

# Biofuel intercropping effects on soil carbon and microbial activity

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**Abstract.** Biofuels will help meet rising demands for energy and, ideally, limit climate change associated with carbon losses from the biosphere to atmosphere. Biofuel management must therefore maximize energy production and maintain ecosystem carbon stocks. Increasingly, there is interest in intercropping biofuels with other crops, partly because biofuel production on arable land might reduce availability and increase the price of food. One intercropping approach involves growing biofuel grasses in forest plantations. Grasses differ from trees in both their organic inputs to soils and microbial associations. These differences are associated with losses of soil carbon when grasses become abundant in forests.

We investigated how intercropping switchgrass (*Panicum virgatum*), a major candidate for cellulosic biomass production, in loblolly pine (*Pinus taeda*) plantations affects soil carbon, nitrogen, and microbial dynamics. Our design involved four treatments: two pine management regimes where harvest residues (i.e., biomass) were left in place or removed, and two switchgrass regimes where the grass was grown with pine under the same two biomass scenarios (left or removed). Soil variables were measured in four 1-ha replicate plots in the first and second year following switchgrass planting. Under switchgrass intercropping, pools of mineralizable and particulate organic matter carbon were 42% and 33% lower, respectively. These declines translated into a 21% decrease in total soil carbon in the upper 15 cm of the soil profile, during early stand development. The switchgrass effect, however, was isolated to the interbed region where switchgrass is planted. In these regions, switchgrass-induced reductions in soil carbon pools with 29%, 43%, and 24% declines in mineralizable, particulate, and total soil carbon, respectively.

Our results support the idea that grass inputs to forests can prime the activity of soil organic carbon degrading microbes, leading to net reductions in stocks of soil carbon. Active microbial biomass, however, is higher under switchgrass, and this microbial biomass is a dominant precursor of soil carbon formation. Future studies need to investigate soil carbon dynamics throughout the lifetime of intercropping rotations to evaluate whether increases in microbial biomass can offset initial declines in soil carbon, and hence, maintain ecosystem carbon stocks.

**Key words:** biofuels; intercropping; *Panicum virgatum*; perennial cropping systems; *Pinus taeda*; preferential substrate utilization; priming effects; soil carbon stocks; soil microbial biomass; switchgrass.

## INTRODUCTION

Given growing concerns related to global climate change and energy independence, there is increasing interest in the production and management of biofuel crops (Hoffert et al. 2002, Pacala and Socolow 2004, Ragauskas et al. 2006). In particular, the development of management regimes for next-generation biofuels, such as switchgrass (*Panicum virgatum* L.), has received some of the greatest attention (NRC 2011, Davis et al. 2012). Next-generation biofuels are advantageous because of their capacity to grow on degraded land; consequently, the production of these feedstocks does not necessarily usurp land already devoted to food production (Sanderson et al. 1996, Davis et al. 2012). Additionally, when grown on degraded land or in place of annual crops, next-generation perennial biofuel crops

often improve these lands, leading to reduced greenhouse gas emissions and increased soil fertility and soil carbon (C) storage (Mehdi et al. 1999, Liebig et al. 2008, Davis et al. 2012, Monti et al. 2012). For example, as much as a 65% increase in soil C has been noted when annual agricultural crop management regimes are converted to switchgrass biofuel production (Tolbert et al. 2002, Monti et al. 2012).

In light of the positive impacts of next-generation biofuels on C sequestration and the potential economic advantages, biofuel intercropping regimes that augment current land-use practices have been proposed (Albaugh et al. 2012, Blazier et al. 2012, Susaeta et al. 2012). One such regime is the intercropping of switchgrass in intensively managed loblolly pine (*Pinus taeda* L.) plantations (Albaugh et al. 2012, Blazier et al. 2012). In these systems, switchgrass is planted in the interbed region (i.e., the zone between rows of planted trees) of pine plantations. This management regime is likely economically advantageous to the landholder (Susaeta

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et al. 2012), but the impacts of such management regimes on soil C stocks, soil nitrogen (N) stocks, and the associated soil microbial communities that both decompose and form soil C, have not been thoroughly examined under conventional or intercropping regimes (NRC 2011, Williams et al. 2011).

Intercropping regimes may affect soil C pools by eliciting a response in the microbial community akin to either priming or preferential substrate utilization (Bradford et al. 2008). With priming the increase in high-quality detrital inputs that intercropped species represent, relative to the main crop species, may lead to increased activity of soil C-degrading microbes, and hence, a subsequent decline in soil C pools (Fontaine et al. 2004, Fontaine and Barot 2005). Alternatively, if preferential substrate utilization (PSU) is occurring, then little change or even an increase in soil C pools is expected because the soil microbial community preferentially uses the higher quality, biofuel inputs (Fontaine and Barot 2005, Blagodatskaya and Kuzyakov 2008). That is, priming increases the decomposition of soil C, whereas PSU decreases soil C decomposition. However, the net outcome of priming vs. PSU seems dependent on the system investigated, particularly the amount of labile C inputs relative to microbial biomass and/or the recalcitrance of standing soil organic C stocks (Blagodatskaya and Kuzyakov 2008, Bradford et al. 2008, Strickland et al. 2011). For example, in forests colonized by grass species, marked declines in surface soil C have been observed in some systems (Strickland et al. 2010, 2011), but not others (Kourtev et al. 2003, Litton et al. 2006). The balance between priming and PSU over the course of a stand's development presumably will determine how biofuel intercropping regimes affect soil C stores, and hence, the efficacy of biofuel intercropping at mitigating soil C loss to the atmosphere.

To examine the effect of biofuel intercropping on soil C stocks, we examined changes in active and stable soil C pools using a novel experiment that examined switchgrass intercropping under two loblolly pine (*Pinus taeda* L.) forest management regimes in the southeastern United States (i.e., conventional management with biomass residuals left in place or biomass removed for biofuel production). Grass invasions of native forests in this region are associated, in general, with declines in soil C stocks (Strickland et al. 2010, 2011, Kramer et al. 2012). We therefore hypothesized that priming is the principal mechanism operating in the intercropping system. Alternatively, if PSU is the principal mechanism, then no change or even an increase in soil C should be associated with switchgrass intercropping. We also assessed soil N pools because changes in soil C stocks typically mirror those for soil N stocks, where net losses (if priming dominates) would indicate a decrease in site fertility (McLauchlan 2006), questioning the sustainability of intercropping regimes. Lastly, we assessed the biomass and catabolic potential of the soil microbes to examine whether changes in soil C and N

pools could be putatively linked to expected shifts in microbial community properties due to grass inputs as expected under priming or PSU. The results of this research highlight how biofuel intercropping management regimes affect soil C, N, and microbial properties.

