



Perennial Grass Bioenergy Cropping on Wet Marginal Land: Impacts on Soil Properties, Soil Organic Carbon, and Biomass During Initial Establishment

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Abstract

The control of soil moisture, vegetation type, and prior land use on soil health parameters of perennial grass cropping systems on marginal lands is not well known. A fallow wetness-prone marginal site in New York (USA) was converted to perennial grass bioenergy feedstock production. Quadruplicate treatments were fallow control, reed canarygrass (*Phalaris arundinaceae* L. Bellevue) with nitrogen (N) fertilizer (75 kg N ha⁻¹), switchgrass (*Panicum virgatum* L. Shawnee), and switchgrass with N fertilizer (75 kg N ha⁻¹). Based on periodic soil water measurements, permanent sampling locations were assigned to various wetness groups. Surface (0–15 cm) soil organic carbon (SOC), active carbon, wet aggregate stability, pH, total nitrogen (TN), root biomass, and harvested aboveground biomass were measured annually (2011–2014). Multi-year decreases in SOC, wet aggregate stability, and pH followed plowing in 2011. For all years, wettest soils had the greatest SOC and active carbon, while driest soils had the greatest wet aggregate stability and lowest pH. In 2014, wettest soils had significantly ($p < 0.0001$) greater SOC and TN than drier soils, and fallow soils had 14 to 20% greater SOC than soils of reed canarygrass + N, switchgrass, and switchgrass + N. Crop type and N fertilization did not result in significant differences in SOC, active carbon, or wet aggregate stability. Cumulative 3-year aboveground biomass yields of driest switchgrass + N soils (18.8 Mg ha⁻¹) were 121% greater than the three wettest switchgrass (no N) treatments. Overall, soil moisture status must be accounted for when assessing soil dynamics during feedstock establishment.

Keywords Marginal soil · Switchgrass · Reed canarygrass · Soil organic carbon · Active carbon · Wet aggregate stability

Abbreviations

CTRL	Fallow control treatment	Q4	Soil moisture/wetness quintile 4
OM	Organic matter	Q5	Soil moisture/wetness quintile 5
Active carbon	Permanganate-oxidizable (labile) carbon	RCG	Reed canarygrass + fertilizer N treatment
Q1	Soil moisture/wetness quintile 1	SOC	Soil organic carbon
Q2	Soil moisture/wetness quintile 2	SWG	Switchgrass treatment
Q3	Soil moisture/wetness quintile 3	SWG N	Switchgrass +fertilizer N treatment
		TN	Total nitrogen

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Introduction

Perennial bioenergy crops are projected to reduce US reliance on fossil fuels, reduce greenhouse gas emissions, and enhance rural economies. In the Northeast US (NE), fallow marginal lands are cited as a primary resource base for the perennial bioenergy sector [5, 93]. Many soils in this region are not well suited (hence marginal [75]) for row crop agriculture due to seasonal water saturation or near saturation, which commonly

results from presence of shallow restrictive layers. Soil moisture status renders about 20% of NE soils poorly or very poorly drained [94]. Though the capacity of perennial grasses to tolerate (and potentially improve) suboptimal soil properties, reduce erosion, sequester soil organic carbon (SOC), and improve soil biodiversity are well known [9, 48, 56, 81], research on the impacts of dedicated bioenergy perennial crops at the field scale under varied soil conditions is yet needed. Additionally, there is little representation of the NE in most reviews of C sequestration potential of bioenergy crops [9, 88]. The extent of potential carbon (C) sequestration as an ecosystem service of perennial bioenergy systems is dependent on soil texture, initial SOC, climate, drainage, cropping history, and management practices [88]. Though conversion of cropland to grassland (pastures or bioenergy plantations) results in accumulation of SOC ([29, 43, 82]; [30]; [28]), an initial loss of SOC is observed when previously uncultivated fallow lands are converted to perennial grasses [2, 16, 19, 88, 92]. Cultivation of undisturbed/untilled soils usually depletes organic carbon stocks by releasing stored carbon to the atmosphere and hence impacts both CO₂ fluxes and soil fertility [12, 28]. Tillage results in significant fracturing of peds, reduced soil aggregation [85, 99], mixing of soil horizons/loss of stratification [21, 72, 104], disruption of plant roots and variations in mycorrhizal communities [34], influences on priming effects through variations in the fresh carbon to nutrient concentration ratios along the soil profile [22], and declines in SOC stocks [6, 39]. This incurred “carbon debt” is often repaid over a period of years or decades before any net sequestration occurs.

Soil moisture is one of the most important environmental controls of plant growth [42, 77, 78, 84] and soil microbial activity, hence affecting both C inputs and outputs of soil [49, 64, 67, 73]. As the rate of decomposition relative to production is low in cold and wet climates, soil C stocks are globally greater in such areas [24, 36, 62, 97]. Switchgrass (*Panicum virgatum* L.) is a common warm-season (C4) perennial selected as a model system for cellulosic biomass production. Apart from placing organic C in deeper soil layers, switchgrass is thought to increase the proportion of stable soil aggregates, which aids in long-term C sequestration [51]. Reed canarygrass (*Phalaris arundinacea* L.) is an herbaceous cool-season (C3) perennial long used for forage production in wetness-prone soils. Because of its adaptation to wet conditions, it is regarded as an invasive species in riparian fringes and wetlands throughout North America [59]. Its capacity to accumulate large amounts of C in biomass within a short-growing season is thought to cause significant alterations in SOC dynamics [7, 83]. Due to differing mechanisms of carbon fixation of the two photosynthetic pathways (Hatch-Slack cycle [C4 plants] vs. Calvin

cycle [C3 plants]), warm-season C4 plants have approximately 50% greater photosynthetic efficiency than cool-season C3 plants [100]. It is generally assumed that C4 grasses also have greater long-term C sequestration potential, due to belowground productivity that is greater than C3 species [1, 3, 13, 43, 44].

Work by Corre et al. [16] at six sites in the Northeast US showed that there was no significant difference in SOC values between C3 and C4 grasses at all depths in moderately drained soil, and conversion of C3 to C4 grasslands resulted in SOC loss that required 16–18 years to recover. Studies with pasture mixtures (perennial C3/C4 grasses and legumes) in well-drained soils in Pennsylvania showed no relationship between changes in soil C and root biomass [87] between 5 and 60 cm. In southeastern Pennsylvania [79], conversion of hayfield/pastureland to switchgrass (and subsequent grazing activities) on well-drained, deep soils did not result in any change (minor loss) in net SOC after 5 years at soil depth increments of 0–5, 5.15, and 15–30-cm depth and there were no differences in SOC levels under different cultivars.

Given that inter-annual changes are small in comparison to the large reservoir of SOC [45, 69], SOC shifts are difficult to measure in the short term. However, the rapid, simple, and inexpensive permanganate-oxidizable carbon assay (more commonly known as active carbon, sometimes abbreviated as POXC) estimates a processed and stabilized pool of labile soil C derived from total microbial biomass [17, 18, 26, 60]. As such, it is sensitive to management and/or environment [17, 50, 60, 96, 101] and may be effective over shorter timeframes as a leading indicator of SOC trends and is accordingly used in soil health analytical assessments [61].

Perennial cropping promotes increased root production and exudates which are thought to promote aggregate formation and SOC stabilization. With reorientation of soil particles through physical and biological [35, 86, 96] disturbances, SOC becomes more protected within aggregates. The extent to which soil aggregates resist disintegration when wetted via simulated rainfall is measured as the wet aggregate stability of the soil, which is also often used as a soil health indicator [61].

