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Physiological shifts in the microbial community drive changes in enzyme activity in a perennial agroecosystem

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Abstract Perennial agroecosystems have the potential to promote plant–microbial linkages by increasing the quantity of root carbon entering the soil. However, an understanding of how perennial cropping systems affect microbial communities remains incomplete. The objective of this study was to determine the potential for a fertilized perennial bioenergy cropping system to impact microbial growth and enzyme activity. Three times throughout the growing season we examined the activity of four enzymes involved in decomposition (ß-glucosidase, ß-xylosidase, cellobiohydrolase, and N-acetyl glucosaminidase) in replicated plots of an annual (corn) and perennial-based (switchgrass) cropping system. We also took simultaneous measurements of microbial biomass and potential rates of microbial respiration and net N mineralization. Microbial biomass was unaffected by cropping system. Mid-summer, however, we observed increases in enzyme activity and potential microbial respiration in the perennial system that were independent of microbial biomass, likely in response to labile carbon inputs. Further, we observed lower net N mineralization, higher microbial biomass nitrogen and higher activity of nitrogen liberating enzymes, which are indicative of a community with high nitrogen demands. Overall, our research demonstrates that perennial agroecosystems can affect the physiological capacity of the microbial community, yielding communities with greater nitrogen retention and greater rates of decomposition as a result of allocation of resources towards enzyme production and nitrogen mining. These results can inform biogeochemical models with respect to the importance of temporally dynamic changes in carbon and nitrogen availability and microbial carbon use efficiency as drivers of enzyme production.

Keywords Enzymes · Microbial biomass · Microbial physiology · Coupled carbon-nitrogen cycling · Landscape Biomass Project · Bioenergy cropping systems

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Introduction

Soil microbial communities play a significant role in global carbon (C) and nitrogen (N) cycling. They are responsible for the majority of organic matter decomposition (Swift et al. 1979) and govern processes of N uptake and loss (Canfield et al. 2010). In addition to

abiotic factors (Baer et al. 2010; Kemmitt et al. 2008), microbial processes depend on linkages with plants. For example, variation in plant inputs arising from differences in species composition, plant productivity, and resource allocation, impact soil microbial activity by altering the quality and quantity of organic matter entering the soil via root and leaf litter and root exudates (Hooper et al. 2000; Kuzyakov 2010; Wardle 2004; Wardle 2002). Thus, the response of microbial communities to plant inputs can have important implications for nutrient cycling and ecosystem functioning.

Much of the research on plant–microbial linkages has occurred in the context of natural ecosystems (Balser and Firestone 2005; Strickland et al. 2009; Waldrop and Firestone 2006), while the role of plant–microbial linkages in agricultural systems is not well studied (de Vries and Bardgett 2012). This is despite the fact that approximately one sixth of the land in the United States is devoted to agriculture (USDA 2007). Moreover, there is increased recognition that microbial communities are critical to the long-term productivity of agricultural ecosystems (Altieri 1999; Brussaard et al. 2007; Heijden and Wagg 2012) and sensitive to land management (Baer et al. 2002; Bandick and Dick 1999; Joergensen and Emmerling 2006; Lagomarsino et al. 2009). Identifying specific plant–microbial linkages, therefore, is key to modeling the fate of C and N in agroecosystems, especially for economies like bioenergy production that seek to balance high energy production with environmental benefits (Jordan et al. 2007).

The significance of plant inputs to soil microbial processes depends in part on C availability, which is often the main factor limiting microbial growth and activity (Burford and Bremner 1975; Fierer et al. 2009; Wang et al. 2003; Wardle 1992; Zak et al. 1994). To gain access to C, microbes synthesize extracellular enzymes that decompose particulate and mineral associated organic matter (Asmar et al. 1994; Burns et al. 2002; Sinsabaugh and Moorhead 1994). In this way, extracellular enzymes are considered the proximal drivers of decomposition and are important for the stabilization of soil organic matter (Six et al. 2006). Enzyme production requires energy, however, so enzyme patterns reflect not only the quantity of C in a system, but the availability of relatively labile C, such as root rhizodeposits (Schimel and Weintraub 2003). For example, previous laboratory and field experiments have demonstrated that additions of cellulose, representing fresh organic matter, can stimulate cellulose-degrading enzymes (Fontaine et al. 2004b) and lignin-degrading enzymes (Talbot and Treseder 2012).