## METHODS

### *Site description*

The site was established in 2008 and is located in Lenoir County on the Lower Coastal Plain Physiographic Region of North Carolina, USA (35° N, 77° W). Soils at this site are classified as either Pantego (fine, loamy, siliceous, semiactive thermic Umbric Paleaquults) or Rains (fine, loamy siliceous, semiactive, thermic Typic Paleaquults) soil series. Prior to study establishment in 2008, the site had been managed as a loblolly pine (*Pinus taeda* L.) plantation, which was planted in 1974. Following harvest of these trees, the site was prepared for the current study. The treatments followed traditional pine plantation re-establishment with harvest residuals (i.e., non-merchantable material from harvest) left in place (+biomass), or "more unconventionally" removed (–biomass) to simulate the use of residual biomass for biofuel production. These two pine establishment treatments were crossed with a switchgrass intercropping treatment (present or absent). The design thus produced four treatments (+biomass/–switchgrass, –biomass/–switchgrass, +biomass/+switchgrass, –biomass/+switchgrass) replicated across four blocks using a complete randomized block design. Additionally, we also assessed plots planted with only switchgrass ( $n=4$ ), established at the same location and having the same site conditions as the other treatments, enabling us to gauge the effect of intercropping vs. standard switchgrass management regimes. All plots were 0.8 ha and located in close proximity to each other.

Pines were established in the winter of 2008. Briefly, sites were V-sheared and bedded using a bulldozer and plow to create a raised planting surface for pines. Weyerhaeuser's (Vanceboro, North Carolina, USA) liquid-suspension based fertilizer (3% N, 6.2% P, 2.5% K, 4.5% Mg, and 2% Ca) was also incorporated into beds to promote seedling root development and establishment. Pines were then planted at  $6.1 \times 1.5$  m ( $20 \times 5$  feet) spacing. For treatments where biomass was removed, an excavator was used to remove residual woody debris after clear-cut harvesting. This removal resulted in an average 1.5 Mg/ha of residual biomass after removal vs. 9.4 Mg/ha when residual biomass was retained (Beauvais 2010).

For treatments where switchgrass was intercropped, additional V-shearing was employed to prepare a 3-m strip between pine rows. Switchgrass (Alamo cultivar) was then planted in early June 2009 at 9 kg/ha of pure live seed (PLS) in six rows spaced 40 cm apart to a depth of 0.6 cm and covered with soil using a modified corn planter. Switchgrass was fertilized with Weyerhaeuser's coated Arborite fertilizer at a rate of 65 kg N, 6.6 kg P,

and 0.24 kg B per hectare during the second growing season (June 2010). Also during this period, switchgrass plots were sprayed with 2,4-D and a post-emergent herbicide (Basagran; BASF, Research Triangle Park, North Carolina, USA) to control competing vegetation. Switchgrass was mowed following the first growing season (March 2010). In 2011, switchgrass was mowed, raked, and baled using standard agricultural equipment. Switchgrass yields in 2010 averaged 3.40, 3.43, and 4.74 Mg/ha and in 2011, averaged 4.98, 3.89, and 4.24 Mg/ha for +biomass/+switchgrass, -biomass/+switchgrass, and switchgrass-only treatments, respectively. Although an annual N-based fertilization regime [ $\sim 65$  kg N per grass hectare] was planned, fertilization did not occur in 2011 due to drought conditions. The switchgrass-only treatment was prepared by 100% V-shearing with remaining debris root raked. The interbeds of treatments that were not intercropped with switchgrass did not receive these preparatory treatments, and a mix of grasses (mainly *Andropogon virginicus*) and weedy annuals dominated the interbed. For more site details see Albaugh et al. (2012).

*Determination of soil characteristics, microbial biomass, microbial community function, and SOC pools*

Soils were collected in 2010 and 2011 to 15-cm depth using a push tube sampler. In both years, we measured microbial variables, and in 2011, we measured soil C pools. All soils were sieved using a 2-mm mesh and then stored at 4°C until analysis. Gravimetric soil moisture, 100% water holding capacity (WHC), and soil pH were determined on all soil samples. Both gravimetric soil moisture and WHC (after wetting to field capacity) were determined by drying soil at 105°C for 24 h. Soil pH (1:1 soil:H<sub>2</sub>O by volume) was determined using a benchtop meter.

Microbial biomass was determined via substrate-induced respiration (SIR), which is expected to be an indicator of total active biomass (Wardle and Ghani 1995), following Strickland et al. (2010). Briefly, SIR was determined using soil slurries (4 g dry mass equivalent soil) that were pre-incubated for 1 h with excess substrate (i.e., autolyzed yeast), followed by a 4-h incubation at 20°C. After 4 h, using a static incubation technique, respiration was determined on an infrared gas analyzer (IRGA; Model LI-7000, Li-Cor Biosciences, Lincoln, Nebraska, USA). Using the same protocol described for SIR, we also examined community function using an abbreviated catabolic response profile (CRP; Degens and Harris 1997) of three C compounds (glucose, glycine, and oxalate). Briefly, 13.51, 1.13, and 9.00 mg/g dry mass soil of glucose, glycine, and oxalate, respectively, were added to soil sourced from each treatment plot, and the resultant respiration due to each compound was measured after a 4-h incubation. Each of these compounds is representative of the major constituents found in root exudates and differ with regards to microbial growth efficiencies (van Hees et al. 2005a).

For example, glucose has greater growth efficiency than oxalate.

Mineralizable C, an estimate of bioavailable C (Fierer et al. 2005), was determined using 60-d C-mineralization assays. Soils were maintained at 20°C and 65% WHC for 60 d with periodic determination of respiration using the same static incubation technique described in the previous paragraph, except soils were incubated for 24 h prior to measuring. Total mineralizable C was estimated by integrating CO<sub>2</sub> production across time.