In this perennial grass field study, we used 4 years (2011, 2012, 2013, and 2014) of soil properties data [SOC (OM derived), active carbon, wet aggregate stability, soil pH; also direct measurements of SOC and TN in 2014] and cumulative aboveground biomass yields to explore (1) the impact of the conversion via tillage of the previously fallow field to perennial grass during the early establishment phase and (2) the degree of soil moisture and vegetation control on soil properties. Finally, the association of soil and crop productivity parameters (cumulative aboveground biomass and root biomass) was also evaluated. Belowground root biomass was also measured annually. The 3-year cumulative values for aboveground biomass and third-year root biomass were used for

analyses, given that switchgrass typically reaches full yield potential only after several growing seasons [55, 65].

We hypothesized that (a) there would be loss in SOC due to the initial plowing, (b) that soil properties and aboveground biomass yields would be influenced by moisture gradient of the field, and (c) that at this early stage, there would be no detectable differences in SOC levels because of plant photosynthetic pathway (C3 vs. C4) or use of N fertilization.

Methods

Experimental Site The primary research site was a 10-ha field at Ithaca, New York, USA (42° N 28.20', 76° W 25.94') (Fig. 1a) with a predominant drainage catena comprised of three soil series: well-drained Canaseraga (coarse-silty, mixed, active, mesic Typic Fragiudept), somewhat poorly drained Dalton (coarse-silty, mixed, active, mesic Aeric Fragiudept), and poorly drained Madalin (fine, illitic, mesic Mollic Endoaqualf). Small areas of associated Rhinebeck (fine, illitic, mesic Aeric Endoaqualfs) and Langford (fine-loamy, mixed, active, mesic Typic Fragiudepts) soils were also present. As reflected in their taxonomic classifications, the Dalton, Canaseraga, and Langford series silt loams are characterized by a dense subsoil fragipan (Fig. 1b). The field topography is undulating, with slopes in the sampled areas varying from 0 and 8%, with a small area with short slopes up to 15% on the eastern edge of the field (Fig. 1b). Perched water tables recur seasonally, resulting from shallow restrictive layers (fragipan and/or dense basal till). The field is marginal for row crop or alfalfa production [75] due to this recurring wetness in many areas. Before perennial grasses were established in July 2011, the field had effectively been fallow for circa 50 years, with occasional mowing (and rarely hay harvest) used to prevent reversion to shrub and tree growth. The site's fallow vegetation in 2011 was dominated by legacy reed canarygrass and mixed forbs, including goldenrod (*Solidago* sp.) and, in the wettest areas, hemp dogbane (*Apocynum cannabinum*).

The total departure from 30-year average values of temperature is seen in Fig. 2a, and that from precipitation is seen in Fig. 2b. The crops were not irrigated. Subsurface tile drains (paralleling the main and lateral legacy surface drainage swales, Fig. 1a) were installed in June 2011 to help ensure trafficability of the wettest areas at harvest. A randomized complete block design was used for 16 large experimental units (denoted A through P, Fig. 1a) that comprised quadruplicate plots of the four cropping treatments: switchgrass (v. Shawnee, a selection from upland ecotype Cave-in-Rock) (SWG), switchgrass + fertilizer N (SWG_N), reed canarygrass (cv. Bellevue) + fertilizer N (RCG), and pre-existing fallow control (CTRL). Where used, the N fertilization rate was 75 kg N ha⁻¹ of ammonium sulfate ((NH₄)₂SO₄), surface-applied once annually in the spring, starting in 2012 for reed

canarygrass + fertilizer and delayed (as is typical for slow-to-establish switchgrass) until 2013 for SWG_N.

Sampling Subplots The trial is unique as the experimental units (each between 0.34 and 0.44 ha) were intentionally laid out to capture the continuum of soil moisture conditions that vary naturally from moderately well drained to poorly drained. In June 2011, the fallow land was prepared for perennial grass production by successively mowing, spraying re-growth with glyphosate herbicide, conventional moldboard plowing (20-cm nominal depth, with variation for unevenness) disking, and harrowing prior to planting. Five permanent sampling subplots were established along the natural moisture gradients of each experimental unit (Fig. 1a) based on an initial intensive survey (June 1–2, 2011) of surface layer volumetric water content measurements by time domain reflectometry (TDR, Campbell Scientific Hydrosense meter using 12-cm sensor rods). The subplot approach thus yielded 80 permanent sampling points where soil (including SOC and other soil health parameters) and harvested aboveground biomass yields (among other parameters) are sampled yearly. Frequent periodic surface soil moisture measurements (by TDR) were used to characterize the relative soil moisture status of each subplot. For each measurement event, a field average volumetric water content of all 80 subplots was calculated, and each subplot's value was normalized relative to the field mean (yielding a “relative soil moisture ratio” for that subplot and time point). These relative values were averaged over the entire study period for each subplot, and each subplot's characteristic wetness (relative to the field average) was thus established over 40 such measurement events cumulatively representing thousands of readings at the site. Finally, these multi-year mean values for the 80 subplots were aggregated into “soil wetness quintiles” for the entire study period (Table S4).

Therefore, 20 subplots of each cropping system and 16 subplots of each wetness quintile (Table 1 (a)) formed the basis of comparison in this study. Given that soil moisture did not vary uniformly among the total population of subplots as divided among cropping treatments, *N* for each wetness quintile under a cropping system varied between 2 and 6 (Table 1 (b)). Thus, the unintended consequence of incorporating greater precision to predict wetness and crop interaction was an unbalanced sample size for each wetness quintile (Table 1 (b)). The driest subplots represented in wetness quintile 5 (Q5) had mean volumetric water contents approximately 0.8 times the field mean, whereas the more variable wettest subplots, wetness quintile 1 (Q1) averaged 1.3 times the field mean [74]. These quintiles were used for categorizing soil moisture status with respect to other parameters.

Calculated for one sampling event (August 2014) but not shown were corresponding values in gravimetric units, based on a subset of soils collected and analyzed for a measurement

