	1.511	0.225
	15-30	0.0012	0.0010	0.0006	0.0009	Depth	2.49	0.071
	30-60	0.0022	0.0006	0.0007	0.0005	H x F	1.714	0.197
	0-60	0.0080	0.0049	0.0042	0.0043	H x F x D	0.137	0.937

1 tailed ANOVA. Items bolded $P < 0.05$

Table S2. Total mass, carbon, and nitrogen stocks (kg m^{-2}) of the low-density (LF) and the high- density (DF) fractions of the soil organic matter. Values are averages for 4 replicated plots. ANOVA results for harvesting and fertilizer treatment effects.

Harvesting frequency:		Annual	Annual	Biannual	Biannual	ANOVA		
Fertilization rate:		0kg N/ha	196 kg N/ha	0kg N/ha	196 kg N/ha		F Value	P Value
	Soil depth (cm)	(kg/m^2)	(kg/m^2)	(kg/m^2)	(kg/m^2)			
DF C	0-5	0.853	0.720	0.891	0.913	Harvest	1.327	0.255
stock	5-15	1.349	1.169	1.329	1.347	Fertilizer	**10.071**	**0.003**
	15-30	1.288	0.982	1.180	1.056	Depth	**16.979**	**0.000**
	30-60	0.973	0.786	1.132	0.740	H x F	0.617	0.417
	0-60	4.463	3.657	4.532	4.056	H x F x D	0.907	0.445
DF N	0-5	0.089	0.072	0.095	0.098	Harvest	**4.608**	**0.037**
stock	5-15	0.137	0.127	0.142	0.140	Fertilizer	**8.671**	**0.005**
	15-30	0.137	0.108	0.157	0.114	Depth	**12.105**	**0.000**
	30-60	0.126	0.104	0.142	0.117	H x F	0.036	0.850
	0-60	0.489	0.412	0.536	0.469	H x F x D	0.386	0.764
LF	0-5	0.102	0.167	0.087	0.137	Harvest	1.739	0.194
mass	5-15	0.049	0.072	0.024	0.034	Fertilizer	2.036	0.160
	15-30	0.021	0.016	0.027	0.014	Depth	**24.462**	**0.000**
	30-60	0.014	0.010	0.013	0.006	H x F	0.232	0.632
	0-60	0.186	0.265	0.151	0.190	H x F x D	0.020	0.996
LF C	0-5	0.115	0.139	0.088	0.107	Harvest	**4.081**	**0.049**
Stock	5-15	0.091	0.136	0.046	0.049	Fertilizer	0.125	0.725
	15-30	0.048	0.029	0.045	0.037	Depth	**8.895**	**0.000**
	30-60	0.048	0.022	0.028	0.025	H x F	0.014	0.908
	0-60	0.302	0.326	0.206	0.218	H x F x D	0.309	0.819
LF N	0-5	0.0041	0.0082	0.0035	0.0052	Harvest	3.371	0.073
stock	5-15	0.0037	0.0072	0.0024	0.0027	Fertilizer	1.292	0.261
	15-30	0.0023	0.0012	0.0025	0.0015	Depth	**6.087**	**0.001**
	30-60	0.0018	0.0012	0.0014	0.0012	H x F	0.619	0.435
	0-60	0.0119	0.0179	0.0098	0.0106	H x F x D	0.441	0.725

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Table S3. Seasonal effects on soil and root carbon and nitrogen stocks (kg m^{-2}) within the biannual harvesting treatment. Values are averages for 4 replicated plots. ANOVA results for harvesting and fertilizer treatment effects.