We used a fractionation method to differentiate between the faster cycling particulate organic matter (POM) and slower cycling mineral-associated soil C and N pools (Schlesinger and Lichter 2001), using the method described in Bradford et al. (2008). Air-dried soil (10 g) from each plot was dispersed with sodium hexametaphosphate via shaking (18 h), and then passed through a 53- $\mu$ m sieve. Mineral-associated material was considered  $<53$   $\mu$ m, and POM material was  $>53$   $\mu$ m. Both soil fractions were dried (105°C) and ball-milled to a fine powder. Percentage C and N were determined using an NA1500 CHN analyzer (Carlo Erba, Milan, Italy). Because switchgrass-derived material is enriched in  $\delta^{13}\text{C}$  compared to pine-derived material, we also determined the  $\delta^{13}\text{C}$  value of these pools using isotope ratio mass spectrometry. Via stable-isotope mixing equations (Fry 2006), we were able to determine the contribution of switchgrass to the soil C pools using the equation: switchgrass-derived C =  $C_{\text{pool}} \times [(^{13}\text{C}_{+\text{sg}} - ^{13}\text{C}_{-\text{sg}}) / (^{13}\text{C}_{\text{sg}} - ^{13}\text{C}_{-\text{sg}})]$ . Where  $C_{\text{pool}}$  is the measured size of the pool,  $^{13}\text{C}_{-\text{sg}}$  is the  $\delta^{13}\text{C}$  value in plots where switchgrass is absent,  $^{13}\text{C}_{+\text{sg}}$  is the  $\delta^{13}\text{C}$  value in plots where switchgrass is present, and  $^{13}\text{C}_{\text{sg}}$  is the  $\delta^{13}\text{C}$  value of switchgrass itself.

We present bed and interbed estimates of soil organic C and N pools per square meter. Block level estimates of bulk density (g/cm<sup>3</sup>; Elliott et al. 1999) for the bed or interbed were used for these estimates as these were significantly or marginally different (bed,  $F_{3,18} = 2.4$ ,  $P < 0.10$ ; interbed,  $F_{3,18} = 3.4$ ,  $P < 0.05$ ). No significant differences in bulk density were noted between treatments in either the bed ( $F_{1,18} = 0.78$ ,  $P = 0.39$ ) or interbed zones ( $F_{1,18} = 1.05$ ,  $P = 0.43$ ). To calculate estimates of soil organic C and N pools at the plot (i.e., hectare) scale, we first calculated the proportion of the hectare that was either bed or interbed, and then summed the amount of organic matter C and N found in both of these zones. These estimates omitted the C and N found in the transition zone between the bed and interbed. The bed zone accounted for  $\sim 25\%$  of a hectare plot and the interbed zone for  $\sim 45\%$ . The remaining 30% of the area represented the unshaded transition zone between the base of the raised beds and the interbed area planted with switchgrass.

*Data analysis*

To assess the effect of pine management regimes and switchgrass intercropping, SIR microbial biomass and

mineralizable C were analyzed across both sample years using linear mixed-effects models (Pinheiro and Bates 2000). For these models, plot identity was nested in block and treated as a random effect. Sampling date, presence/absence of pine biomass residuals, and the presence/absence of switchgrass were all treated as main effects and allowed to interact. These analyses were performed in the freeware statistical package R (R Development Core Team 2012). Functional assessments of the microbial community, i.e., CRP, as influenced by biomass and switchgrass treatments were analyzed using permutational MANOVA and visualized using nonmetric multidimensional scaling (NMDS) in the Primer package (Clarke and Gorley 2006). Regression analysis was used to assess relationships between the contribution of switchgrass to soil C and N pools and the changes in the sizes of these pools. Significant treatment differences were considered at  $P < 0.05$ , but because estimates of soil organic matter can be quite heterogeneous, we considered  $P < 0.10$  to be marginally significant (Carney et al. 2007, Strickland et al. 2010). When necessary, data were log-transformed to meet assumptions of normality.

## RESULTS

### *Soil C and N stocks*

**Hectare-scale analysis.**—At the hectare scale, POM C (approximately one-third of total soil C) declined by 33% in the presence of intercropped switchgrass ( $F_{1,9} = 8.73$ ,  $P < 0.05$ ; Fig. 1A), whereas mineral-associated soil C (approximately two-thirds of total soil C) was unaffected by the presence of switchgrass ( $F_{1,9} = 1.87$ ,  $P = 0.20$ ; Fig. 1B). The effect on POM C was similar to that observed in the switchgrass monoculture (Fig. 1a). Neither pool was affected by the presence/absence of biomass residuals or by a biomass  $\times$  switchgrass interaction. However, because of the significant decrease in POM C, total soil C (POM + mineral) declined by 24% ( $F_{1,9} = 5.21$ ,  $P < 0.05$ ; Fig. 1C), again similar to the observation for switchgrass monoculture. Soil N exhibited a similar pattern, with switchgrass-induced declines of 40% in POM N ( $F_{1,9} = 12.23$ ,  $P < 0.01$ ; Fig. 1D), but no decrease in mineral-associated N ( $F_{1,9} = 0.85$ ,  $P = 0.38$ ; Fig. 1E). The decrease in POM N was on par with values observed under switchgrass monoculture (Fig. 1D). A marginally significant decrease of 21% in total N ( $F_{1,9} = 3.85$ ,  $P = 0.08$ ; Fig. 1F) was noted. Soil N was not affected by the presence/absence of biomass or by a biomass  $\times$  switchgrass interaction at the hectare scale.

**Zone-scale analysis.**—The decline in POM seemed to be a direct result of switchgrass planting, as evidenced by a switchgrass  $\times$  zone interaction for both POM C ( $F_{1,12} = 24.49$ ,  $P < 0.001$ ) and POM N ( $F_{1,12} = 14.56$ ,  $P < 0.01$ ). That is, in the bedded zone (i.e., with trees) the presence of switchgrass in the plots had no effect on either POM C ( $F_{1,9} = 0.85$ ,  $P = 0.38$ ) or POM N ( $F_{1,9} = 0.32$ ,  $P = 0.59$ ). In fact, switchgrass-derived C only accounted for  $\sim 0.9\%$  of POM C in the bedded zone

(Table 1). However, in the interbed where switchgrass was planted, switchgrass was associated with a 43% and 51% decline in both POM C ( $F_{1,9} = 24.81$ ,  $P < 0.001$ ; Table 1) and N ( $F_{1,9} = 21.29$ ,  $P < 0.01$ ; Table 2), respectively. Switchgrass-derived C only accounted for  $\sim 11\%$  of POM C in the interbed zone (Table 1). Additionally, in the interbed there were negative relationships between the proportion of switchgrass-derived C in the POM pool and the size of the POM C ( $F_{1,6} = 4.64$ ,  $P = 0.07$ ; adj.  $r^2 = 0.34$ ;  $y = -0.17x + 4.68$ ) and N pools ( $F_{1,6} = 6.99$ ,  $P < 0.05$ ; adj.  $r^2 = 0.46$ ;  $y = -0.01x + 0.14$ ), suggesting that changes in POM C and N were directly associated with switchgrass inputs (Fig. 2).