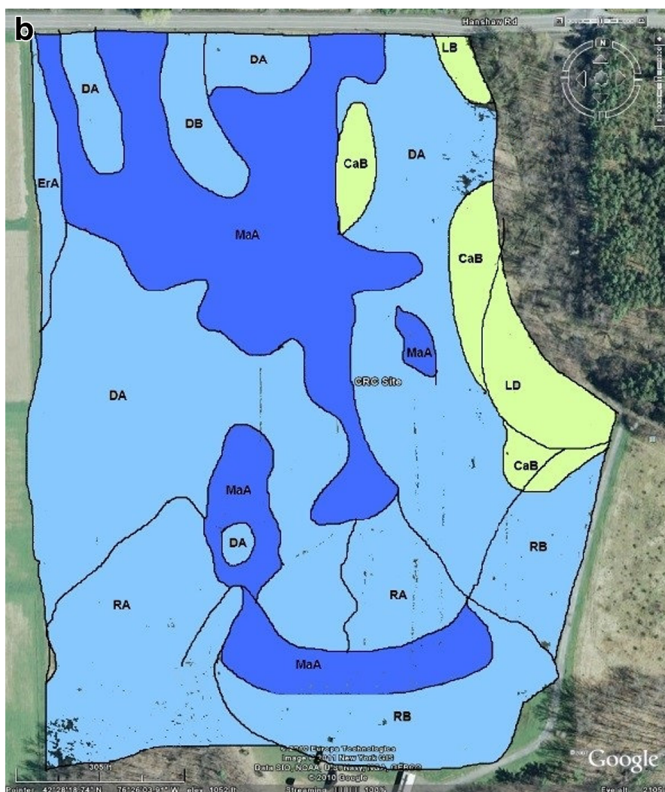
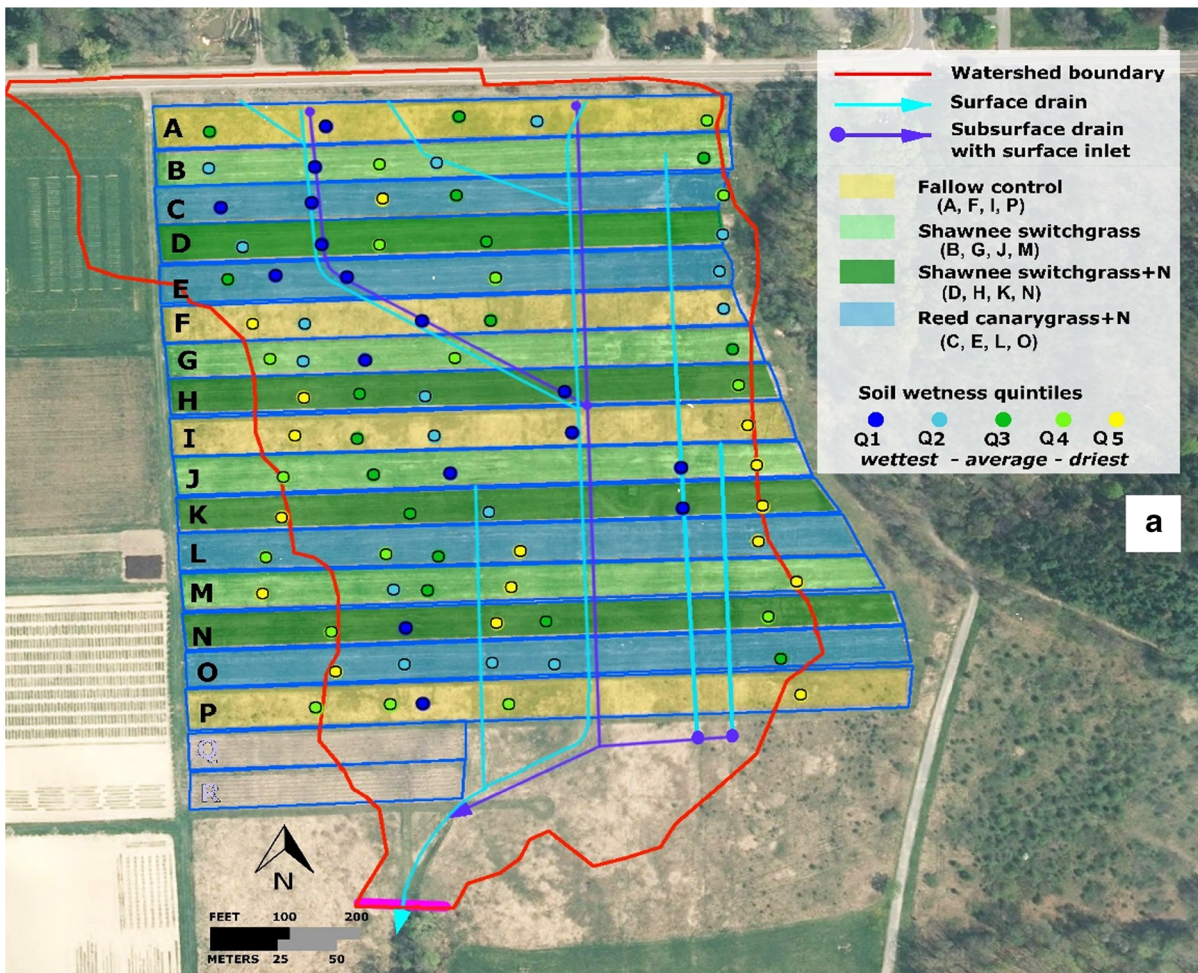


Fig. 1 a Field site layout depicting experimental units: fallow control, switchgrass, switchgrass + fertilizer 75 kg N ha^{-1} , reed canarygrass + fertilizer 75 kg N ha^{-1} . Each experimental unit comprises of five sampling subplots which were classified in quintiles Q1 to Q5 based on their mean relative soil moisture levels. Modified from July 2017 GoogleEarth image. **b** Soil series mapping adapted from Cline and Bloom [15]: CaB Caneseraga 3–8% slopes, DA Dalton 0–3% slopes, DB Dalton 3–8% slopes, LB Langford 3–8% slopes, LD Langford 8–15% slopes, MaA Madalin 0–3% slopes. (Moderately well drained (yellow); somewhat poorly drained (light blue); poorly drained (dark blue))

event in 2014. With these data, the volumetric water content values (TDR) were regressed against values of the gravimetric water content, and the equation was used to compute the mean water content values corresponding to the high, mid, and low TDR moisture quintiles from the field. The multi-year mean growing season gravimetric water contents corresponding to Q1 (highest), Q3, and Q5 (lowest) were 0.5, 0.4, and 0.3 g g^{-1} , respectively. As the gravimetric soil moisture equivalent from a single time point would not be able to correctly reflect the variability of the soil moisture content over the study period, we have instead used the quintile-based relative volumetric wetness to compare SOC levels. Soil texture of the 80 subplots, grouped as wetness quintiles, is presented in Table 2.

Sampling Protocol Soil sampling in June 2011 took place prior to any tillage and thus represented the preconversion

fallow state. Soils from the surface Ap layer were sampled each year in June/July. A flat shovel was used to dig to 15-cm depth at two locations equidistant (1.2 m) from the center of each subplot (as marked by a permanent subplot flag). Approximately 4 kg of soil was dug from each of the two locations, mixed, and composited in a bucket, with circa 1 kg transferred to labeled polyethylene bag. The soils were initially air-dried and later oven-dried at $55 \text{ }^\circ\text{C}$ for several days until constant weight was achieved. The soil samples were stored for later processing.

Biomass Sampling For this study, aboveground yield from each subplot was determined for the years 2012, 2013, and 2014 using hand-harvesting of replicate 1-m^2 quadrants, followed by crop vs. weed separation, weighing, and dry matter analysis. Results were added to obtain a cumulative yield value for each subplot used in the analysis. For belowground biomass, the soil bags were dried and weighed, and coarse roots were removed by handpicking and kept separately. Root crowns were not sampled. The roots were then passed through a 2-mm sieve to remove associated dirt particles and then weighed. The procedure (of non-washing and handpicking) was undertaken to preserve soil samples for further analysis, avoiding destructive processing. This approach recovers $\sim 60\%$ of the root mass typically obtained through more extensive soil washing techniques [54]. The coarse root biomass estimation from each subplot for the years 2012,