Sampling month:		July	July	November	November	3 way ANOVA		
Harvesting frequency:		Biannual	Biannual	Biannual	Biannual			
Fertilization rate:		0kg N/ha	196 kg N/ha	0kg N/ha	196 kg N/ha		F Value	P Value
	Soil Depth (cm)	(kg/m^2)	(kg/m^2)	(kg/m^2)	(kg/m^2)			
Soil C stock	0-5	1.000	0.766	0.978	1.020	Harvest	2.971	0.091
	5-15	1.600	1.493	1.375	1.396	Fertilizer	**5.316**	**0.025**
	15-30	1.429	1.483	1.225	1.093	Depth	**14.914**	**0.000**
	30-60	1.276	0.847	1.160	0.765	H x F	0.245	0.623
	0-60	5.305	4.588	4.737	4.274	H x F x D	0.570	0.638
Soil N Stocks	0-5	0.097	0.076	0.098	0.104	Harvest	3.191	0.080
	5-15	0.159	0.161	0.145	0.143	Fertilizer	2.378	0.130
	15-30	0.149	0.169	0.159	0.115	Depth	**17.799**	**0.000**
	30-60	0.159	0.145	0.144	0.118	H x F	1.091	0.302
	0-60	0.565	0.552	0.546	0.480	H x F x D	2.171	0.104
Root Biomass	0-5	0.748	0.658	0.914	0.885	Harvest	2.481	0.122
	5-15	0.439	0.921	1.134	0.981	Fertilizer	0.96	0.332
	15-30	0.415	0.903	0.523	0.609	Depth	1.925	0.138
	30-60	0.196	0.735	0.899	0.411	H x F	**5.535**	**0.023**
	0-60	1.797	3.216	3.470	2.886	H x F x D	1.141	0.342
Root C stock	0-5	0.044	0.049	0.061	0.067	Harvest	1.59	0.213
	5-15	0.027	0.057	0.057	0.055	Fertilizer	1.109	0.298
	15-30	0.018	0.048	0.030	0.036	Depth	**2.882**	**0.045**
	30-60	0.016	0.030	0.044	0.018	H x F	2.302	0.136
	0-60	0.104	0.184	0.191	0.176	H x F x D	0.327	0.806
Root N Stock	0-5	0.0010	0.0008	0.0015	0.0016	Harvest	**7.585**	**0.008**
	5-15	0.0004	0.0010	0.0014	0.0013	Fertilizer	1.218	0.275
	15-30	0.0003	0.0008	0.0006	0.0009	Depth	**5.121**	**0.004**
	30-60	0.0002	0.0006	0.0007	0.0005	H x F	0.900	0.347
	0-60	0.002	0.003	0.004	0.004	H x F x D	0.504	0.681

Table S4. Seasonal effects on total mass, carbon, and nitrogen stocks (kg m^{-2}) of the low-density (LF) and the high-density (DF) fractions of the soil organic matter within the biannual harvesting treatment. Values are averages for 4 replicated plots. ANOVA results for harvesting and fertilizer treatment effects.

Sampling month: Harvesting frequency: Fertilization rate:		July Biannual 0kg N/ha	July Biannual 196 kg N/ha	November Biannual 0kg N/ha	November Biannual 196 kg N/ha	ANOVA	F Value	P Value
	Soil depth (cm)	(kg/m^2)	(kg/m^2)	(kg/m^2)	(kg/m^2)			
DF-C stock	0-5	0.920	0.670	0.891	0.913	Harvest	**4.515**	**0.039**
	5-15	1.566	1.403	1.329	1.347	Fertilizer	**8.144**	**0.006**
	15-30	1.408	1.456	1.180	1.056	Depth	**17.359**	**0.000**
	30-60	1.370	0.825	1.132	0.740	H x F	0.617	0.417
	0-60	5.264	4.355	4.532	4.056	H x F x D	0.636	0.595
DF-N stock	0-5	0.094	0.072	0.095	0.098	Harvest	3.494	0.068
	5-15	0.157	0.157	0.142	0.140	Fertilizer	2.865	0.097
	15-30	0.148	0.168	0.157	0.114	Depth	**19.495**	**0.000**
	30-60	0.160	0.145	0.142	0.117	H x F	0.897	0.348
	0-60	0.560	0.541	0.536	0.469	H x F x D	2.134	0.108
LF mass	0-5	0.101	0.140	0.087	0.137	Harvest	0.100	0.753
	5-15	0.026	0.060	0.024	0.034	Fertilizer	**4.321**	**0.043**
	15-30	0.009	0.012	0.027	0.014	Depth	**55.322**	**0.000**
	30-60	0.007	0.005	0.013	0.006	H x F	0.407	0.527
	0-60	0.142	0.217	0.151	0.190	H x F x D	0.00	0.817
LF C stock	0-5	0.080	0.096	0.088	0.107	Harvest	0.204	0.653
	5-15	0.034	0.090	0.046	0.049	Fertilizer	1.907	0.174
	15-30	0.020	0.026	0.045	0.037	Depth	**16.537**	**0.000**
	30-60	0.029	0.022	0.028	0.025	H x F	0.962	0.334
	0-60	0.164	0.234	0.206	0.218	H x F x D	0.785	0.508
LF N stock	0-5	0.0034	0.0046	0.0035	0.0052	Harvest	0.184	0.670
	5-15	0.0018	0.0047	0.0024	0.0027	Fertilizer	2.041	0.160
	15-30	0.0009	0.0012	0.0025	0.0015	Depth	**8.524**	**0.000**
	30-60	0.0020	0.0009	0.0014	0.0012	H x F	0.735	0.396
	0-60	0.008	0.011	0.010	0.011	H x F x D	0.721	0.544