Total C and N showed similar patterns as POM material (Tables 1 and 2). That is, the decline in both total C ( $F_{1,12} = 9.53$ ,  $P < 0.01$ ) and total N ( $F_{1,12} = 5.74$ ,  $P < 0.05$ ) could be explained by a switchgrass  $\times$  zone interaction. Specifically, the bedded zone was unaffected by the presence of switchgrass in the plot, but its presence in the interbed led to declines in total C and N. In contrast, only a zone effect was noted for the amount of mineral-associated C ( $F_{1,12} = 6.47$ ,  $P < 0.05$ ; Table 1) and N ( $F_{1,12} = 8.32$ ,  $P < 0.05$ ; Table 2), with the interbed having greater amounts of C and N compared to the bed and switchgrass-derived C contributing relatively little to the mineral C pool ( $\sim 1.5\%$ ; Table 1).

Presence/absence of biomass residuals also interacted with zone for both POM C ( $F_{1,12} = 20.34$ ,  $P < 0.001$ ) and N ( $F_{1,12} = 7.55$ ,  $P < 0.05$ ). When biomass was present, no differences in POM C ( $F_{1,9} = 2.09$ ,  $P = 0.18$ ) or N ( $F_{1,9} = 1.27$ ,  $P = 0.29$ ) were found between zones (i.e., bed or interbed). However, when biomass residuals were removed, POM C ( $F_{1,9} = 11.72$ ,  $P < 0.01$ ) and N ( $F_{1,9} = 5.31$ ,  $P < 0.05$ ) were both greater in the interbed compared to the bed.

The presence/absence of biomass residuals also interacted with zone for both total C ( $F_{1,12} = 10.49$ ,  $P < 0.01$ ) and N ( $F_{1,12} = 5.29$ ,  $P < 0.05$ ). In both instances, this interaction was due to similar amounts of C and N in both zones when biomass was retained, but C and N increased in the interbed by 26% and 16%, respectively, and decreased in the bed by 14% and 17%, respectively, when biomass was removed.

### *Bioavailable C*

**Hectare-scale analysis.**—At the hectare scale, bioavailable C declined from 2010 to 2011 ( $F_{1,21} = 8.50$ ,  $P < 0.01$ ). Additionally, a significant sampling date  $\times$  biomass residuals interaction ( $F_{1,21} = 5.96$ ,  $P < 0.05$ ) was noted, as well as a main effect of switchgrass ( $F_{1,21} = 19.16$ ,  $P < 0.001$ ). The interaction between sampling date and the presence/absence of biomass was due to similar amounts of bioavailable C in both years when biomass was removed. When biomass was retained, greater bioavailable C was found in 2010 and less in 2011. The main effect of switchgrass intercropping tended to be associated with an overall decline in

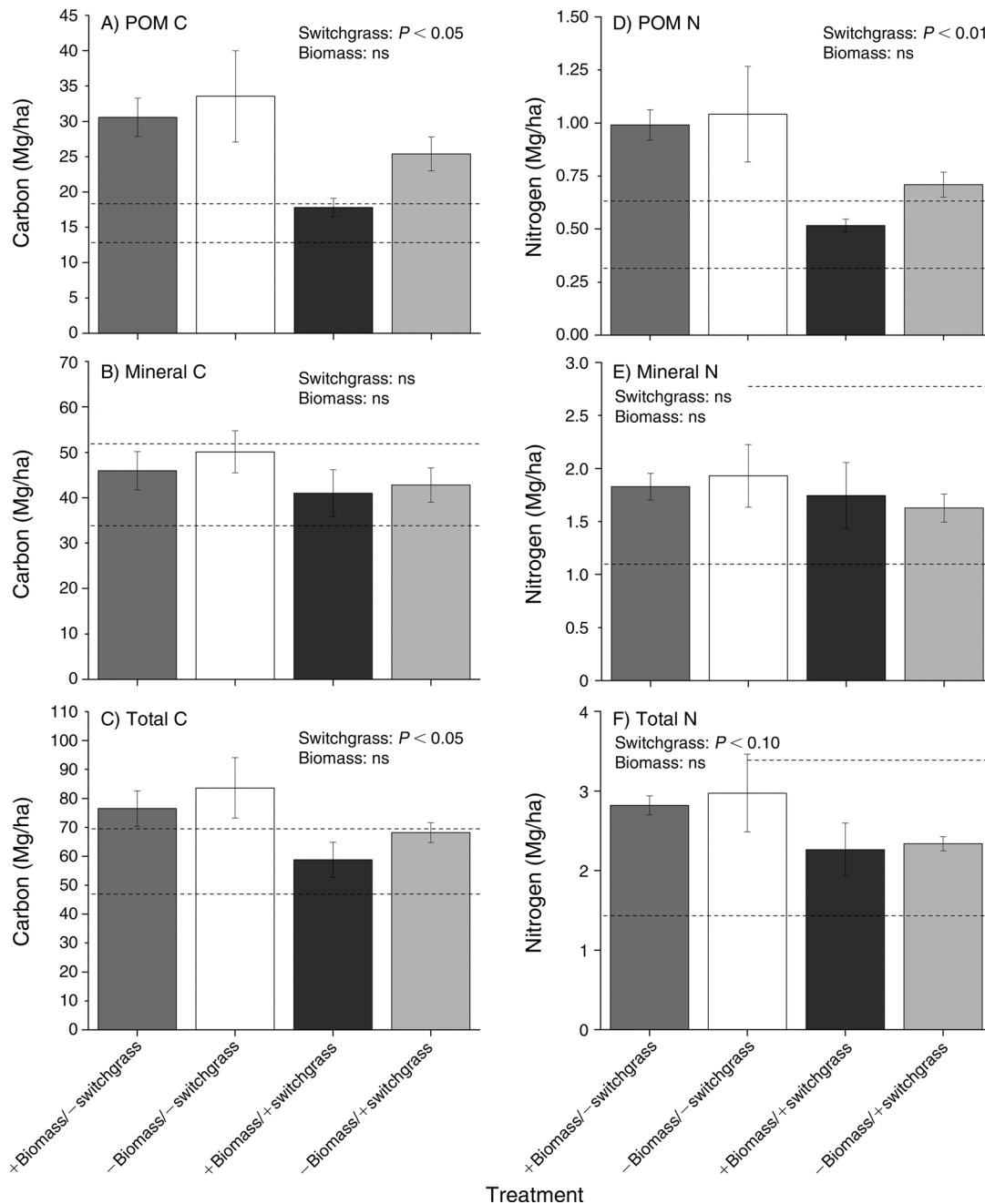


FIG. 1. Particulate organic matter (POM), mineral, and total (A–C) soil C and (D–F) soil N pools in the presence/absence of loblolly pine (*Pinus taeda*) biomass residuals and in the presence/absence of switchgrass (*Panicum virgatum*) biofuel plantings ( $n = 4$ ). Treatments followed traditional pine plantation re-establishment with harvest residuals left in place (+biomass) or removed (–biomass), crossed with a switchgrass intercropping treatment (present or absent; +switchgrass or –switchgrass) to produce four treatments. Linear mixed-effects model results for switchgrass and biomass removal levels are shown. There was no significant (ns) switchgrass  $\times$  biomass interaction for any pool. The dotted lines represent the 95% confidence interval for plots planted only in switchgrass. Bars represent means  $\pm$  SE.

