

RESEARCH ARTICLE

Effects of nitrogen fertilization and bioenergy crop type on topsoil organic carbon and total Nitrogen contents in middle Tennessee USA

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Abstract

Nitrogen (N) fertilization affects bioenergy crop growth and productivity and consequently carbon (C) and N contents in soil, it however remains unclear whether N fertilization and crop type individually or interactively influence soil organic carbon (SOC) and total N (TN). In a three-year long fertilization experiment in switchgrass (SG: *Panicum virgatum* L.) and gamagrass (GG: *Tripsacum dactyloides* L.) croplands in Middle Tennessee USA, soil samples (0–15cm) were collected in plots with no N input (NN), low N input (LN: 84 kg N ha⁻¹ yr⁻¹ in urea) and high N input (HN: 168 kg N ha⁻¹ yr⁻¹ in urea). Besides SOC and TN, the above-ground plant biomass was also quantified. In addition to a summary of published root morphology data based on a separated mesocosm experiment, the root leachable dissolved organic matter (DOM) of both crops was also measured using archived samples. Results showed no significant interaction of N fertilization and crop type on SOC, TN or plant above-ground biomass (ABG). Relative to NN, HN (not LN) significantly increased SOC and TN in both crops. Though SG showed a 15–68% significantly higher ABG than GG, GG showed a 9.3–12% significantly higher SOC and TN than SG. The positive linear relationships of SOC or TN with ABG were identified for SG. However, GG showed structurally more complex and less readily decomposed root DOM, a larger root volume, total root length and surface area than SG. Collectively, these suggested that intensive N fertilization could increase C and N stocks in bioenergy cropland soils but these effects may be more likely mediated by the aboveground biomass in SG and root chemistry and morphology in GG. Future studies are expected to examine the root characteristics in different bioenergy croplands under the field fertilization experiment.

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Introduction

Perennial switchgrass (SG: *Panicum virgatum* L) and gamagrass (GG: *Tripsacum dactyloides* L) are two important bioenergy crops that are common alternative energy sources for sustainable replacement of fossil fuels [1–3]. Added together with other cellulosic biofuel crops, these dedicated energy crops will contribute to more than 30% of biofuel plant biomass in the coming decades [2, 4]. N fertilizers generally increase bioenergy crop yields [5, 6], but many studies report highly varied magnitudes and signals of soil C and N contents in response to N fertilization [7–10]. Few studies have compared the root traits in different bioenergy crops and no study has investigated the role of root traits in mediating bioenergy crop and soil responses to N fertilization. Elucidating the effects of fertilization on plant and soil C and N dynamics will provide fundamental knowledge needed to develop effective strategies to improve soil quality, C sequestration, agricultural productivity, and climate change adaptation [11–13].

Past studies showed no consistent pattern of N fertilization effect on SOC and TN contents. N fertilizations can enhance SOC and TN contents by 9–45% in SG croplands [8, 14–20]. Under an intensive N fertilization regime (e.g., 180 kg N ha⁻¹ yr⁻¹), SOC enhancement is reported due to C accretion from elevated root C input and reduced input of particulate organic C [7, 21]. In another study, both inorganic and manure N fertilizations can improve SOC sequestration capacity in SG croplands [15], which is associated with elevated shoot and root biomass [22]. In other studies, N fertilizations, however, show no significant effects on SOC pools in a soil profile (0–100cm) at a fertilization rate between 0 and 220 kg N ha⁻¹ yr⁻¹ [17, 23, 24]. A similar conclusion was reached in a fertilization experiment of short-term period of 2–3 years after SG establishment [20]. On the other hand, little change in soil TN under fertilization can be derived based on the N budget for annual SG production, which was closely balanced with 6.3 g N m⁻² removed by harvest of aboveground biomass and 6.7 g N m⁻² supplied by fertilization [25]. Though not common, N fertilizations can also diminish SOC and TN stocks, and this effect is particularly evident in the stable, mineral-associated C and N pools at depths greater than 15 cm [8]. To our best knowledge, no studies have reported soil C and N storages in response to N fertilization in GG croplands. It also remained unknown whether there was significant interaction of N fertilization and bioenergy crop type on SOC and TN stocks.

The large variations of SOC and TN in response to N fertilization are typically attributed to the perennial nature of bioenergy crops and their deep-rooted growth form [18]. Relative to SG, GG is reported to possess larger roots, higher root biomass and volume [26], total root length and surface area [27]. This contrasting root morphology may favor accrual of SOC and TN [28, 29]. Besides the root morphology, plant litter and root chemistry of bioenergy crops may also influence SOC and TN changes under N fertilization. Gil and Fick [30] identified a higher plant biomass C:N ratio for GG than other crops, which correlated strongly with lower net N mineralization and losses thus favoring C and N sequestrations [31]. The *in situ* root chemistry (C:N) of bioenergy crops is rarely quantified but our recent work found that both plant shoot and root C:N differed largely between SG and GG based on a mesocosm experiment. These studies focused on plant traits, but provided little information of C and N changes in soil simultaneously so that understanding the interaction of soil and bioenergy crop is hindered.

Knowing the abundance of humic-like or protein-like compounds will offer information of chemical recalcitrance [32, 33], but this analysis has not been conducted for root of bioenergy crops. A study revealed that the structural-tissue-dominated slow turnover root C pool concentrated at surface soil horizons in a prairie [34] and this indicated a strong linkage of root chemistry with soil C and N storage. However, no study has simultaneously quantified soil

response and root traits (e.g., morphology and chemistry) in order to explore how root traits likely moderate soil responses in bioenergy crops. Using a bioenergy crop field experiment in Middle Tennessee, we investigate the effects of N fertilization on the elemental characteristics of plant and soil C and N in two bioenergy croplands (SG and GG). N fertilization represents the primary management practice in our research plots with no tillage, plowing, or minor mechanical disturbance applied during the experimental period.

Given the different nature of SG and GG roots (i.e., chemistry and morphology), we first hypothesize that there is a significant interaction of N fertilization and bioenergy crop type on SOC, TN and plant aboveground biomass such that N fertilization-enhanced SOC, TN and plant aboveground biomass was more pronounced in GG than SG. Alternatively, there is only significant N fertilization effect. In that scenario, we establish the second hypothesis that the N fertilization effect will be significant only under a high fertilization rate because the low fertilization effect can be masked due to large variations in field measurements. Based on a mesocosm experiment examining the two bioenergy crop (SG and GG) seedlings' characteristics, we set up the third hypothesis that the root leachable dissolved organic matter (DOM) is more structurally complex and less easily decomposed for GG than SG because GG root is larger based on the published data synthesis of root morphology in the same mesocosm study. Although we lack fertilization treatment in the mesocosm study, the root morphology and chemistry of the two bioenergy crops are compared and linked to SOC and TN changes in response to N fertilization.