1 tailed ANOVA. Items bolded $P < 0.05$

TABLE S5

					Annual				Biannual			
Bulk Density		Initial			0 kg N/ha		196 kg N/ha		0 kg N/ha		196 kg N/ha	
(g/cm³)	**Depth**	Average	*S.E.*	**Depth**	Average	*S.E.*	Average	*S.E.*	Average	*S.E.*	Average	*S.E.*
	0-10cm	1.131	*0.037*	**0-5cm**	1.697	*0.256*	1.522	*0.185*	1.664	*0.227*	1.656	*0.261*
	10-25cm	1.301	*0.030*	**5-15cm**	1.791	*0.101*	1.579	*0.216*	1.707	*0.156*	1.645	*0.098*
	25-50cm	1.328	*0.016*	**15-30cm**	1.854	*0.070*	1.686	*0.082*	1.678	*0.146*	1.690	*0.107*
	50-100cm	1.284	*0.009*	**30-60cm**	1.851	*0.084*	1.732	*0.082*	1.709	*0.077*	1.737	*0.065*
Soil C%												
(wt. percent)	**Depth**	Average	*S.E.*	**Depth**	Average	*S.E.*	Average	*S.E.*	Average	*S.E.*	Average	*S.E.*
	0-10cm	1.104	*0.070*	**0-5cm**	1.141	*0.032*	1.129	*0.080*	1.176	*0.057*	1.232	*0.075*
	10-25cm	0.555	*0.050*	**5-15cm**	0.804	*0.050*	0.826	*0.048*	0.805	*0.041*	0.849	*0.030*
	25-50cm	0.228	*0.029*	**15-30cm**	0.480	*0.041*	0.400	*0.036*	0.487	*0.047*	0.431	*0.023*
	50-100cm	0.089	*0.012*	**30-60cm**	0.184	*0.017*	0.155	*0.012*	0.226	*0.018*	0.147	*0.013*
Soil C Stock												
(kg C/m²)	**Depth**	Average	*S.E.*	**Depth**	Average	*S.E.*	Average	*S.E.*	Average	*S.E.*	Average	*S.E.*
	0-10cm	1.252	*0.079*	**0-5cm**	0.968	*0.047*	0.859	*0.062*	0.978	*0.027*	1.020	*0.061*
	10-25cm	1.083	*0.097*	**5-15cm**	1.440	*0.070*	1.305	*0.049*	1.375	*0.090*	1.396	*0.077*
	25-50cm	0.758	*0.095*	**15-30cm**	1.336	*0.118*	1.011	*0.059*	1.225	*0.113*	1.093	*0.092*
	50-100cm	0.568	*0.077*	**30-60cm**	1.022	*0.091*	0.808	*0.070*	1.160	*0.092*	0.765	*0.063*

Table S5: Initial and end-of-season values for each treatment by depth for bulk density, soil C%, and soil C stock.

TABLE S6

	Annual Harvest				Twice-annual (July samples)			
Soil Depth (cm)	196 kg N/ha		0 kg N/ha		196 kg N/ha		0 kg N/ha	
Drying Temp	50°C	105°C	50°C	105°C	50°C	105°C	50°C	105°C
0-5cm	9075.3	9053.7	4212.5	4196.7	10158.8	10107.2	11426.7	11371.2
5-15cm	10488.2	10446.9	10618.9	10558.9	14685.2	14626.3	18385.6	18307
15-30cm	13284.3	13201.1	14535.7	14358.6	15311.1	15236.7	12200.6	12122.3
30-60cm	11885.1	11782.9	8552.3	8499	9298.8	9238.8	17282.7	17161

Weight in mg.

	percent difference between 50C and 105C			
	0-5cm	5-15cm	15-30cm	30-60cm
h1 401	-0.2%	-0.4%	-0.6%	-0.9%
h1- 408	-0.4%	-0.6%	-1.2%	-0.6%
july 401	-0.5%	-0.4%	-0.5%	-0.6%
july 408	-0.5%	-0.4%	-0.6%	-0.7%

Table S6. Soil dry weight comparison between 50 and 105 °C.