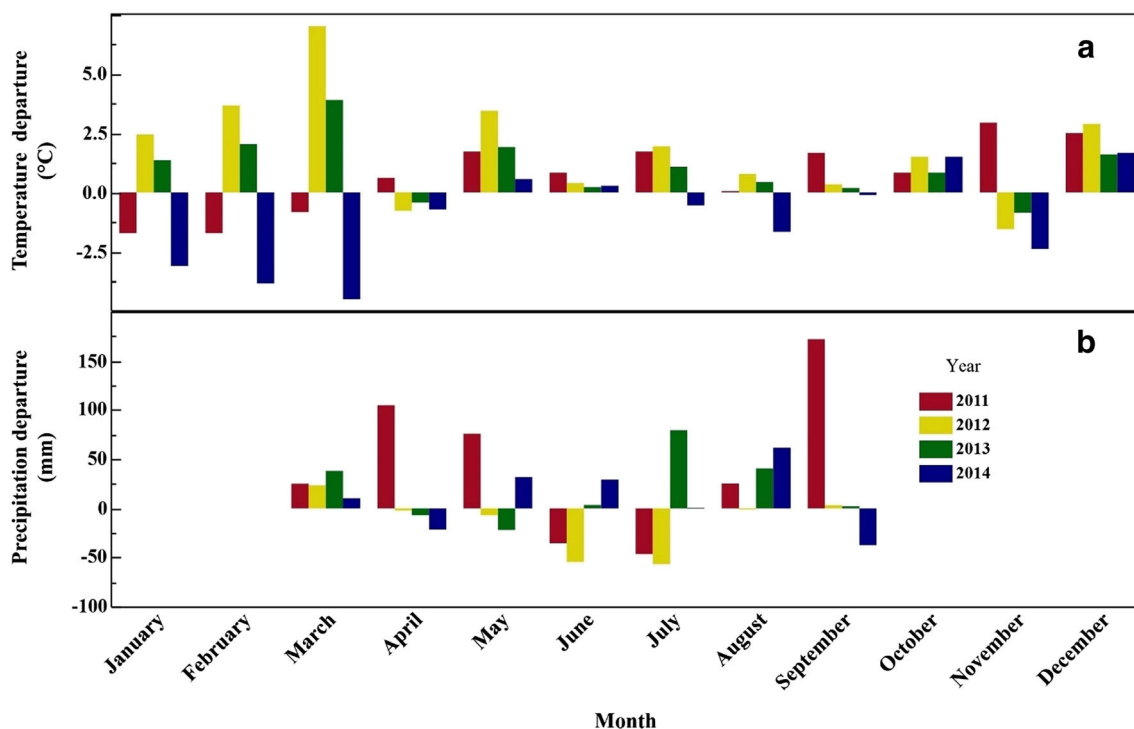


Fig. 2 Monthly departure of **a** air temperature and **b** precipitation from the 30-year mean (1981–2010) values for the 4-year study period (2011–2014) at Cornell University, Ithaca, obtained from the Northeastern

Regional Climate Center database. The precipitation data spans the growing season for both reed canarygrass and switchgrass (March–September)

Table 1 (a) Distribution schematic for the 80 subplots of the quadruplicate cropping systems and (b) distribution schematic of the number of subplots in each wetness quintile for each cropping system while the fallow control (CTRL), reed canarygrass + fertilizer 75 kg N ha⁻¹ (RCG), switchgrass (SWG), and switchgrass + fertilizer 75 kg N ha⁻¹ (SWGN) are the cropping systems; the wetness quintiles vary between Q1 (wettest) and Q5 (driest)

(a)		Distribution of subplots at the field site							
Cropping system	Multi-year wetness quintile rank (Q1, wettest–Q5, driest)	Multi-year wetness quintile rank (Q1, wettest–Q5, driest)							
		RCG	SWG	SWGN	Q1	Q2	Q3	Q4	Q5
CTRL									
20		20	20	20	16	16	16	16	16
(b)		Distribution of wet quintile representatives for each cropping system							
Multi-year wetness quintile rank (Q1, wettest–Q5, driest)		CTRL	RCG	SWG	SWGN				
		Q1	Q2	Q3	Q4				
Q1		5	3	4	4				
Q2		6	2	4	4				
Q3		4	5	3	4				
Q4		2	6	5	3				
Q5		3	4	4	5				

2013, and 2014 are presented in Figs. S9a, b. These conservative underestimates have been used for correlations with soil properties.

Laboratory Analyses Each soil sample (dried, with roots removed) was passed through a soil mill (Dynacrush DC-5 Soil Crusher, Custom Laboratory Eqpt, Orange City, Florida) with 10-mesh sieve (2-mm openings) three times before being finally sieved with a 1.8-m sieve, in accordance with Garten and Wullschlegler [25]. The coarse fragments were then weighed to help when converting analytical results (which based on fine soil only) to a realistic areal basis, since said fragments remain present during bulk density determinations. The mineral soil fraction (including any fine roots that passed the sieve) was thus prepared, and elemental C and N analysis was carried out on oven-dried (60 °C) 0.4 g soil samples by combustion infrared detection [LECO TruMac CN, (LECO Corp., St. Joseph, MI) with analytical precision of 0.01 mg or 0.3% RSD (whichever is greater) for N and 0.01 mg or 0.4% RSD for C (whichever is greater)]. Without access to LECO during the first year, the loss on ignition (LOI) method was used to estimate OM for 2011 samples, and OM estimations for all years were subsequently determined. For LOI

method, mass losses from ignition (2 h at 500 °C) were determined gravimetrically on oven-dried (105 °C for at least 4 h) ground and sieved soil samples. New York State empirical relationships of fractional OM content = 0.7(LOI)–0.23 (R² = 0.94) and OM = 1.724 (SOC) have been used for calculations of % C [61]. SOC results presented here as 3-year comprehensive data and as change from baseline levels are derived OM values, while direct elemental analysis SOC values from 2014 (presented in Fig. S1a, b) have been used for correlating with other parameters.

As pH values of all soil samples were below 7, the total soil C was considered equivalent to SOC [10, 70], with no carbonate presence assumed, which was further confirmed using 5 M HCl that resulted in no effervescence on a subset of 16 soil samples. SOC data reported here on a mass basis (g kg⁻¹), as the switchgrass establishment was still in process after 3 years, and post-establishment bulk density measurements had not yet been done by 2014. Soil pH and texture analyses were carried on as outlined in Moebius-Clune et al. [61]. Active carbon analysis was carried out via permanganate oxidation and spectrophotometry [17, 61, 101], and wet aggregate stability was measured using a sprinkle infiltrometer that steadily rains on a sieve containing a known weight of soil aggregates between 0.25 and 2 mm [61].

Table 2 Mean soil texture analysis results for surface soils (0–15 cm) of 80 subplots grouped by soil wetness quintile. Mean values and corresponding standard errors. Wetness quintiles not sharing the same lowercase letter within a texture class are significantly different at *p* < 0.05

Wetness quintile rank	Sand (%)	Silt (%)	Clay (%)
Q1	13.6 ± 0.7, c	68.2 ± 0.7, a	18.2 ± 0.9, a
Q2	15.7 ± 0.8, c	67.4 ± 0.7, ab	17 ± 0.9, a
Q3	16 ± 0.7, bc	67 ± 0.7, ab	17 ± 0.9, a
Q4	19 ± 0.8, ab	66.4 ± 0.8, ab	15 ± 0.9, a
Q5	20 ± 0.7, a	65 ± 0.7, b	15.4 ± 0.9, a

Statistical Analyses Hierarchical linear mixed-effects models were fit using SOC (OM derived), active carbon, wet aggregate stability, soil pH for all years (2011, 2012, 2013, 2014), 2014 SOC (measured directly), 2014 TN, and 3-year cumulative aboveground biomass as response variables, to analyze the impact of soil moisture and cropping system on these variables. In all the models, wetness quintile rank, cropping system, and the interaction between wetness quintile rank and cropping system were fixed effects, with subplot nested within experimental unit treated as random effect. In model selection, the interaction between fixed effects was removed when

insignificant (p value > 0.05). Changes in soil properties [SOC (OM derived), active carbon, wet aggregate stability, soil pH] from the baseline were calculated by subtracting initial (2011) values from values after 3 years (2014) for each sampling location (subplot). Similar structured linear mixed-effects models were used to explain differences among moisture quintiles or cropping systems for changes in these soil parameters from baseline. Additionally, to understand whether changes from baseline were significant within a moisture quintile or cropping system, paired t tests were performed. Post hoc treatment comparisons were made by using Tukey's HSD method to control for multiple comparisons.

Pearson correlation coefficients were computed between the analyzed variables of 2014 [SOC (measured directly), TN, active carbon, wet aggregate stability], edaphic factors (silt content, clay content, and soil pH), and cumulative aboveground biomass and 2014 root biomass. All statistical analyses were carried out using JMP Pro 12 (SAS Inc., Cary, NC).