Materials and methods

Site description, soil and plant sample collections

Initially established in 2011, the bioenergy crop field fertilization experiment is located at the Tennessee State University (TSU) Main Campus Agriculture Research and Education Center (AREC) in Nashville, TN, USA (Lat. 36.12° N, Long. 36.98° W, elevation 127.6 m above sea level). Prior to the establishment of switchgrass and gamagrass croplands, the land use type was the mowed grassland for several decades. No fertilizers were applied during the prior land use. Climate in the region is a warm humid temperate climate with an average annual temperature of 15.1°C, and total annual precipitation of 1200 mm [35]. The crop type and N fertilization treatments were included in a randomized block design [27, 36]. The two crop types were *Alamo* SG (*Panicum virgatum* L.) and GG (*Tripsacum dactyloides* L.). The three N levels included no N fertilizer input (NN), low N fertilizer input (LN: 84 kg N ha⁻¹ yr⁻¹ as urea), and high N fertilizer input (HN: 168 kg N ha⁻¹ yr⁻¹ as urea), and each treatment had four replicated plots with a dimension of 3 m × 6 m. The low N fertilization rate was determined as the optimum N rate to maximize cellulosic ethanol production in established northern latitude grasslands [37]. The high N rate doubled the low rate in order to create appreciable gap and detectable effect between the two levels. The fertilizer was manually applied in June or July each year after cutting the grass. The soil series for the plots is Armour silt loam soil (fine-silty, mixed, thermic Ultic *Hapludalfs*) with acidic soil pH (i.e., 5.97) and intermediate organic matter content of 2.4% [36, 38].

In the fertilization experiment, soil samples (0–15 cm) were collected from 12 plots (2 crops × 3 N inputs × 2 replicated plots) on June 6, 2015. Within each plot, 24 cores were randomly collected using a spatially explicit sampling design [39] and a total of 288 soil cores were obtained in 12 plots. This soil sampling design has been used to quantify the spatial heterogeneity of soil microbial biomass, SOC and TN in the same experiment [36, 40], and in a former study [41]. The soil samples were transported to the TSU lab in a cooler filled with ice packs and were then subsequently stored at 4 °C until analysis. Visible roots and rocks were removed

from the samples, and soil samples were then passed through a 2 mm soil sieve. SOC and TN concentrations were analyzed using a Costech 4010 elemental analyzer (Costech analytical technologies Inc., Valencia, CA, USA). Although 24 samples were collected and analyzed in each replicated plot (i.e., used to map soil C and N spatial heterogeneity in another manuscript), the mean values of SOC, TN, and C:N were obtained in each plot and applied in the ANOVA test in order to avoid the artificial effect of pseudo-replication [42]. This generated 12 samples (3 fertilization \times 2 crop \times 2 replicate).

Harvesting of SG and GG aboveground (ABG) biomass was conducted twice in four replicated plots under each of three fertilization treatments during June to October in 2014 and 2015. This resulted in 24 samples in each year (2 crops \times 3 N inputs \times 4 replicated plots). At each harvest, plants were cut 7 inches above the ground using a Carter Mfg. Co plot harvester with flail cutters and mounted module capable collecting biomass fresh weights in the field. In each plot, subsamples of fresh biomass per unit area were dried to constant weight at 70°C using an Oven King industrial capacity dryer (Washington Industrial Corp. Seattle, WA, USA) to determine dry biomass. The unit of biomass was expressed as Mg ha⁻¹. To analyze biomass C and N concentrations, subsamples of dry biomass in 2014 and 2015 were selected and one composited sample was obtained by equal weight of sample for each crop under each fertilization treatment (i.e., NN, LN, and HN). This generated 6 samples (3 fertilization \times 2 crop). Plant samples were analyzed for C and N concentrations using a Costech 4010 elemental analyzer (Costech analytical technologies Inc., Valencia, CA, USA).

The mesocosm experiment, root sample collection and analysis

The root materials of SG and GG were obtained from the historical archived samples collected from a mesocosm experiment [27]. Briefly, the experiment was conducted in the greenhouse of the TSU campus farm in 2015 when the SG and GG seedlings were planted in tree pots for three months. Before planting, seeds were germinated in potting mix (FafardH #2 mix). At the 3- to 4-leaf stage, seedlings were transplanted into 15-cm wide \times 41-cm high tree pots (Stuewe and Sons, <https://www.stuewe.com/products/treepots.php>), each containing 6 kg of soil. The pot pH was set at 6.5, which is similar to the acidic soil pH in the field fertilization experiment (i.e., 5.97). Each treatment was replicated eight times. After 3 months, root samples were cut from below the soil surface and rinsed thoroughly with DI water. Root samples were dried in 70°C to constant weight. The root traits including surface area, length, and biomass productivity were compared between SG and GG and the results have been published formerly [27].

For this study, eight replicated root samples for both crops were selected to analyze the abundance and components of dissolved organic matter (DOM) leached from root samples. This generated 16 samples. This analysis was conducted at the Molecular Eco-Geochemistry laboratory of University of Alabama. For DOM leaching, root powders were mixed with carbon-free ultrapure water at a ratio of around 1:8 by mass for most samples. If slurry-like mixture appeared at this ratio, extra water was added until a clear liquid layer appeared. The mixtures were constantly agitated for 42 hours on an orbital shaker at 300 rpm, followed by centrifugation at 4,000 rpm for 20 minutes. The upper liquid layer was carefully transferred to a new vial using a pipette and the leachable DOM in these samples was further analyzed for absorbance and fluorescence properties (i.e., Excitation-Emission Matrix coupled with Parallel Factor Analysis), following the analytical methods described in detail in former publications [33, 43].

Here we briefly described the procedures on how to conduct the DOM absorbance and fluorescence property analysis. The absorbance of DOM was analyzed using a UV-1800

Shimadzu spectrophotometer, and the spectra from the wavelength of 190 to 670 nm at a 1 nm interval were collected. Three-dimensional fluorescence excitation-emission matrices (EEM) were analyzed using a Horiba Jobin-Yvon Fluoromax-3 spectrofluorometer, with the reading collected at excitation wavelengths from 240 to 500 nm at 5 nm intervals and emission wavelengths from 280 to 538 nm at 3 nm intervals. The EEM spectra were corrected for blanks, the inner filter effect, and the manufacturer's correction factors and subsequently normalized relative to the area under the water Raman peak [44]. A series of optical indices are calculated to interpret DOM source and compositional characteristics: 1) slope ratio (S_R) of absorbance of 275–295nm over 350–400nm, which is negatively correlated with DOM molecular weight [45, 46]; 2) the ratio of E2: E3 (ratio of absorbance at 250 to 365 nm), which decreases as DOM molecular size increases [47]; 3) fluorescence index (FI), for which lower values are thought to represent larger, structurally more complex compounds usually produced from terrestrial plant decay [48]; 4) the ratio of C to T, which indicates the relative amount of humic-like (recalcitrant) versus protein-like (labile) compounds in a sample [32]; and 5) humification index (HIX), for which greater values correspond to an increasing degree of humification [49, 50].