Given the stoichiometric requirements of microorganisms, it is not possible to fully understand plant effects on microbial C cycling without considering how C concentrations affect N availability (Barrett and Burke 2002; Fontaine et al. 2004a; Reich et al. 2006; Sinsabaugh et al. 2008). For example, Phillips and colleagues (2010) found that microbes in relatively C-rich environments like the rhizosphere become N-limited such that energy from labile root exudates is used to produce enzymes that mineralize N-rich organic matter. Depending on the dynamics between plant and microbial uptake, mineralized N is retained by the microbial community through rapid immobilization (Kuzyakov et al. 2000; Vitousek and Matson 1984). In these cases, turnover rates of the microbial biomass increase but microbial activity associated with decomposition remains stable (Kuzyakov et al. 2000). At other times increased microbial activity can lead to faster rates of SOM decomposition ("priming") and subsequent release of plant available N (Bengtson et al. 2012; Dijkstra et al. 2009; Jingguo and Bakken 1997; Phillips et al. 2010) as a result of increased predation on bacteria or competition for N by plants, for example (Clarholm 1985; Griffiths and Robinson 1992; Kuzyakov et al. 2000).

Perennial cropping systems, such as those proposed for cellulosic bioenergy production, may promote plant–microbial linkages because of their extensive root networks and allocation of belowground C. The development of perennial root systems during grassland restoration represents a significant source of C inputs to soils that stimulates microbial biomass and activity and can change community composition (Allison et al. 2005; Bach et al. 2010; Baer et al. 2010; Barrett and Burke 2000; Camill et al. 2004; McKinley et al. 2005). Carbon additions from perennial root systems may also affect microbial communities in cultivated soils. Previous work in annual agroecosystems has demonstrated that greater C additions from the application of organic residues, cover crops (Bandick and Dick 1999), or diverse crop rotations (Dodor and Tabatabai 2002, 2003) increase microbial biomass and enzyme activity as compared to conventionally managed cropping systems. However,

N addition has been shown to suppress microbial growth, an effect that is most pronounced in ecosystems that receive high rates of N fertilization over long durations like agricultural ecosystems of the US cornbelt (Liebig et al. 2002; Treseder 2008). Therefore, microbial response to greater root C in intensively managed perennial agroecosystems remains largely unknown.

Resolving the dominant plant–microbial linkages in agroecosystems is critical to our ability to model the biogeochemical outcomes of these ecosystems. To partially fill this knowledge gap, the goal of this study was to discern short-term potential for plant–microbial linkages associated with C and N cycling in a perennial agroecosystem managed intensively for bioenergy production. Specifically, we tested whether microbial communities associated with a perennial cropping system differed in biomass, enzyme activity and potential mineralization rates as compared to an annual cropping system. We predicted that the perennial crop, with greater allocation of C belowground, would stimulate the activity of enzymes associated with C and N cycling. We predicted that changes in enzyme activity would be coupled to microbial biomass, and increase with increasing microbial biomass in the perennial agroecosystem. Furthermore, we predicted that microbial growth would result in concurrent increases in microbial respiration and N retention, as the utilization of C would increase microbial demand for N and reduce net N mineralization rates in the perennial system relative to the annual agroecosystem (Baer et al. 2002, 2010; Barrett and Burke 2000; Camill et al. 2004).

Methods

Study site

The study was conducted as part of the Landscape Biomass Project at the Uthe Research & Demonstration Farm, Boone County, Iowa, USA (41°55′N, 93°45′W) during the 2011 growing season (1 June–23 August). Soils at the site are classified as fine-loamy Hapludoll Mollisols and follow a topographic gradient. The slope on the summit and floodplain is ~0.5 % and the side slope is ~2.5 %. For the soils sampled in this study there were no significant changes in soil organic carbon (average 17.2 g kg^{-1}), total soil N (average of 1.42 g kg^{-1}), bulk density (average of 1.57 g cm^{-3}), or soil texture (average of 49.4 % silt + clay) (Ontl et al. 2013). Prior to study initiation, the upland was managed under a corn–soybean rotation, with corn in rotation before the establishment of the research plots. Total precipitation at the site from 1 April to 31 October 2011 was 644 mm, which was slightly below the 20-years average of 662 mm. Mean annual temperature was 8.9 °C and average daily temperatures were within the range of 20 year data for the period of the study (Ontl et al. 2013).