Results

We first present yearly trends of SOC (OM derived), active carbon, wet aggregate stability, and soil pH as impacted by moisture or cropping system as well as the change in these parameters from 2011 baseline levels. Whenever results are expressed based on *wetness quintiles*, parameter values are averaged over all cropping systems; conversely, when represented based on *cropping systems*, results are averaged over all wetness quintiles. This is because the interaction of cropping system \times wetness quintile rank was not a significant effect for any soil parameter during any sampling year nor for changes from preplow baseline. Thereafter, we present cumulative aboveground biomass. Finally, we present relationships among soil properties (2014 only) and cumulative aboveground biomass and 2014 root biomass. SOC (measured directly), TN, and yearly root biomass data are presented in supplementary information.

Soil Organic Carbon, Active Carbon, Total N, Wet Aggregate Stability, Soil pH from Baseline to 2014 SOC levels in the wettest soils (Q1) were significantly ($p < 0.0001$) greater than in soils of the drier quintiles (Q2, Q3, Q4, Q5) during each sampling year (Fig. 3a, Table S5) with the only exception being Q2 soils in 2013. Among the cropping systems, SOC values were significantly ($p = 0.02$ and 0.03) greater for CTRL soils than for that of RCG in 2012 and SWGN in 2013 (Fig. 3b, Table S5), respectively. Decrease in SOC from 2011 to 2014 was significant ($p < 0.0001$) for each moisture quintile group and cropping system (Table S6a). Though the loss from baseline levels was not significant among the wetness quintiles, the loss in CTRL was significantly ($p = 0.009$) lower than those of SWG and SWGN (Fig. 4a, b, Table S6b). In 2014, mean SOC (direct measurement) values varied between

29.6 ± 4 (Q5) and 39.5 ± 6.5 g C kg⁻¹ (Q1) (Fig. S1a), those of Q1 being significantly ($p < 0.0001$) greater than the soils of all other wetness quintiles (Fig. S1a, Table S7 and S8). SOC varied between 31.2 ± 1.4 (SWGN) and 37.4 ± 1 g C kg⁻¹ (CTRL), for soils of the different cropping systems, with CTRL being significantly ($p = 0.03$) greater than SWG and SWGN (Fig. S1b, Tables S7 and S8). In 2014, TN values varied between 2.9 ± 0.3 (Q5) and 3.9 ± 0.6 g kg⁻¹ (Q1), with those of Q1 soils being significantly ($p < 0.0001$) greater than all the other wetness quintiles (Fig. S1c, Table S7 and S8). Cropping system was not a significant main effect impacting 2014 TN (Table S7).

The wettest Q1 soils displayed greater active carbon values than those of drier soils during different years, being significantly greater than those of the driest quintile (Q5) for the years 2011 ($p = 0.04$), 2012 ($p = 0.0001$), 2013 ($p = 0.04$), and 2014 ($p = 0.001$) (Fig. 3c, Table S5). Among the cropping systems, active carbon was significantly greater in CTRL than all other treatments in 2012 ($p = 0.0002$) (Fig. 3d, Table S5). Increases in active carbon over time were significant for CTRL and SWG cropping systems only; change from baseline in RCG or SWGN or any of the five wetness groups (Q1, Q2, Q3, Q4, and Q5) was not significant (Table S6a). Though the change from baseline levels was not significant among the wetness quintile groups, the increase in CTRL was significantly ($p = 0.007$) greater than those of SWG and SWGN (Fig. 4c, d, Table S6b).

Wet aggregate stability values for the driest Q5 soils were greatest among the wetness quintiles during all years and were significantly ($p = 0.03$) greater than those of Q1 in 2013 (Fig. 3e, Table S5). Cropping system was a significant main effect impacting wet aggregate stability, with CTRL being greater than all other treatments in 2012 ($p = 0.0001$), 2013 ($p = 0.0003$), and 2014 ($p = 0.005$) (Fig. 3f and Table S5). Loss in wet aggregate stability of soils from baseline levels for each moisture quintile and cropping system (except CTRL) was significant (Table S6a). Though loss from baseline levels was not significant among the wetness quintile groups, that for CTRL was significantly ($p = 0.01$) lower than those for RCG and SWGN (Fig. 4e, f, Table S6b).

Soil pH was significantly ($p < 0.0001$) lower in the driest soils (Q5), than soils of all wetter quintiles (Q4, Q3, Q2, Q1) for all sampling years (Fig. 3g, Table S5). Among the cropping systems, CTRL was significantly ($p = 0.008$) greater than RCG in 2012, significantly ($p < 0.0001$) greater than all other treatments (RCG, SWG, and SWGN) in 2013, and significantly ($p = 0.009$) greater than SWGN in 2014 (Fig. 3h and Table S5). Reduction in soil pH from baseline levels was significant for each moisture quintile and cropping system (Table S6a) group, but it did not vary significantly among wetness quintiles nor the cropping system groups (Fig. 4g, h, Table S6b).

Cumulative Harvestable Standing Biomass and Root Biomass

The interaction of cropping system and moisture quintile was significant ($F = 2.2$, $p = 0.02$), (Table S7). Cumulative

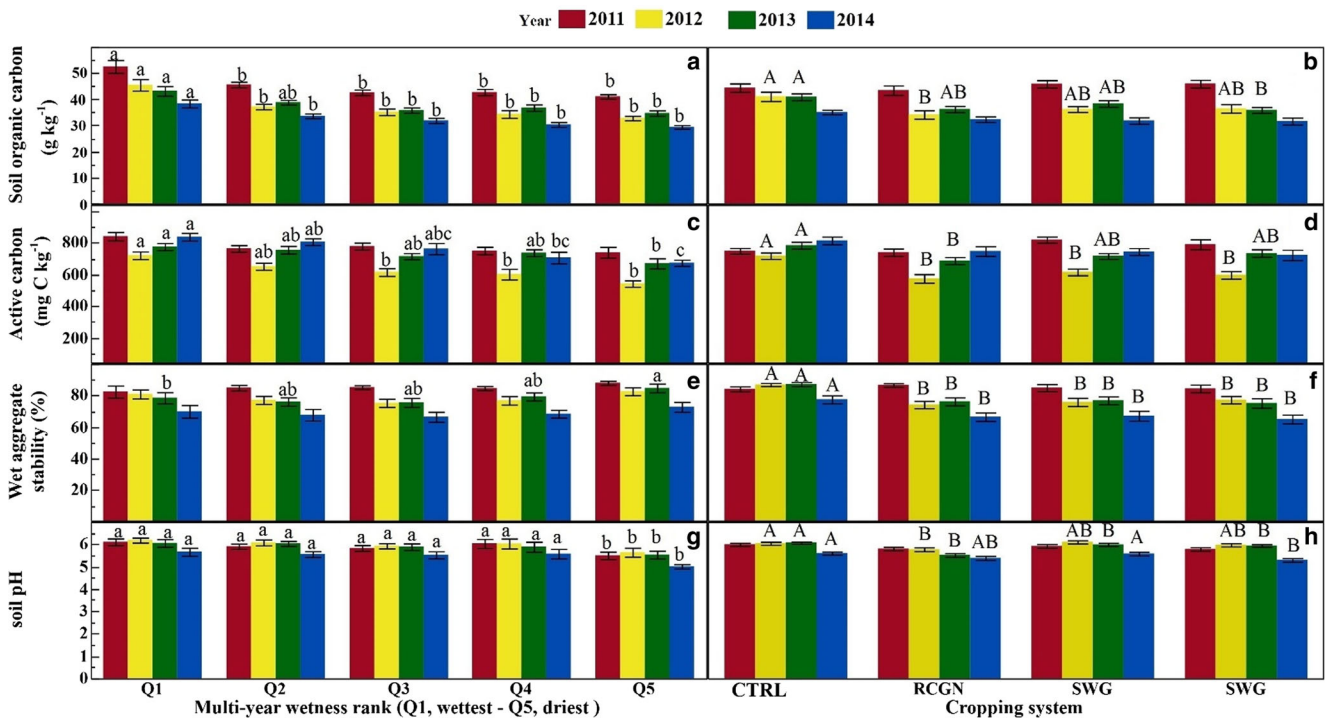


Fig. 3 Mean values of soil surface (0–15 cm) parameters: SOC (OM derived) for wetness quintiles (a) and cropping systems (b), active carbon for wetness quintiles (c) and cropping systems (d), wet aggregate stability for wetness quintiles (e) and cropping systems (f), and soil pH for wetness quintiles (g) and cropping systems (h) for the years 2011–2014. Error bars represent standard errors; *n* varies between 2 and 6 for each wetness quintile and *n* = 20 for each cropping system from the field set up of 80 subplots within 16 experimental units of