The parallel factor analysis (PARAFAC) was conducted in MATLAB using the DOMFluor toolbox described in detail by [51], and the final model was validated using the split-half analysis [52]. Based on fluorescence excitation-emission matrix [32, 53], the PAFRAC model was used to identify three components—C1 and C2 representing protein-like DOM and C3 representing humic-like DOM (S1 Table). Given that tyrosine-like DOM is found to be the first component to decrease in leaf leachate during the senescence [54], a high percent tyrosine-like DOM and low tryptophan-like DOM in a sample indicate more labile SOM to microbial degradation.

Statistical analysis

Two-way analysis of variance (ANOVA) was used to test the main and interactive effects of N fertilization and crop type on SOC, TN, and C: N, and plant ABG biomass in 2014 and 2015. Tukey HSD *Post hoc* tests were conducted to compare the means when a main or interactive effect is significant. To conduct the ANOVA, the original data was log transformed if it violated equal variance assumption. The regression plots between SOC, TN and plant ABG biomass were also obtained for SG and GG. One-way ANOVA was used to examine the effect of crop type on the indices of DOM leached from root (S_R , E2: E3, FI, and HIX; CT, Tyrosine-like DOM, Tryptophan-like DOM and Humic DOM). These analyses were conducted using R [55]. The significance level was set at $P < 0.1$. This threshold *p-value* was selected to accommodate the likely high variability of initial soil C and N contents at the beginning of the experiment.

Results

SOC, TN, C: N, and ABG biomass under fertilization in SG and GG

There was no significant interactive effect of fertilization and crop type on SOC, or TN ($P > 0.1$; Table 1). There were significant effects of fertilization and crop type on SOC and TN ($P < 0.1$; Table 1). Post hoc tests indicated that relative to NN, LN insignificantly increased SOC and TN by 2.5% and 2.8%, and HN significantly increased SOC and TN by 15% and 17%, respectively (Table 2). Relative to SG, GG showed a significantly 9.3% higher SOC and 12% higher TN (Table 2). Last, no significant fertilization or crop type effect on C: N was detected, but their interaction effect is significant ($P < 0.1$; Table 1).

There was no significant fertilization effect or interaction of fertilization and crop type on ABG, but there was significant effect of crop type on ABG in both collections in 2014 and 2015

Table 1. *p*-values of two-way ANOVA statistical tests on the main and interactive effects of N fertilization and crop type on SOC, TN and C: N as well as aboveground plant biomass (ABG) in 2014 and 2015 under three fertilization treatments in SG and GG croplands at the fertilization experiment in TSU AERC in Nashville, TN, USA.

Variable	N fertilization	Crop	Crop×N fertilization
SOC	0.072	0.082	0.878
TN	0.057	0.049	0.429
C: N	0.401	0.163	0.059
ABG (2014)	0.434	0.097	0.821
ABG (2015)	0.144	0.025	0.463

Bold numbers denote $P < 0.1$.

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($P < 0.1$; Table 1). Post hoc tests indicated a significantly higher ABG by 15–68% in SG than GG (Fig 1). The regression plots of SOC, TN and ABG biomass showed stronger linear relationship of SOC or TN with ABG for SG ($R^2 > 0.86$) than those for GG ($R^2 < 0.41$) (Fig 2).

DOM chemistry of SG and GG roots

Both S_R and FI values were significantly higher for SG than GG samples (Fig 3), indicating a lower molecular weight and structural complexity of DOM from SG than from GG. The mean values in C:T and HIX were lower and the mean value in E2: E3 was higher for SG than GG, but no statistically significant differences were detected for these indices (Fig 3). The percent tyrosine-like DOM was significantly higher and the percent tryptophan-like DOM was lower in SG than GG, however, percent humic-like DOM little differed between SG and GG (Fig 3).

Discussion

N fertilization enhanced SOC and TN concentrations in SG and GG

Based on our results, we rejected the first hypothesis that the fertilization and crop type interactively influenced SOC and TN. However, we found that N fertilization significantly increased SOC and TN in both SG and GG croplands. This was likely due to the minimal management and mechanical disturbance in our plots, which minimized soil decomposition due to less exposure of below surface soil to air, consequently diminished soil C and N losses in favor of soil C and N accumulations in perennial bioenergy feedstock grasslands [56–58]. In

Table 2. Mean (\pm SE) SOC and TN concentrations (%), and C: N as well as their respective coefficients of variance (CV, %) under three fertilization treatments in SG and GG croplands at the fertilization experiment in TSU AERC in Nashville, TN, USA.

Crop	N Fertilization	SOC		TN		C: N	
		Mean \pm SE	CV	Mean \pm SE	CV	Mean \pm SE	CV
		%	%	%	%	%	
SG	NN	1.48 \pm 0.005 ^a	0.45	0.13 \pm 0.002 ^a	1.86	11.05 \pm 0.09 ^a	1.13
	LN	1.56 \pm 0.09 ^a	7.91	0.15 \pm 0.01 ^a	11.23	10.57 \pm 0.27 ^a	3.61
	HN	1.72 \pm 0.03 ^b	2.32	0.17 \pm 0.0003 ^b	0.26	10.27 \pm 0.18 ^a	2.45
GG	NN	1.66 \pm 0.11 ^a	9.30	0.16 \pm 0.007 ^a	6.08	10.10 \pm 0.23 ^a	3.15
	LN	1.66 \pm 0.14 ^a	11.52	0.16 \pm 0.02 ^a	14.04	10.62 \pm 0.22 ^a	2.95
	HN	1.89 \pm 0.07 ^b	5.48	0.18 \pm 0.004 ^b	3.51	10.40 \pm 0.13 ^a	1.73

SG: switchgrass; GG: gamagrass; NN: No N input; LN: Low N fertilizer input (84 kg N ha⁻¹ yr⁻¹)

HN: High N fertilizer input (168 kg N ha⁻¹ yr⁻¹)