bioavailable C, which was similar to the decline noted for the switchgrass monoculture (Fig. 3).

**Zone-scale analysis.**—Only a marginally significant switchgrass  $\times$  zone interaction ( $F_{1,36} = 3.04$ ,  $P = 0.09$ ) was observed for bioavailable C (Table 1). This

interaction was due to no change in the bedded zone, while bioavailable C declined in the interbed when associated with switchgrass. A main effect of time was noted ( $F_{1,36} = 9.56$ ,  $P < 0.01$ ), with a decline in bioavailable C from 2010 to 2011. The main effect of

switchgrass presence was also associated with a decline in bioavailable C ( $F_{1,9} = 11.11$ ,  $P < 0.01$ ).

#### *Microbial biomass*

*Hectare-scale analysis.*—At the hectare-plot scale, active microbial biomass (i.e., SIR) increased from 2010 to 2011, and a significant sampling year  $\times$  switchgrass interaction was noted ( $F_{1,21} = 5.42$ ,  $P < 0.05$ ). This interaction was due to the fact that the presence of switchgrass had no effect on SIR biomass in 2010, but in 2011 SIR biomass tended to be greater when switchgrass was present (Fig. 4). Additionally, a significant main effect of switchgrass was noted ( $F_{1,21} = 4.87$ ,  $P < 0.05$ ), as well as a marginally significant biomass  $\times$  switchgrass interaction ( $F_{1,21} = 3.21$ ,  $P = 0.09$ ). The biomass  $\times$  switchgrass interaction was due to greater SIR biomass when both switchgrass and biomass were present compared to lower SIR when biomass residuals were removed, regardless of the presence of switchgrass (Fig. 4). This interaction suggests that the removal of biomass residuals may mediate the influence of switchgrass on SIR biomass.

*Zone-scale analysis.*—Examining SIR biomass within zones (bed vs. interbed), we noted significant sampling year  $\times$  zone  $\times$  switchgrass ( $F_{1,36} = 7.16$ ,  $P < 0.05$ ) and sampling year  $\times$  biomass  $\times$  switchgrass ( $F_{1,36} = 4.62$ ,  $P < 0.05$ ) interactions. To examine these three-way interactions, we examined treatment effects within each sampling year. For the 2010 sampling year, no significant main effects or interactions were noted (Table 1). However, in 2011, significant biomass  $\times$  switchgrass ( $F_{1,9} = 6.99$ ,  $P < 0.05$ ) and zone  $\times$  switchgrass ( $F_{1,12} = 9.19$ ,  $P < 0.05$ ) interactions were noted (Table 1). The biomass  $\times$  switchgrass interaction was due to similar SIR biomass when biomass was removed regardless of the presence of switchgrass, but when both switchgrass and biomass were present SIR biomass increased, and when only biomass residuals were present SIR biomass decreased. These responses match those observed at the hectare scale and may be driven by the switchgrass inputs priming the decomposition of the harvest residue material and so increasing the resources available to microbes. The zone  $\times$  switchgrass interaction was due to no effect of switchgrass in the bed, but switchgrass presence was associated with an increase in SIR biomass in the interbed.

#### *Community function*

Microbial community function, as assessed by catalytic response profiling (CRP), showed significant zone  $\times$  sampling date ( $F_{1,45} = 5.29$ ,  $P < 0.01$ ) and zone  $\times$  switchgrass interactions ( $F_{1,45} = 3.31$ ,  $P < 0.05$ ). The first of these interactions was due to differences in CRP between sampling dates in the interbeds ( $F_{1,21} = 5.64$ ,  $P < 0.01$ ), but not the beds ( $F_{1,21} = 1.47$ ,  $P = 0.23$ ). In the interbeds, communities collected in 2011 tended to have greater respiration on oxalate, while those collected in 2010 tended to have greater respiration on glycine and

glucose. The zone  $\times$  switchgrass interaction was due to a switchgrass effect in the interbed ( $F_{1,21} = 4.38$ ,  $P < 0.05$ ), but not the bed ( $F_{1,21} = 1.07$ ,  $P = 0.32$ ). Specifically, when switchgrass was present, communities tended to exhibit lower respiration on glucose and oxalate, but respiration on glycine was unaffected (Fig. 5).

#### DISCUSSION

Land management regimes for the production of biofuels are of increased interest because of growing concerns related to energy production and global climate change (Hoffert et al. 2002, Pacala and Socolow 2004, Ragauskas et al. 2006). Given these concerns and interest in maximizing the amount of land that can be used for biofuel production, novel management regimes have been suggested (Albaugh et al. 2012, Susaeta et al. 2012). One such management regime is to intercrop perennial grasses in forest plantations (Albaugh et al. 2012). However, the effect that intercropping has on soil communities and soil C and N pools is relatively unknown (Williams et al. 2011, Nair et al. 2012). Here, using a large-scale experiment designed to examine the effects of intercropping a perennial grass, we found that the presence of switchgrass leads to a priming effect, which induces marked declines in soil C and N pools, as well as change in the activity and function of soil microbial communities.