quadruplicate cropping system. Different letters above bars indicate significant differences (*p* < 0.05) for each parameter between different wetness (in lowercase) or cropping system groups (in uppercase) for a specific year; differences are not between different years or among each group for different years. No letters indicate absence of significant differences among groups. Whenever expressed as wetness quintiles, values are averaged over cropping systems and when represented as cropping systems are averaged over wetness quintiles

aboveground biomass (Fig. 5) across wetness groups for the four cropping systems varied between 7.2 ± 1.2 (SWG, Q2) and 18.8 ± 1.4 Mg ha⁻¹ (SWG, Q5), with aboveground biomass for SWG Q5 significantly greater than SWG, Q2. However, regardless of wetness level, there was no significant difference between the cumulative aboveground biomass of control and RCG (Fig. 5, Table S8). The yearly (2012, 2013, 2014) coarse root biomass are presented in Table S9a, b, grouped in accordance to their wetness quintiles and cropping systems.

Relationships Between 2014 Soil Properties, Cumulative Aboveground, and 2014 Root Biomass SOC (direct measurement) of soils was positively correlated to root biomass, (*r* = 0.3, *p* = 0.002), but was not correlated to cumulative aboveground biomass. It was also positively, but weakly correlated to soil pH (*r* = 0.3, *p* = 0.002), silt content (*r* = 0.2, *p* = 0.05), and clay content (*r* = 0.2, *p* = 0.07) and negatively to sand content (*r* = -0.4, *p* = 0.001). TN of soils was positively, but weakly correlated to 2014 root biomass (*r* = 0.2, *p* = 0.03), silt content (*r* = 0.2, *p* = 0.04) and clay content (*r* = 0.3, *p* = 0.02), and soil pH (*r* = 0.2, *p* = 0.04) and negatively to sand content (*r* = -0.4, *p* < 0.0001). Active carbon of soils was weakly

correlated to root biomass (*r* = 0.2, *p* = 0.1), soil pH (*r* = 0.4, *p* = 0.0002), and aggregate stability (*r* = 0.3, *p* = 0.004) and strongly to SOC (*r* = 0.7, *p* < 0.0001) and TN (*r* = 0.6, *p* < 0.0001); it was not associated with sand, silt, or clay contents or cumulative aboveground biomass. Aggregate stability of soils was correlated to SOC (*r* = 0.5, *p* < 0.0001), TN (*r* = 0.4, *p* < 0.0001), and 2014 root biomass (*r* = 0.3, *p* = 0.02); it was not associated with soil pH, sand, silt, or clay contents. Correlations are presented in Fig. 6. Additionally, loss in SOC (OM derived), active carbon, and aggregate stability of soils from pre-plow baseline levels were all correlated {(*r* = 0.3, *p* = 0.02), (*r* = 0.3, *p* = 0.01), (*r* = 0.2, *p* = 0.03), respectively} to 2014 root biomass, but not to cumulative aboveground biomass (results not shown).

Discussion

Influences on SOC and TN It is unsurprising that the wettest Q1 soils displayed significantly greater SOC in comparison to drier (less wet) soils, during any sampling year (Fig. 3a). Additionally, the driest soils (Q5) displayed the lowest SOC value during any sampled year. Thus, in the moisture range of

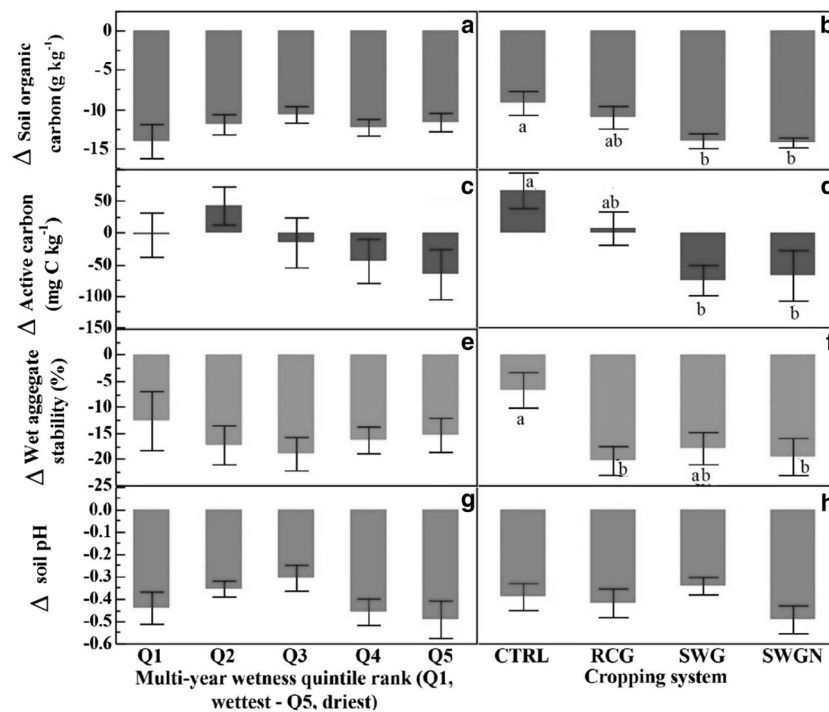


Fig. 4 Mean values of change of soil surface (0–15 cm) parameters from 2011 to 2014: Δ SOC for wetness quintiles (a) and cropping systems (b), Δ active carbon for wetness quintiles (c) and cropping systems (d), Δ wet aggregate stability for wetness quintiles (e) and cropping systems (f), and Δ soil pH for wetness quintiles (g) and cropping systems (h). Error bars represent standard errors; n varies between 2 and 6 for each wetness quintile and $n = 20$ for each cropping system from the field set up of 80

subplots with 16 experimental units of quadruplicate cropping system. Different letters above bars indicate significant differences ($p < 0.05$) among groups for each parameter; no letters indicate absence of significant differences among groups. Whenever expressed as wetness quintiles, values are averaged over cropping systems and when represented as cropping systems are averaged over wetness quintiles

0.3 g g^{-1} (Q5) to 0.5 g g^{-1} (Q1), results from our field indicate that wettest and driest soils were reservoirs of the greatest and smallest SOC pool, respectively, as is well understood [36, 64,

97] based on how saturated vs. dry conditions affect C mineralization. Conditions of seasonal saturation result in decreased OM mineralization, while increased OM inputs occur during