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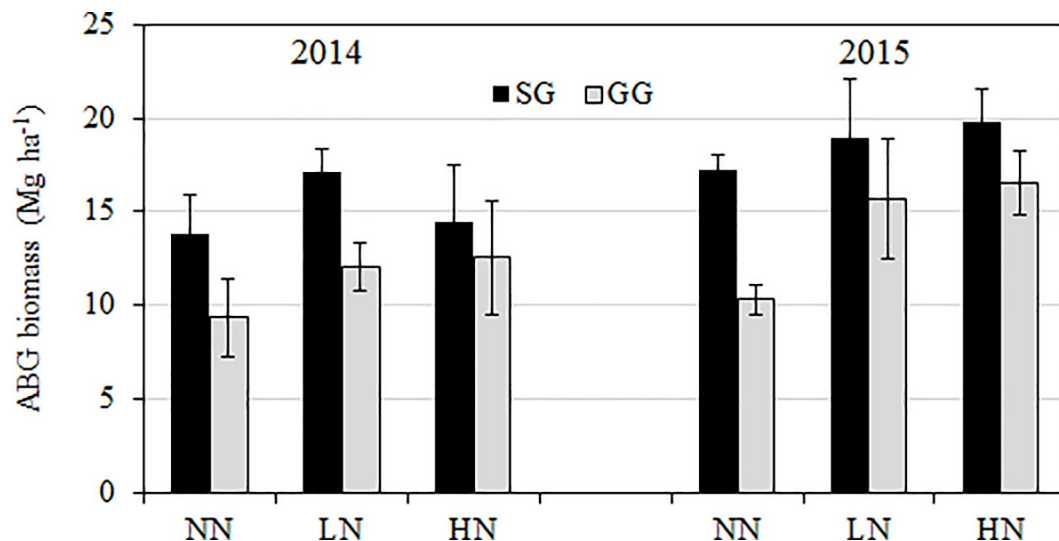


Fig 1. Mean (\pm SE) plant ABG biomass (Mg ha^{-1}) under three fertilization treatments (e.g. NN, LN and HN) in SG and GG croplands in 2014 and 2015. There was only significant crop type effect on ABG biomass in each collection year (Table 1). Each bar represents a mean value of four replicates ($n = 4$). NN: no N input; LN: low N input (84 kg N ha^{-1} in urea); HN: high N input (168 kg N ha^{-1} in urea); ABG: aboveground.

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other bioenergy croplands with more common practices, i.e., tillage, plowing and mechanical movement as implemented in conventional croplands (e.g., wheat and corn), more pronounced soil C and N losses may occur due to greater soil decomposition and likely reverse the net SOC and TN gains to net losses. Furthermore, N fertilization could significantly depress soil respiration, microbial biomass and extracellular oxidases' activities [59, 60], which led to slow turnover of soil C and N cycles and overall SOC and TN accretions [59].

The fertilizer-elevated aboveground biomass yield and the belowground rhizodeposits may also have contributed to the SOC and TN sequestrations by supplying additional amounts of C and N to the soil [7, 56, 61]. This interpretation is supported by the fertilization-enhanced aboveground plant biomass in SG (10–15%) and GG (52–61%) as measured in the same year (e.g., 2015) in this study. Though the belowground root biomass was not qualified in the current fertilization experiment due to technical difficulty (e.g., more than 100cm deep soil excavation and much wider horizontal digging), our literature review showed that bioenergy crop root biomass were not responsive to N fertilization [23, 62]. Despite the less response of total root biomass, both SG and GG possessed a significant volume and mass of coarse root and recalcitrant root exudate to soil [7, 63–65], which had longer residence times in soil in favor of soil C sequestration [66].

N fertilization effects on SOC and TN depend on fertilization rate

Results from this study supported our second hypothesis that relative to no fertilizer input, fertilization resulted in substantial SOC and TN enrichments only at the relatively high N application rates ($168 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) and less likely so at the low fertilization rates ($84 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). This finding contrasts with other studies that have demonstrated negative effects of relatively high fertilization rates on soil C and N storage [7, 67]. These negative effects were interpreted as a result of high fertilizer input causing more abundant soil bacteria and low fertilizer use efficiency, which elevated the C and N losses from the soil system [67]. Stewart, Follett [7] documented significant soil C and N accretions even when amended with a relatively low fertilizer