We expected that switchgrass intercropping would either lead to an increase or little change in soil C via PSU or a decline in soil C via priming. We observed evidence that suggests priming is the primary mechanism affecting soil C under the intercropping regimes. When switchgrass was intercropped with loblolly pine (and when grown in monoculture), significant decreases in bioavailable C, POM C, and total soil C pools were noted. The decrease in total soil C is primarily due to the decrease in the POM pool. Mineral-associated soil C did not significantly decline under switchgrass, and switchgrass-derived C was not a major constituent of this pool (Table 1). This is likely due to the slower turnover, and hence, response times of mineral-associated C (Schlesinger and Lichten 2001, Grandy and Robertson 2007). Declines in both POM and total soil C/ha, with switchgrass intercropping, averaged 32.6% and 20.7%, respectively. These findings contrast with observations of an increase in soil C when land is converted to switchgrass management regimes (Liebig et al. 2008, Monti et al. 2012). However, this is not an unusual finding when considering the context of our sites vs. those of other studies. That is, most studies that have observed an increase in soil C associated with switchgrass management regimes are established on degraded sites or those that were historically under annual crop cultivation regimes (Monti et al. 2012). Such a change, not dissimilar to transitioning from till to no-till agriculture (Reeves 1997, Tolbert et al. 2002), in management is likely to lead to an increase in soil C because these sites are not near their C saturation

TABLE 1. Soil C pools (mean  $\pm$  SE) for loblolly pine (*Pinus taeda*) biomass residuals and switchgrass (*Panicum virgatum*) treatments ( $n = 4$ ) in the bed and interbed zones, and plots containing switchgrass monoculture.

Zone and treatment	POM C (kg/m <sup>2</sup> )	Switchgrass-derived POM C (%)	Mineral C (kg/m <sup>2</sup> )	Switchgrass-derived mineral C (%)
<b>Bed</b>				
+Biomass/-switchgrass	3.5 $\pm$ 0.3	0	5.8 $\pm$ 0.4	0
-Biomass/-switchgrass	2.6 $\pm$ 0.6	0	5.9 $\pm$ 0.7	0
+Biomass/+switchgrass	3.8 $\pm$ 0.5	1.0 $\pm$ 0.5	6.0 $\pm$ 0.2	1.9 $\pm$ 0.6
-Biomass/+switchgrass	3.3 $\pm$ 0.7	0.8 $\pm$ 0.8	4.8 $\pm$ 0.7	0.5 $\pm$ 0.5
<b>Interbed</b>				
+Biomass/-switchgrass	4.8 $\pm$ 0.6	0	7.0 $\pm$ 0.9	0
-Biomass/-switchgrass	6.0 $\pm$ 1.2	0	7.8 $\pm$ 0.7	0
+Biomass/+switchgrass	1.8 $\pm$ 0.2	13.8 $\pm$ 1.2	5.8 $\pm$ 1.1	2.2 $\pm$ 1.5
-Biomass/+switchgrass	3.8 $\pm$ 0.2	7.6 $\pm$ 1.8	6.8 $\pm$ 0.8	1.5 $\pm$ 0.6
Switchgrass only	2.2 $\pm$ 0.2	22.4 $\pm$ 6.0	6.1 $\pm$ 0.7	3.6 $\pm$ 2.8

Notes: Mineralizable C and substrate-induced respiration (SIR) biomass are given for both the 2010 and 2011 sampling dates. Treatments followed traditional pine plantation re-establishment with harvest residuals left in place (+biomass) or removed (-biomass), crossed with a switchgrass intercropping treatment (present or absent; +switchgrass or -switchgrass) to produce four treatments. Particulate organic matter is abbreviated as POM.

potential (Six et al. 2002, Stewart et al. 2007). Our sites are instead established on soils rich in organic C and that have been under active forest management for over 30 years (Albaugh et al. 2012). Under these conditions the introduction of a perennial grass, which represents a potentially more labile C input to these systems (Blazier et al. 2012), may set the stage for a priming effect and hence loss of soil C (Fontaine et al. 2004, Bradford et al. 2008). Such a scenario parallels declines in POM and total soil C observed in secondary forests, invaded by C<sub>4</sub> grasses, in the southeastern United States (Strickland et al. 2010, 2011).

Slight differences in management practices across our treatments (other than switchgrass planting) are unlikely to explain the observed declines in soil C, although we stress that the effects of site preparation on soil properties are important to assess given that they are an integral part of any land management regime (Oldfield et al. 2014). For example, V-shearing is a soil disturbance that could lead to a loss of soil C, akin to tillage effects. Yet all treatments were V-sheared and the expectation for tilling is that subsequent events, like the additional V-shearing in the switchgrass plots, have

diminishing influence on soil C declines (Brevik 2012). Further, fertilization in managed forests tends to increase soil C (Johnson and Curtis 2001, Jandl et al. 2007), but we observed declines under switchgrass treatments despite the additional fertilization they received in 2010. Finally, we did not assess biomass and input quality for treatments that were not planted with switchgrass, meaning that there is the potential that priming was also occurring in these plots. However, given that total pools of POM and mineral associated C were lower in the presence of switchgrass suggests that, at the very least, priming had a stronger influence on soil C stocks in plots where switchgrass was being deliberately grown as biofuel.

Perhaps the most compelling arguments that switchgrass was the ultimate cause of the observed declines in soil C and N are the negative relationships between the POM pools and the proportion of switchgrass-derived C found in them (Fig. 2). Specifically, these relationships suggest that the more switchgrass C entering below-ground food webs, the smaller the POM pool. These inputs most probably entered the soil via root pathways given that aboveground switchgrass biomass was

TABLE 2. Soil N pools (mean  $\pm$  SE) for pine biomass residuals and switchgrass treatments ( $n = 4$ ) in the bed and interbed zones, and plots containing switchgrass monoculture.

Zone and treatment	POM N (g/m <sup>2</sup> )	Mineral N (g/m <sup>2</sup> )	Total N (g/m <sup>2</sup> )
<b>Bed</b>			
+Biomass/-switchgrass	106.2 $\pm$ 12.6	228.2 $\pm$ 22.5	334.3 $\pm$ 33.0
-Biomass/-switchgrass	80.6 $\pm$ 24.0	228.8 $\pm$ 43.9	309.4 $\pm$ 67.4
+Biomass/+switchgrass	113.6 $\pm$ 9.2	249.7 $\pm$ 22.6	363.2 $\pm$ 23.9
-Biomass/+switchgrass	94.1 $\pm$ 19.8	173.2 $\pm$ 24.2	267.3 $\pm$ 15.5
<b>Interbed</b>			
+Biomass/-switchgrass	161.2 $\pm$ 21.1	279.7 $\pm$ 32.0	440.9 $\pm$ 41.1
-Biomass/-switchgrass	186.7 $\pm$ 44.2	302.0 $\pm$ 42.5	488.7 $\pm$ 76.6
+Biomass/+switchgrass	51.6 $\pm$ 7.6	249.2 $\pm$ 57.3	300.8 $\pm$ 62.0
-Biomass/+switchgrass	105.3 $\pm$ 14.6	265.3 $\pm$ 31.7	370.7 $\pm$ 24.0
Switchgrass only	67.5 $\pm$ 11.6	276.3 $\pm$ 61.1	343.7 $\pm$ 71.2

TABLE 1. Extended.