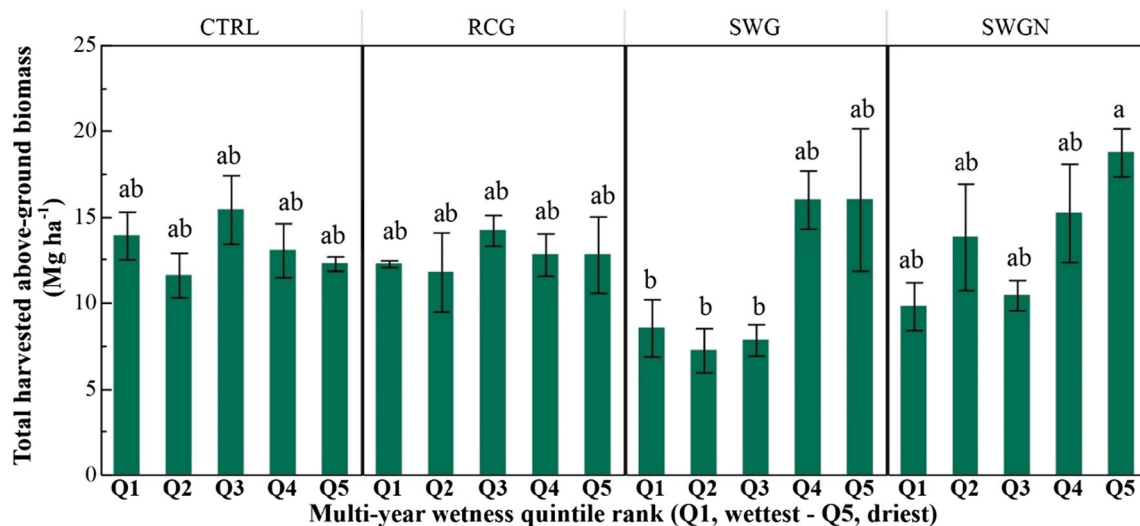


Fig. 5 Mean values of cumulative harvested aboveground biomass from 2012 to 2014 across all wetness quintiles (Q1 wettest–Q5 driest) of the four cropping systems: fallow control (CTRL), reed canarygrass + fertilizer 75 kg N ha^{-1} (RCG), switchgrass (SWG), and switchgrass + fertilizer 75 kg N ha^{-1} (SWGN). Error bars represent standard errors; n

varies between 2 and 6 for each wetness quintile of the quadruplicate cropping systems design covering 80 subplots from the 16 experimental units. Different letters above bars indicate significant differences ($p < 0.05$) in aboveground biomass among the moisture-crop groups

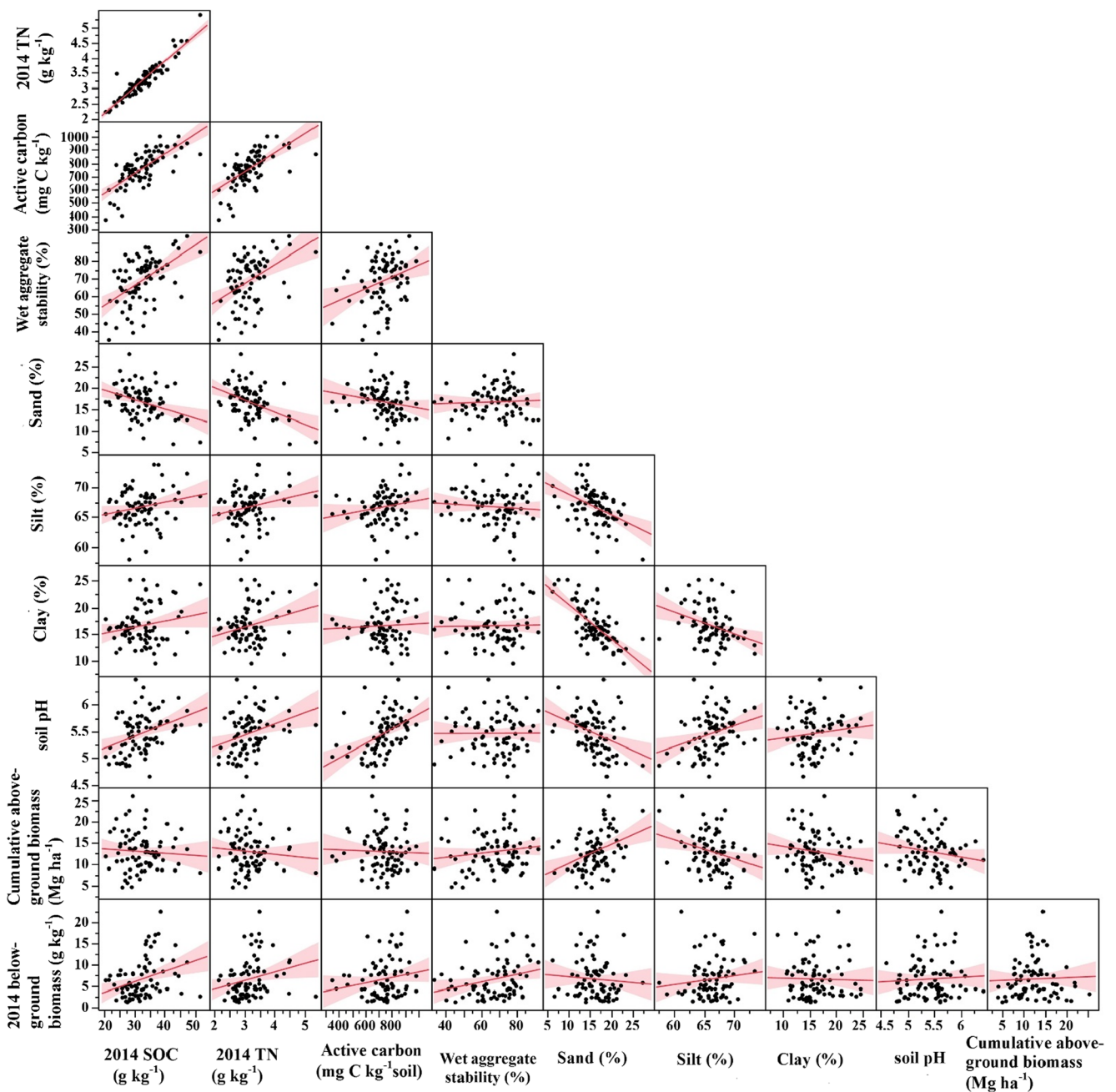


Fig. 6 Scatter plot matrices displaying relationships between analyzed variables of 2014 -SOC (direct measurement), TN, active carbon, wet aggregate stability, cumulative harvested aboveground biomass, and

2014 root biomass and the edaphic factors, clay contents, sand contents, and soil pH ($n = 80$)

the growing season (especially during midsummer when moisture is limiting in drier soils, as in [42, 64]).

SOC loss from 0 to 15-cm surface soil (Fig. 3b, and compared to fallow control in 2014, and S1b) associated with reed canarygrass and switchgrass establishment (including mowing, spraying, plowing, disking, and harrowing) is consistent with other perennial grass establishment studies, where SOC either decreased or remained unchanged [10, 16, 33, 57, 58, 71, 79, 87, 95] during similar or longer timeframe of grass establishment. Stockfisch et al. [91] reported significant

reduction of total SOC with moldboard plowing of long-term minimally tilled silt loam soils. Davidson and Ackerman [19] concluded that up to 40% of the original Ap SOC stocks could be lost within the first 5 years when uncultivated lands are brought into cultivation. Our study indicates 25–31% SOC loss in 3 years for the plots planted to the grasses. Hence, our hypothesis of SOC loss following plowing is supported. In addition to induced direct losses, the low biomass inputs during the seeding year (2011) and during the slow multi-year establishment phase of switchgrass

[2, 16, 68, 105] have probably also contributed to the observed lower SOC levels in switchgrass plots. Furthermore, in an annually harvested bioenergy system, SOC recovery/buildup may require a longer time than an unharvested system where C inputs from aboveground biomass remain in place, enabling faster recovery [89].