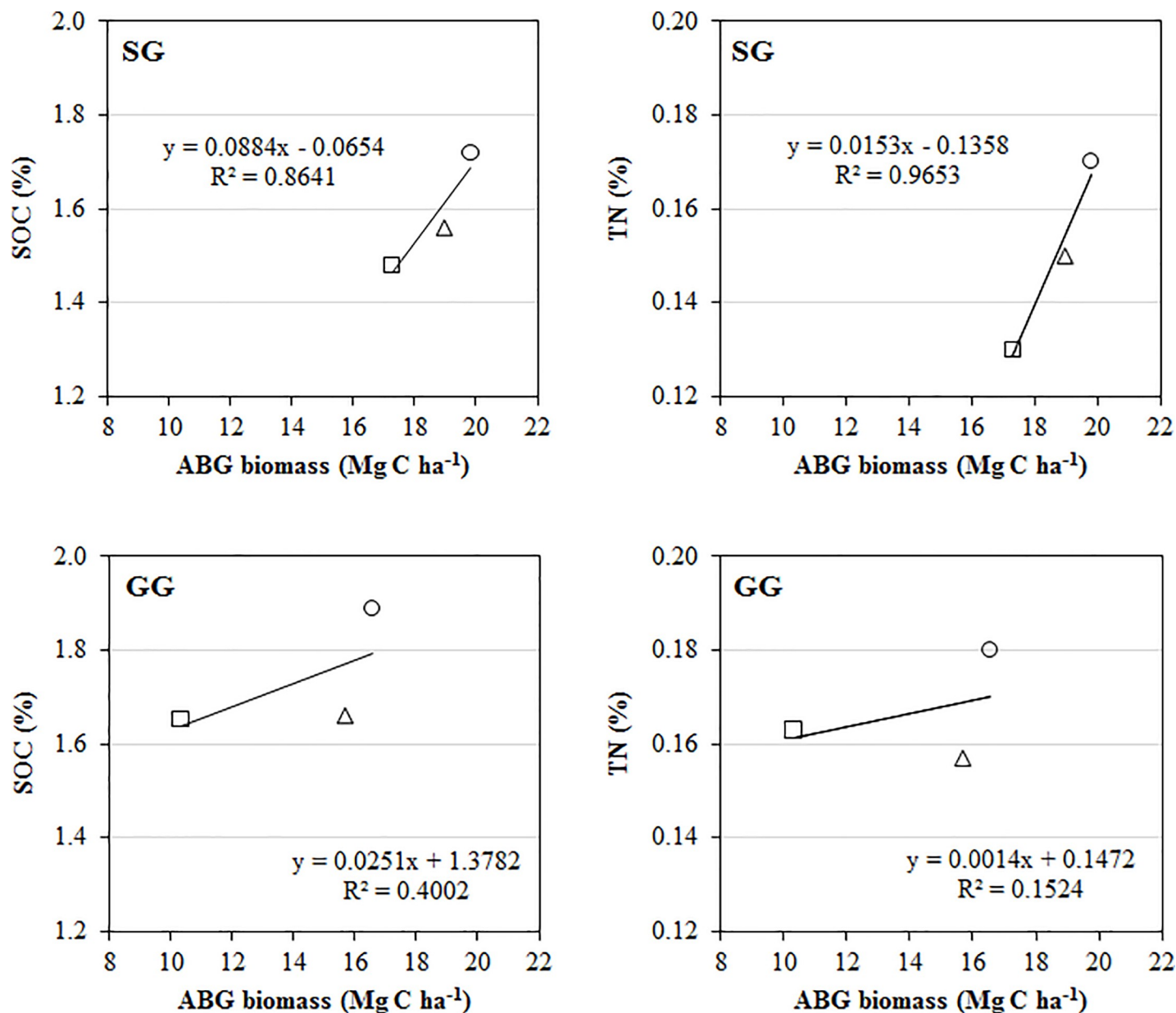


Fig 2. Regression plots of mean SOC, TN and plant ABG biomass under NN (□ square), LN (Δ triangle) and HN (○ circle) in SG and GG croplands. Plant ABG biomass was referred to the collection in 2015 only. NN: no N input; LN: low N input (84 kg N ha⁻¹ in urea); HN: high N input (168 kg N ha⁻¹ in urea); ABG: aboveground.

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input rate similar to this study. These accretions were likely driven by a greater return of aboveground and belowground plant materials to soil after harvesting. Collectively, a threshold fertilizer input rate may exist in our research plots as to their effects on soil C and N storage, but precautions should be taken when different soil and plant types need to be accounted for. Nevertheless, a wide spectrum of fertilization intensity of up to 300 kg N ha⁻¹ has been reported in published studies [68, 69], future studies should examine the N fertilization effects in a wide range of fertilization input rates. From a pragmatic perspective, given the pressing need for minimizing the adverse impacts of agriculture on environment, a recommendation is

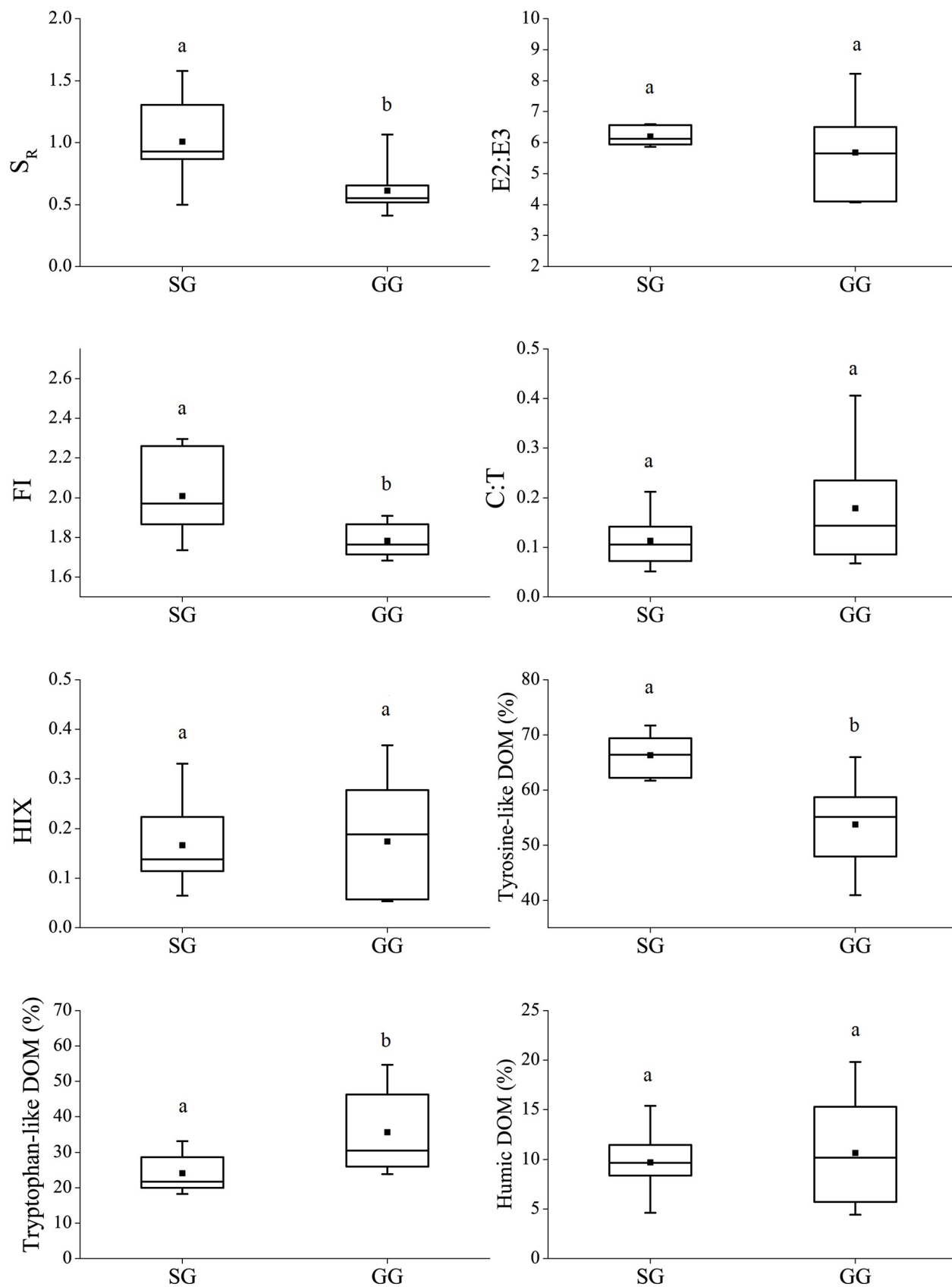


Fig 3. Boxplots of DOM source-composition indices (SR, FI and HIX) and percentage of tryptophan-like compound in leachable DOM in root of SG and GG (N = 8). For each panel, the different lowercase letters denote significant difference between SG and GG ($P < 0.05$). Boxplots show medians (line), means (dot), 1st and 3rd quartiles (box, interquartile range or *IQR*), upper and lower extremes (whiskers). The whiskers were determined as equal to or less extreme than 1.5 times *IQR* against 1st and 3rd quartiles, respectively. The definitions of indices and compounds were defined in the *Methods* section.

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to adopt a sustainable agricultural practice and the important measure is to lower the use of N and other fertilizers [70]. Therefore, it is imperative to elucidate whether a lower-end threshold of fertilizer input exists so that the fertilizer use would continuously benefit both crop productivity and soil fertility with less adverse impact on environment.