FIGURE S1

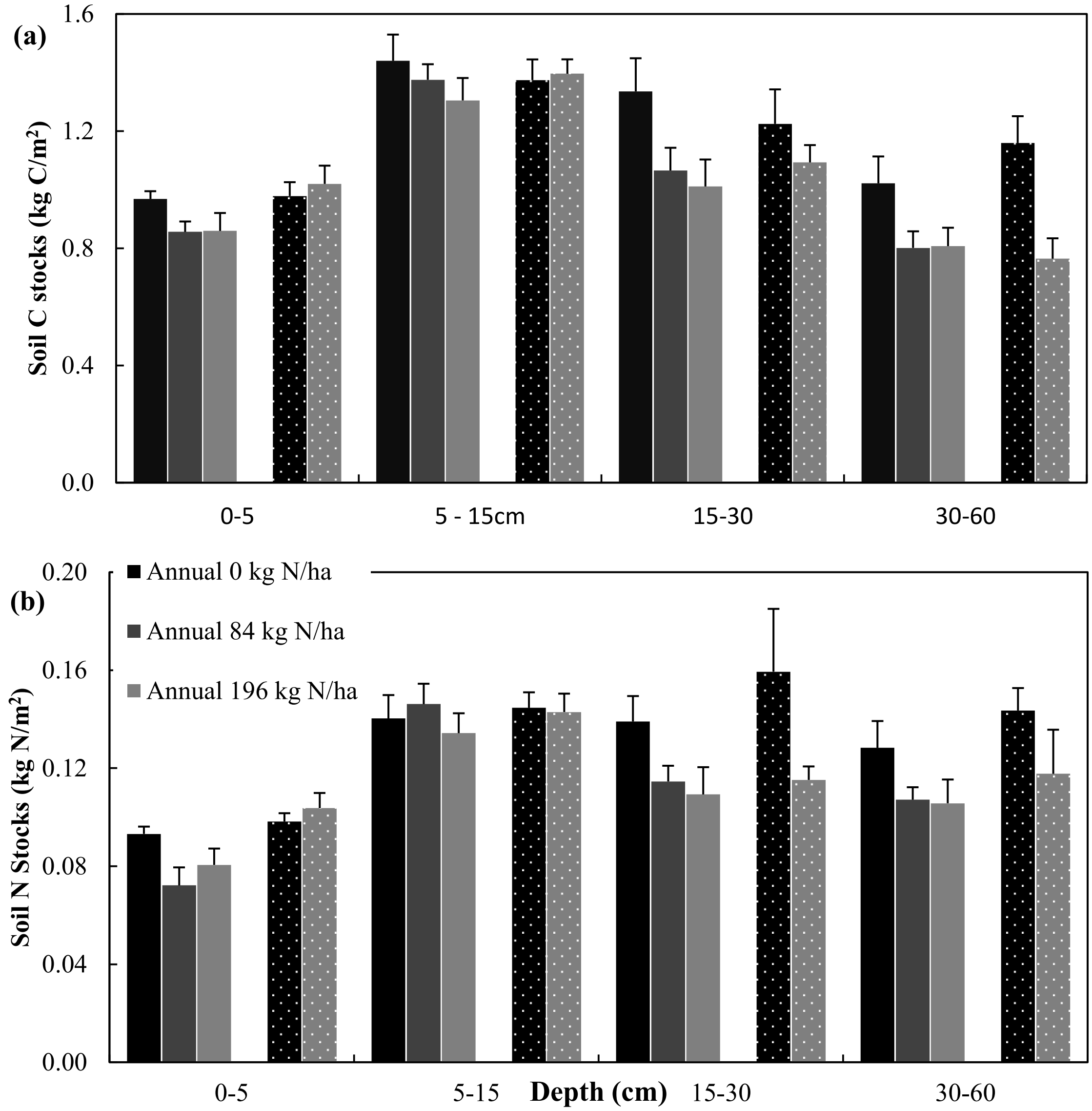

Figure S1: Depth effect of harvesting and fertilizer treatments on soil N and C stocks