However, the notable concurrent decrease in SOC levels in the fallow control plots (Figs. 3b and 4b) was surprising (and resulted in repeated rechecking of sampling and analysis records). We subsequently recognized multiple factors that potentially contributed to this observed decline. First, it was recognized that maintaining the control plots in a strictly unmowed fallow condition since 2011 represented a shift from earlier historical treatments in which occasional mowing (e.g., every 1–2 years) took place, which would have stimulated greater biomass production and surface OM deposition in the prior regime. (Historical aerial imagery suggests that annual mowing had been discontinued sometime between 2005 and 2009, as 2009 imagery showed decreased vegetative uniformity at the site. Thus, a shift to lower rates of surface deposition occurred prior to the study's onset.) Second, while the subsurface drains installed in 2011 were not extensive in terms of field coverage, their impact by draining the “bottom of the bowl” at the site would have helped bring about drier average conditions somewhat earlier in the spring and later in the fall. OM losses associated with hydrologic modification, such as drain installation, has been reported as a disadvantage of such systems [11, 53, 102]. Third, annual climatic factors may have also contributed, as the growing seasons of 2011–2014 were drier than the 30-year average (Fig. 2b). Net loss of carbon from grasslands during drought months is often observed [63, 87]. The mean annual temperature departure (especially the non-growing season, October to March) during the study period (Fig. 2a) was greater than the 30-year average, a factor that has been observed to contribute to SOC losses [87]. The first two factors (management and drainage) would be expected to result in new lower equilibrium SOC levels, with the warmer and drier conditions in 2011 to 2014 also contributing in the same direction.

The lack of significant difference between C3 reed canarygrass and C4 switchgrass (fertilized and unfertilized) for SOC values during any sampling year (Figs. 3b, 4b and S1b), suggest that (at least in the short term) plant photosynthetic pathway had little impact on SOC dynamics, consistent with other studies [16, 52]. The absence of significant differences between SOC levels of switchgrass and switchgrass + fertilizer indicates that moderate (i.e., lower than those used to maximize crop yields, here 75 kg ha⁻¹) N fertilization rates were not (again in this short timeframe) either beneficial or

detrimental for SOC, as noted by other researchers [40, 90]. Our hypothesis that there would be no near-term difference in SOC levels as consequences of plant photosynthetic pathway or N fertilization are thus supported. The positive correlation of 2014 SOC levels to both clay and silt contents is consistent with many reports that OM is protected through associations with these particles ([31, 36, 66, 80, 86]; [106]; [4, 27]), improving long-term SOC accrual and stabilization. As TN is closely associated with SOC and is an important constituent of OM, it displayed similar trends, consistent with other studies (e.g., [64], which showed greater TN accrual in wetter restored prairie soil).

Influences on Active Carbon Although moisture regime was not a significant main effect on the percent change in active carbon from baseline levels, the greatest loss in the driest soils was reflective of moisture control of active carbon pools (Fig. 4c), also evidenced in the wettest soils (Q1) having values that were significantly greater than the driest soils for each sampling year (Fig. 3c). The effects of the 2011 plowing on active carbon were reflected both in the levels in subsequent years (Fig. 3c, d) and in their change from baseline (Fig. 4c, d). Rapid recovery to baseline values in the reed canarygrass plots appeared tied to the rapid crop establishment, in contrast to the lag in the slow-to-establish switchgrass treatments (Fig. 4d). Thus, effects of rapidly established vegetation appeared to be key in offsetting losses initiated by plowing. Increases in active carbon concurrent with decreasing SOC in some treatments (especially during 2013 and 2014, Fig. 3a–d) were indicative of a much quicker recovery of active carbon in comparison to SOC. The faster dynamics substantiates its correlation to non-mineral-associated fast-cycling C pools. The strong positive association of active carbon with SOC is as expected and as reported in other studies [17, 50].

Influences on Wet Aggregate Stability As aggregates between 0.25 and 2 mm were the basis of stability analysis, trends reported here were indicative of soil quality/soil health changes, as large aggregates of this range are more sensitive to management effects, while smaller aggregates (<0.25 mm) are related to older and more stable forms of SOC [98]. Thus, decreases in wet aggregate stability values from baseline were primarily reflective of plowing and alteration in management practices and hydrological regimes. Significantly greater wet aggregate stability of soils of fallow control soils in comparison to other treatments during all post-plowing years (Fig. 3f) could be indicative of the dominant influence of plowing. The positive correlation of aggregate stability to SOC is consistent with Grandy and Robertson [28] and Tiemann and Grandy [96] in silt-loam soils, in contrast to others who found no association [14, 96]. Similar to our results, Kibet et al. [41] found that low (60 and 120 kg

N ha^{-1}) rates of N application did not adversely affect aggregate stability, in contrast to decreases in soil macroaggregates under switchgrass at much greater N application rates (202 kg N ha^{-1} , [37]).

Influences on Soil pH The consistently lower soil pH in the driest soils (Q5) during each sampling year was reflective of the soil series (Fig. 1b), with these soils most likely to have eroded and been more strongly leached during prior use landscape position being key to the overarching wetness levels (drainage class) and associated soil pH levels. Subsequent pH changes correlated with fertilizer application were evident in 2014, when unfertilized switchgrass soils displayed significantly greater pH than that of fertilized switchgrass soils (Fig. 3h). The decrease in soil pH with perennial grass plantings (Fig. 4h) is similar to the modest drop in topsoil pH reported in a 5-year switchgrass study [81].

Influences on Cumulative Harvested Aboveground Biomass Harvestable biomass yields of reed canarygrass, switchgrass, and switchgrass + N treatments are comparable to other studies in this region [23, 32]. The greater yields of switchgrass in the drier quintiles substantiate the better early establishment response of the upland variety of switchgrass, *Shawnee* (with or without N fertilization). At this stage, we found no difference in the cumulative harvested aboveground biomass between switchgrass and switchgrass + N. This is consistent with the findings of Hong et al. [32], which shows that N response is variable for switchgrass in the NE, with some sites showing no yield benefit for added N (at least during establishment), while others, such as the 5-year screening trial for perennial grasses in New York state by Fick et al. [23] showing that N fertilization ($30\text{--}130 \text{ kg ha}^{-1}$) significantly improved yields, especially on poorly drained soils.

Relationships Between Soil and Crop Productivity Parameters

As expected, due to annual harvest removals, there was no correlation between harvested yields and SOC or active carbon. The positive association of SOC, TN, active carbon, and wet aggregate stability with root biomass—and absence of association with cumulative aboveground yields—reinforces the understanding that belowground biomass is of greater importance for SOC dynamics ([25, 36, 48]; [76]; [33, 103]). Additionally, higher root biomass correlations to lower SOC loss, lower active carbon loss, and lower wet aggregate stability loss (results not shown) substantiate it further. However, in addition to the positive influence of root productivity on SOC accretion, attendant counterproductive mechanisms could also result in SOC losses. Perennial grasses with more root biomass produce more exudates which increase microbial decomposition of older OM by the process of positive priming [8, 20, 46, 47, 96]. However, root-mediated OM

decomposition is often short term, and root biomass ultimately plays a key role in accrual of SOC in the longer term, offsetting those losses within one growing season [46] potentially explaining lower SOC loss being correlated to greater root biomass.

Implications and Conclusions

Overall, soil moisture status (drainage class and associated landscape position) is a key governing variable that needs to be accounted for when assessing soil properties and SOC dynamics resulting from establishment of perennial bioenergy crops. Three years after planting, the loss of SOC (from former fallow) incurred by conventional moldboard plowing, altered management, and hydrological regimes had not been repaid under reed canarygrass or slower-to-establish upland switchgrass on wetness-prone marginal land. The use of minimal tillage (such as no till or zone till) may be a useful way to avoid incurring this observed SOC debt if resulting crop establishment is satisfactory. Additional time will be needed for SOC recovery at this site, which is being monitored accordingly (substantial biomass inputs in switchgrass treatments since 2014 may help accelerate this recovery). In contrast, the more rapid recovery of the relatively dynamic pool of active carbon suggests that it may be useful as a moisture-sensitive leading indicator of changing SOC dynamics in a shorter timeframe. Research quantifying soil respiration and SOC turnover through time in long-term experiments is needed to better understand plant-soil interactions affecting soil C dynamics in such seasonally wet soils.

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