SOC and TN storage correlate with different plant traits

Our analysis of leachable DOM from root supported the third hypothesis that GG root contained higher molecular weight and more structurally complex compound than SG root. This result indicates that GG root would be less readily decomposed compared to SG root. SG is known to have a lower specific root length (i.e., root length per unit root biomass) [27, 71] and GG to have larger coarse root biomass and volume [26]. Given the contrasting root chemistry and morphology between SG and GG, one expected to see a relatively short turnover time for SG root and much longer turnover time for GG root [34]. The slow turnover GG root favored long-term SOC and TN sequestrations [34, 72, 73], likely due to more root-derived organic matter in mineral-associated soil fractions [74] and thus offering explanation of the greater SOC and TN stocks in GG than SG as observed in this study. The assumption is that the contrasting root characteristics observed in the mesocosm experiment will remain under the field fertilization experiment. This is likely true because of similar soil pH in the mesocosm and field experiments, which play a key role in root growth and development [75].

On the other hand, stronger linear relationships of SOC and TN with aboveground plant biomass was identified for SG and less so for GG. Given the significantly greater aboveground plant biomass of SG than GG, these results indicate that the contributions of aboveground plant biomass to belowground soil C and N stocks via litterfall input and turnover were stronger in SG than GG. Considering the aforementioned relationship of GG root with soil C and N storage, our results revealed that the plant traits that contributed to the soil C and N sequestrations varied with bioenergy crop species. It was the aboveground plant biomass of SG and the root of GG that have showed likely associations with their respective soil C and N sequestrations. Despite the long known beneficial role of bioenergy crops on soil C, this study highlighted the need to further elucidate the role of different plant traits (e.g., aboveground vs. belowground) in regulating soil C and N sequestration [76].

Conclusions

This study demonstrated that relative to no fertilizer input, intensive N fertilization (e.g., HN) could significantly increase SOC and TN in bioenergy cropland surface soils (0–15 cm). Meanwhile, GG showed significantly higher SOC and TN and significantly lower aboveground biomass than SG. There were strong positive linear relationships of SOC and TN with aboveground biomass in SG, and structurally more complex and less readily decomposed root DOM in GG. This suggested that the intensive N fertilization induced C and N accumulations in soil may be more likely mediated by the aboveground biomass in SG and root chemistry and morphology in GG. Future studies should examine the root characteristics in different bioenergy croplands under the field fertilization experiment.

Supporting information

S1 Table. Characteristics of the three fluorescence components identified by PARAFAC model and their attributed sources. The modeling method was described in *Method* section. (DOCX)

S1 Data. Dataset of SOC, TN and C: N, plant aboveground (ABG) biomass, and root DOM chemistry. (XLSX)

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References

1. Gelfand I, Sahajpal R, Zhang X, Izaurralde RC, Gross KL, Robertson GP. Sustainable bioenergy production from marginal lands in the US Midwest. *Nature*. 2013; 493(7433):514–7. <https://doi.org/10.1038/nature11811> PMID: 23334409
2. Monti A, Barbanti L, Zatta A, Zegada-Lizarazu W. The contribution of switchgrass in reducing GHG emissions. *Global Change Biology Bioenergy*. 2012; 4(4):420–34.
3. Wright L, Turhollow A. Switchgrass selection as a “model” bioenergy crop: A history of the process. *Biomass and Bioenergy*. 2010; 34(6):851–68.

4. Tulbure MG, Wimberly MC, Owens VN. Response of switchgrass yield to future climate change. *Environmental Research Letters*. 2012; 7(4).
5. Lemus R, Lal R. Bioenergy crops and carbon sequestration. *Critical Reviews in Plant Sciences*. 2005; 24(1):1–21.
6. Pedroso GM, Hutmacher RB, Putnam D, Six J, van Kessel C, Linquist BA. Biomass yield and nitrogen use of potential C4 and C3 dedicated energy crops in a Mediterranean climate. *Field Crop Res*. 2014; 161:149–57.
7. Stewart CE, Follett RF, Pruessner EG, Varvel GE, Vogel KP, Mitchell RB. N fertilizer and harvest impacts on bioenergy crop contributions to SOC. *Global Change Biology Bioenergy*. 2016; 8(6):1201–11.
8. Valdez ZP, Hockaday WC, Masiello CA, Gallagher ME, Robertson GP. Soil Carbon and Nitrogen Responses to Nitrogen Fertilizer and Harvesting Rates in Switchgrass Cropping Systems. *Bioenergy Res*. 2017; 10(2):456–64.
9. Bransby DI, McLaughlin SB, Parrish DJ. A review of carbon and nitrogen balances in switchgrass grown for energy. *Biomass Bioenerg*. 1998; 14(4):379–84.
10. Li JW, Jian SY, de Koff JP, Lane CS, Wang GS, Mayes MA, et al. Differential effects of warming and nitrogen fertilization on soil respiration and microbial dynamics in switchgrass croplands. *Global Change Biology Bioenergy*. 2018; 10(8):565–76.
11. Tiemann LK, Grandy AS. Mechanisms of soil carbon accrual and storage in bioenergy cropping systems. *Global Change Biology Bioenergy*. 2015; 7(2):161–74.
12. Turner MG. Landscape ecology: What is the state of the science? *Annu Rev Ecol Evol Syst*. 2005; 36:319–44.
13. Groffman PM, Hardy JP, Fisk MC, Fahey TJ, Driscoll CT. Climate Variation and Soil Carbon and Nitrogen Cycling Processes in a Northern Hardwood Forest. *Ecosystems*. 2009; 12(6):927–43.
14. Springer TL. Effect of Nitrogen Fertilization and Residual Nitrogen on Biomass Yield of Switchgrass. *Bioenergy Res*. 2017; 10(3):648–56.
15. Lee DK, Owens VN, Doolittle JJ. Switchgrass and soil carbon sequestration response to ammonium nitrate, manure, and harvest frequency on conservation reserve program land. *Agronomy Journal*. 2007; 99(2):462–8.
16. Sainju UM, Singh HP, Singh BP. Soil Carbon and Nitrogen in Response to Perennial Bioenergy Grass, Cover Crop and Nitrogen Fertilization. *Pedosphere*. 2017; 27(2):223–35.
17. Heggenstaller AH, Moore KJ, Liebman M, Anex RP. Nitrogen Influences Biomass and Nutrient Partitioning by Perennial, Warm-Season Grasses. *Agronomy Journal*. 2009; 101(6):1363–71.
18. Stewart CE, Follett RF, Pruessner EG, Varvel GE, Vogel KP, Mitchell RB. Nitrogen and harvest effects on soil properties under rainfed switchgrass and no-till corn over 9 years: implications for soil quality. *Global Change Biology Bioenergy*. 2015; 7(2):288–301.
19. Kering MK, Butler TJ, Biermacher JT, Mosali J, Guretzky JA. Effect of Potassium and Nitrogen Fertilizer on Switchgrass Productivity and Nutrient Removal Rates under Two Harvest Systems on a Low Potassium Soil. *Bioenergy Res*. 2013; 6(1):329–35.
20. Ma Z, Wood CW, Bransby DI. Soil management impacts on soil carbon sequestration by switchgrass. *Biomass and Bioenergy*. 2000; 18(6):469–77.
21. Rasmussen PE, Allmaras RR, Rohde CR, Roager NC. Crop Residue Influences on Soil Carbon and Nitrogen in a Wheat-Fallow System1. *Soil Science Society of America Journal*. 1980; 44(3):596–600.
22. Garten CT, Brice DJ, Castro HF, Graham RL, Mayes MA, Phillips JR, et al. Response of "Alamo" switchgrass tissue chemistry and biomass to nitrogen fertilization in West Tennessee, USA. *Agriculture Ecosystems & Environment*. 2011; 140(1–2):289–97.
23. Kibet LC, Blanco-Canqui H, Mitchell RB, Schacht WH. Root biomass and soil carbon response to growing perennial grasses for bioenergy. *Energy, Sustainability and Society*. 2016; 6(1):1.
24. Lai L, Kumar S, Osborne S, Owens VN. Switchgrass impact on selected soil parameters, including soil organic carbon, within six years of establishment. *Catena*. 2018; 163:288–96.
25. Garten CT, Smith JL, Tyler DD, Amonette JE, Bailey VL, Brice DJ, et al. Intra-annual changes in biomass, carbon, and nitrogen dynamics at 4-year old switchgrass field trials in west Tennessee, USA. *Agriculture Ecosystems & Environment*. 2010; 136(1–2):177–84.
26. Clark RB, Alberts EE, Zobel RW, Sinclair TR, Miller MS, Kemper WD, et al. Eastern gamagrass (*Tripsacum dactyloides*) root penetration into and chemical properties of claypan soils. *Plant and Soil*. 1998; 200(1):33–45.