Total C (kg/m <sup>2</sup> )	Mineralizable C (mg/m <sup>2</sup> )		SIR microbial biomass (mg·m <sup>-2</sup> ·h <sup>-1</sup> )	
	2010	2011	2010	2011
9.4 ± 0.7	92.1 ± 22.7	73.6 ± 18.5	0.13 ± 0.02	0.38 ± 0.02
8.5 ± 1.3	77.5 ± 9.8	65.0 ± 19.3	0.15 ± 0.03	0.62 ± 0.10
9.8 ± 0.5	87.2 ± 12.2	47.6 ± 7.8	0.14 ± 0.02	0.42 ± 0.02
8.1 ± 0.3	71.7 ± 16.5	46.8 ± 6.4	0.15 ± 0.03	0.41 ± 0.05
11.8 ± 1.3	98.8 ± 26.2	84.1 ± 9.7	0.14 ± 0.02	0.30 ± 0.01
13.9 ± 1.7	79.1 ± 11.7	91.1 ± 28.1	0.13 ± 0.02	0.44 ± 0.07
7.6 ± 1.2	65.0 ± 3.5	33.4 ± 7.5	0.13 ± 0.02	1.33 ± 0.45
10.7 ± 0.8	56.1 ± 2.2	60.8 ± 11.4	0.10 ± 0.01	0.51 ± 0.19
8.3 ± 0.8	93.6 ± 16.9	48.6 ± 3.5	0.17 ± 0.03	0.78 ± 0.16

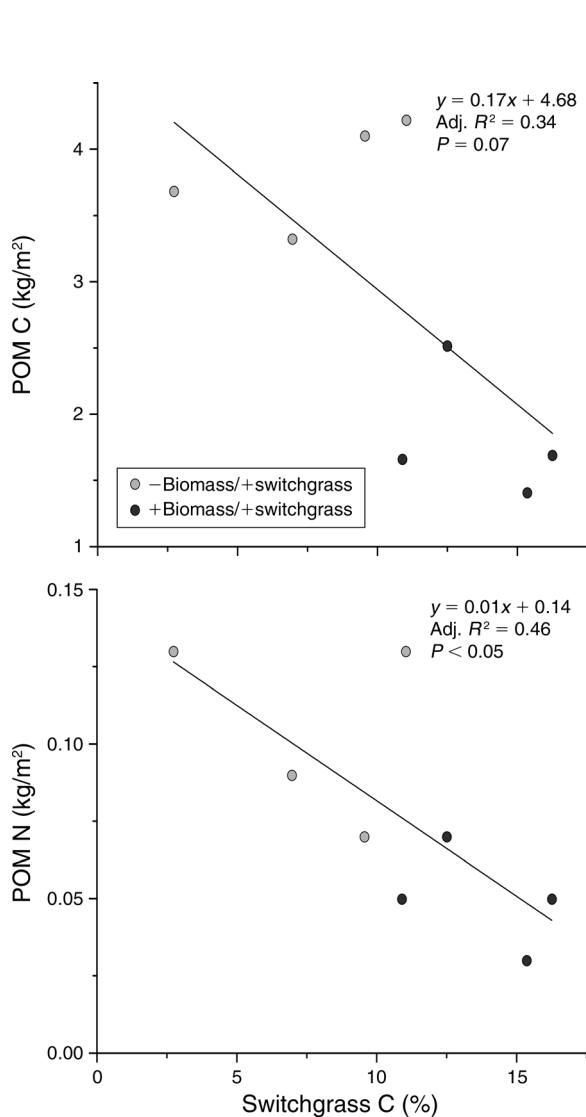


FIG. 2. Relationships between the proportion of switchgrass-derived C in the POM pool and the amount of POM C and N in the interbed zone.

harvested. Notably, all of the declines in soil C were spatially isolated to the interbed zones where switchgrass was planted and where switchgrass-derived C had the greatest influence on POM C (Table 1), further supporting our inference that switchgrass presence is the most plausible driver of soil C losses, and not other management differences across treatments.

Switchgrass effects on soil C appeared mediated via positive effects on active microbial biomass. Specifically, microbial biomass was higher where there was switchgrass (i.e., the interbed zones) in 2011, suggesting that switchgrass caused priming of microbial activity and so enhanced decomposition of soil C (Fontaine et al. 2004). However, it is worth noting that while active microbial biomass is higher under switchgrass, this microbial biomass is a dominant precursor of soil carbon formation and may lead to an increase in soil C in the

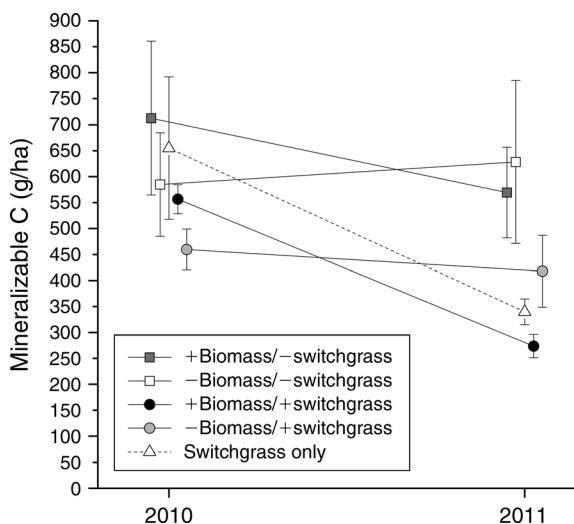


FIG. 3. Mineralizable C, an estimate of bioavailable C, in the presence/absence (+/-) of pine biomass residuals and in the presence/absence of switchgrass biofuel plantings (n = 4) for the 2010 and 2011 sampling years. Also shown are plots planted only in switchgrass. Points represent means ± SE.