27. Dzantor EK, Adeleke E, Kankarla V, Ogunmayowa O, Hui D. Using Coal Fly Ash Agriculture: Combination of Fly Ash and Poultry Litter as Soil Amendments for Bioenergy Feedstock Production. *Coal Combustion and Gasification Products*. 7, 33–39, doi: 104177/CCGP-D-15-000021 2015.
28. Gross KL, Pregitzer KS, Burton AJ. Spatial Variation in Nitrogen Availability in 3 Successional Plant-Communities. *Journal of Ecology*. 1995; 83(3):357–67.
29. Wang LX, Okin GS, Caylor KK, Macko SA. Spatial heterogeneity and sources of soil carbon in southern African savannas. *Geoderma*. 2009; 149(3–4):402–8.
30. Gil JL, Fick WH. Soil nitrogen mineralization in mixtures of eastern gamagrass with alfalfa. *Agronomy Journal*. 2001; 93(4):902–10.
31. Woo DK, Quijano JC, Kumar P, Chaoka S, Bernacchi CJ. Threshold dynamics in soil carbon storage for bioenergy crops. *Environ Sci Technol*. 2014; 48(20):12090–8. <https://doi.org/10.1021/es5023762> PMID: 25207669
32. Helms JR, Stubbins A, Perdue EM, Green NW, Chen H, Mopper K. Photochemical bleaching of oceanic dissolved organic matter and its effect on absorption spectral slope and fluorescence. *Mar Chem*. 2013; 155:81–91.
33. Lu YH, Edmonds JW, Yamashita Y, Zhou B, Jaegge A, Baxley M. Spatial variation in the origin and reactivity of dissolved organic matter in Oregon-Washington coastal waters. *Ocean Dynam*. 2015; 65(1):17–32.
34. Dietzel R, Liebman M, Archontoulis S. A deeper look at the relationship between root carbon pools and the vertical distribution of the soil carbon pool. *SOIL*. 2017; 3(3):139–52.
35. Deng Q, Aras S, Yu CL, Dzantor EK, Fay PA, Luo YQ, et al. Effects of precipitation changes on above-ground net primary production and soil respiration in a switchgrass field. *Agriculture Ecosystems & Environment*. 2017; 248:29–37.
36. Li J, Guo C, Jian S, Deng Q, Yu C-L, Dzantor KE, et al. Nitrogen Fertilization Elevated Spatial Heterogeneity of Soil Microbial Biomass Carbon and Nitrogen in Switchgrass and Gamagrass Croplands. *Sci Rep-Uk*. 2018; 8(1):1734.
37. Jungers JM, Sheaffer CC, Lamb JA. The Effect of Nitrogen, Phosphorus, and Potassium Fertilizers on Prairie Biomass Yield, Ethanol Yield, and Nutrient Harvest. *Bioenerg Res*. 2015; 8(1):279–91.
38. Yu CL, Hui DF, Deng Q, Wang JM, Reddy KC, Dennis S. Responses of corn physiology and yield to six agricultural practices over three years in middle Tennessee. *Sci Rep-Uk*. 2016; 6.
39. Li J. Sampling Soils in a Heterogeneous Research Plot. *J Vis Exp* (), e58519, doi: 103791/58519(2018) 2018.
40. Li J, Jian S, Lane CS, Guo C, Lu Y, Deng Q, et al. Nitrogen Fertilization Restructured the Spatial Patterns of Soil Organic Carbon and Nitrogen in Switchgrass and Gamagrass Croplands in Tennessee USA. *Scientific Reports*. Accepted. 2019.
41. Li JW, Richter DD, Mendoza A, Heine P. Effects of land-use history on soil spatial heterogeneity of macro- and trace elements in the Southern Piedmont USA. *Geoderma*. 2010; 156(1–2):60–73.
42. Hurlbert SH. Pseudoreplication and the Design of Ecological Field Experiments. *Ecological Monographs*. 1984; 54(2):187–211.
43. Lu YH, Bauer JE, Canuel EA, Chambers RM, Yamashita Y, Jaffe R, et al. Effects of land use on sources and ages of inorganic and organic carbon in temperate headwater streams. *Biogeochemistry*. 2014; 119(1–3):275–92.
44. Cory RM, McKnight DM. Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinones in dissolved organic matter. *Environmental science & technology*. 2005; 39(21):8142–9.
45. Helms JR, Stubbins A, Ritchie JD, Minor EC, Kieber DJ, Mopper K. Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. *Limnol Oceanogr*. 2008; 53(3):955–69.
46. Shang P, Lu YH, Du YX, Jaffe R, Findlay RH, Wynn A. Climatic and watershed controls of dissolved organic matter variation in streams across a gradient of agricultural land use. *Sci Total Environ*. 2018; 612:1442–53. <https://doi.org/10.1016/j.scitotenv.2017.08.322> PMID: 28903173
47. De Haan H, De Boer T. Applicability of light absorbance and fluorescence as measures of concentration and molecular size of dissolved organic carbon in humic Lake Tjeukemeer. *Water Research*. 1987; 21(6):731–4.
48. McKnight DM, Boyer EW, Westerhoff PK, Doran PT, Kulbe T, Andersen DT. Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnol Oceanogr*. 2001; 46(1):38–48.