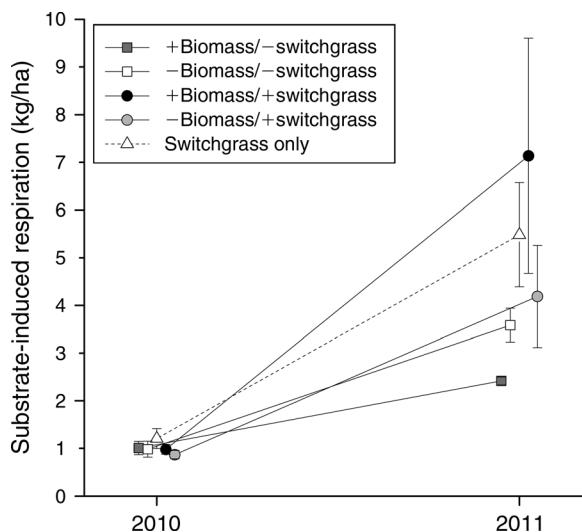


FIG. 4. Substrate-induced respiration, an estimate of active microbial biomass, in the presence/absence (+/–) of pine biomass residuals and in the presence/absence of switchgrass biofuel plantings ( $n = 4$ ) for the 2010 and 2011 sampling years. Also shown are plots planted only in switchgrass. Points represent means  $\pm$  SE.

long term (Bradford et al. 2013). Microbial communities are often influenced by changes in plant community composition, especially when these changes lead to alterations in the quality and quantity of available substrates (Lauber et al. 2008, 2009, Liang et al. 2012). The idea that switchgrass altered substrate inputs is supported by the shifts in microbial community func-

tion. Specifically, microbial communities associated with switchgrass mineralized oxalic acid to a lesser extent than communities not associated with switchgrass. Oxalic acid is a common low molecular weight C compound associated with the root exudates of forests and is also associated with low energy returns to soil microbes assimilating it (van Hees et al. 2005a, b, Frey et al. 2013). The shift away from oxalic acid toward glucose usage under switchgrass may then indicate that microbial communities under switchgrass derive more energy from glucose (Fig. 5), a common rhizodeposit on which microbes can grow efficiently (Bradford et al. 2013), explaining the increases in active microbial biomass and consequent declines in soil C.

Rather than a priming effect, switchgrass could have altered other soil properties that might have stimulated microbial activity. This is not outside of the realm of possibilities and is still in line with our inferences that switchgrass increased microbial activity leading to a decline in soil C and N pools, albeit the mechanism differs. However, we found no effect of switchgrass on either soil moisture ( $F_{1,53} = 0.19$ ,  $P = 0.67$ ) or pH ( $F_{1,53} = 3.99$ ,  $P = 0.051$ ) in the interbed, the zone where switchgrass effects were noted, albeit a marginal effect on pH was noted. It seems then that our data are most consistent with the inference that switchgrass-derived C inputs promote declines in soil C and N pools.

Our data leave outstanding questions about whether declines in surficial soil C under switchgrass early in its cropping translate to declines in soil C across depth and time. Developing such understanding of how switchgrass interacts with the soil environment more broadly,

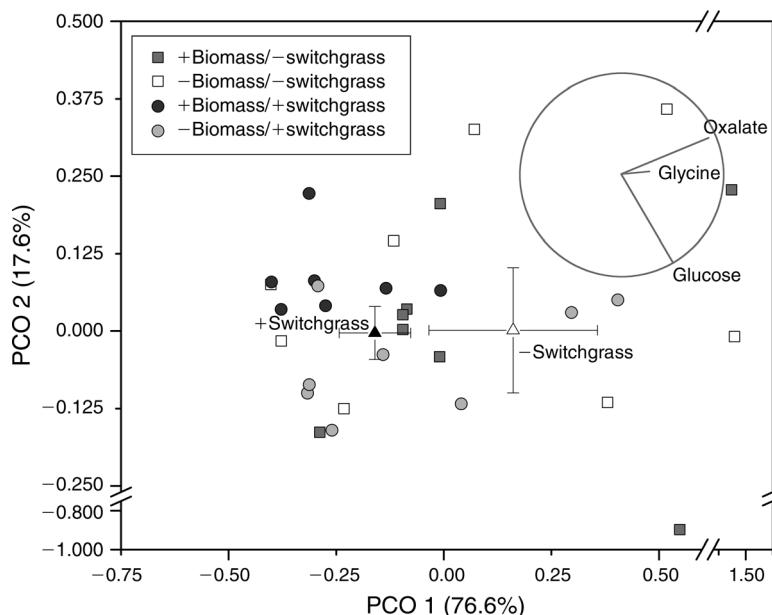


FIG. 5. Principle coordinate analysis of microbial community function in the interbed as assessed via catabolic response profiling using glucose, glycine, and oxalic acid. The centroids  $\pm$  SE are shown for those treatments where switchgrass was present (+) and absent (–). Also shown (circle insert) are the vectors associated with mineralization of the three compounds. When switchgrass was absent, communities tended to have higher respiration on glucose and oxalic acid.

and as the intercropped stands mature, might help identify management strategies to mitigate soil C losses. For example, switchgrass presence might shift pine stands from a tight N cycling system to a more open N cycling system, given that the nutrient acquisition strategy of switchgrass differs to that of pine (Phillips et al. 2012). This shift might then explain why declines in POM associated N were significantly related to switchgrass-derived C inputs (Fig. 2). An improved understanding of whether a mixing of nutrient-acquisition strategies in intercropped stands leads to a decrease in soil C will be vital to management decisions concerning where and under what site conditions switchgrass should be grown from a C, N, and microbiological standpoint (Kramer et al. 2012, Bradford et al. 2013, Phillips et al. 2013, Averill et al. 2014).

The results of our research highlight that managing switchgrass for biofuel production can have negative effects on some soil properties at least initially (this study focused on the first two years after study establishment), leading to declines in soil C and N pools (Fig. 1). However, intercropping switchgrass in loblolly pine plantations is a potentially economically profitable endeavor (Susaeta et al. 2012) and may have equivocal or potentially beneficial effects on other sustainability components, such as biological diversity (Riffell et al. 2012). What is unknown, however, is whether or not loss of soil C will continue as the system further develops, particularly given that active microbial biomass is higher under switchgrass and this microbial biomass is a dominant precursor of soil carbon formation (Grandy and Neff 2008, Bradford et al. 2013), and whether or not the cost associated with such declines outweigh the gains associated with biofuel production, such as a reduced reliance on fossil fuels.

In conclusion, we found that the intercropping of switchgrass is associated with reductions in soil C and N pools during the first two years of establishment, likely because switchgrass primes the activity of the soil microbial community (i.e., induces a priming effect). Future research needs to focus on the specific mechanism(s) responsible for these declines, how it potentially varies with soil depth, and how the system changes over time as the intercropped pine-switchgrass system matures. Such an understanding of the interaction between switchgrass and soil communities will be vital to determining the efficacy of its widespread use as a next generation biofuel.

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