49. Ohno T. Response to comment on "Fluorescence inner-filtering correction for determining the humification index of dissolved organic matter". *Environ Sci Technol*. 2002; 36(19):4196–.
50. Hu Y, Lu YH, Edmonds JW, Liu CK, Wang S, Das O, et al. Hydrological and land use control of watershed exports of dissolved organic matter in a large arid river basin in northwestern China. *J Geophys Res-Biogeophys*. 2016; 121(2):466–78.
51. Stedmon CA, Bro R. Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial. *Limnology and Oceanography: Methods*. 2008; 6(11):572–9.
52. Murphy KR, Stedmon CA, Graeber D, Bro R. Fluorescence spectroscopy and multi-way techniques. *PARAFAC. Analytical Methods*. 2013; 5(23):6557–66.
53. Lu YH, Bauer JE, Canuel EA, Yamashita Y, Chambers RM, Jaffe R. Photochemical and microbial alteration of dissolved organic matter in temperate headwater streams associated with different land use. *J Geophys Res-Biogeophys*. 2013; 118(2):566–80.
54. Wheeler KI, Levia DF, Hudson JE. Tracking senescence-induced patterns in leaf litter leachate using parallel factor analysis (PARAFAC) modeling and self-organizing maps. *Journal of Geophysical Research: Biogeosciences*. 2017; 122(9):2233–50.
55. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vinnna, Austria. 2015; ISBN 3-900051-07-0, URL <http://www.R-project.org/>.
56. Ma Z, Wood CW, Bransby DI. Impacts of soil management on root characteristics of switchgrass. *Biomass and Bioenergy*. 2000; 18(2):105–12.
57. Le X, Hui D, Dzantor E. Characterizing Rhizodegradation of the Insecticide Bifenthrin in Two Soil Types. *Journal of Environmental Protection*. 2011; 2(7):940–6.
58. Anderson EK, Aberle E, Chen C, Egenolf J, Harmoney K, Kakani VG, et al. Impacts of management practices on bioenergy feedstock yield and economic feasibility on Conservation Reserve Program grasslands. *GCB Bioenergy*. 2016; 8(6):1178–90.
59. Jian S, Li J, Chen J, Wang G, Mayes MA, Dzantor KE, et al. Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: A meta-analysis. *Soil Biology and Biochemistry*. 2016; 101:32–43.
60. Ward D, Kirkman K, Hagenah N, Tsvuura Z. Soil respiration declines with increasing nitrogen fertilization and is not related to productivity in long-term grassland experiments. *Soil Biology and Biochemistry*. 2017; 115:415–22.
61. Adkins J, Jastrow JD, Morris GP, Six J, de Graaff M-A. Effects of switchgrass cultivars and intraspecific differences in root structure on soil carbon inputs and accumulation. *Geoderma*. 2016; 262:147–54.
62. Jung JY, Lal R. Impacts of nitrogen fertilization on biomass production of switchgrass (*Panicum Virgatum* L.) and changes in soil organic carbon in Ohio. *Geoderma*. 2011; 166(1):145–52.
63. Jaron A, Aislinn J. Switchgrass Root Decomposition Impacts on Soil Carbon Sequestration. College of Arts and Sciences Presentations 37 http://scholarworksboisestateedu/as_14/37 2014.
64. An Y, Ma YQ, Shui JF. Switchgrass root exudates have allelopathic potential on lettuce germination and seedling growth. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science*. 2013; 63(6):497–505.
65. Rangel-Castro JI, Killham K, Ostle N, Nicol GW, Anderson IC, Scrimgeour CM, et al. Stable isotope probing analysis of the influence of liming on root exudate utilization by soil microorganisms. *Environmental Microbiology*. 2005; 7(6):828–38. <https://doi.org/10.1111/j.1462-2920.2005.00756.x> PMID: 15892702
66. Trumbore S. Age of soil organic matter and soil respiration: Radiocarbon constraints on belowground C dynamics. *Ecological Applications*. 2000; 10(2):399–411.
67. Zhu S, Vivanco JM, Manter DK. Nitrogen fertilizer rate affects root exudation, the rhizosphere microbiome and nitrogen-use-efficiency of maize. *Applied Soil Ecology*. 2016; 107:324–33.
68. Lu CQ, Tian HQ. Global nitrogen and phosphorus fertilizer use for agriculture production in the past half century: shifted hot spots and nutrient imbalance. *Earth Syst Sci Data*. 2017; 9(1):181–92.
69. Potter P, Ramankutty N, Bennett EM, Donner SD. Global Fertilizer and Manure, Version 1: Nitrogen Fertilizer Application. Palisades, NY: NASA Socioeconomic Data and Applications Center (SEDAC); 2011.
70. Cui ZL, Zhang HY, Chen XP, Zhang CC, Ma WQ, Huang CD, et al. Pursuing sustainable productivity with millions of smallholder farmers. *Nature*. 2018; 555(7696):363–+. <https://doi.org/10.1038/nature25785> PMID: 29513654
71. de Graaff MA, Six J, Jastrow JD, Schadt CW, Wulschleger SD. Variation in root architecture among switchgrass cultivars impacts root decomposition rates. *Soil Biology & Biochemistry*. 2013; 58:198–206.

72. Matamala R, González-Meler MA, Jastrow JD, Norby RJ, Schlesinger WH. Impacts of Fine Root Turnover on Forest NPP and Soil C Sequestration Potential. *Science*. 2003; 302(5649):1385–7. <https://doi.org/10.1126/science.1089543> PMID: 14631037
73. De Deyn GB, Cornelissen JH, Bardgett RD. Plant functional traits and soil carbon sequestration in contrasting biomes. *Ecol Lett*. 2008; 11(5):516–31. <https://doi.org/10.1111/j.1461-0248.2008.01164.x> PMID: 18279352
74. Austin EE, Wickings K, McDaniel MD, Robertson GP, Grandy AS. Cover crop root contributions to soil carbon in a no-till corn bioenergy cropping system. *Global Change Biology Bioenergy*. 2017; 9(7):1252–63.
75. Robles-Aguilar AA, Pang J, Postma JA, Schrey SD, Lambers H, Jablonowski ND. The effect of pH on morphological and physiological root traits of *Lupinus angustifolius* treated with struvite as a recycled phosphorus source. *Plant and Soil*. 2019; 434(1):65–78.
76. Gregory AS, Dungait JAJ, Shield IF, Macalpine WJ, Cunliffe J, Durenkamp M, et al. Species and Genotype Effects of Bioenergy Crops on Root Production, Carbon and Nitrogen in Temperate Agricultural Soil. *Bioenergy Res*. 2018; 11(2):382–97.