Experimental design

To assess the impact of perennial agroecosystems on microbial biomass and activity, we sampled from experimental plots of two cellulosic bioenergy cropping systems, or agroecosystem types, consisting of no-till cultivation of an annual crop (continuous corn, *Zea mays* L.; "annual") and a perennial monoculture (switchgrass, *Panicum virgatum* L, cv: 'Cave-In-Rock'; "perennial"). Both crops were replicated three times on three topographic positions (summit, back slope, and toe slope) following a randomized complete block design ($n = 3$). Each experimental plot was ~0.05 ha. The experiment was established in May 2009, at which point the perennial crop of switchgrass was planted. In 2011, the corn crop was planted and corn and switchgrass plots were fertilized on 10 May. Nitrogen fertilization was based on nutrient demands of crops (Vogel et al. 2002) and was applied at a rate of 168 kg urea-N ha^{-1} for corn and 134 kg urea-N ha^{-1} for switchgrass. Both cropping systems received 56 kg P_2O_5 ha^{-1} and 112 kg KCl ha^{-1}. Corn grain was harvested in October by combining followed by a second pass with a flail chopper to clear the stover. Switchgrass was harvested after the first hard frost in November to ensure translocation of N into roots (Wilson et al. 2012). At harvest, maximum aboveground biomass was removed, leaving ~10 % of the aboveground biomass for both corn and switchgrass.

Soil samples were collected from the 18 experimental plots on 1 June (spring), 13 July (mid summer), and 23 August (late summer) 2011. On each sampling date, ten randomly distributed soil cores (2.2 cm in diameter × 15 cm depth) were collected from each plot and composited, then sieved to 4-mm in the lab. Our study focused on bulk soil to ensure any changes we observed were detectable at the plot scale and,

therefore, interpretable at scales relevant to land management.

Microbial biomass and soil characteristics

Each composited sample was analyzed for microbial biomass C (MBC) and microbial biomass N (MBN), extractable nitrate (NO_3^-) and ammonium (NH_4^+), salt-extractable organic C, soil moisture, and pH. Microbial biomass C and N were measured using direct chloroform-fumigation-extraction (modified from Vance et al. 1987). Extracts were analyzed for non-purgeable organic C and total N via combustion catalytic oxidation (Shimadzu TOC-L analyzer, Shimadzu Corporation, Columbia, Maryland, USA), and conversion factors of 0.45 for C and 0.54 for N were used to convert organic C and N to microbial biomass (Brookes et al. 1985; Vance et al. 1987). Salt-extractable organic C was measured as non-purgeable organic C in the unfumigated 0.5 M K_2SO_4 extracts. Similarly, unfumigated samples were used to measure extractable NO_3^- and NH_4^+ concentrations via spectrophotometry (BioTek Synergy HT plate reader, BioTek Instruments, Inc., Winooski, VT, USA; Hood-Nowotny et al. 2010). Soil pH was measured in a 1:1 ratio of soil and water. Gravimetric moisture content was measured as water mass loss upon drying at 105 °C to a constant weight, and used to determine soil moisture and soil wet-weight to dry-weight ratios.

Extracellular enzymes

We analyzed the potential activity of four hydrolytic enzymes involved in carbon and nitrogen cycling: ß-1,4-glucosidase (BG, which hydrolyzes cellobiose into glucose), ß-1,4-xylosidase (BX, which degrades hemicellulose), cellobiohydrolase (CB, an exocellulase), and ß-N-acetyl-glucosaminidase (NAG, which breaks down chitin and peptidoglycan, and hydrolyses glucosamine from chitobiose). Potential enzyme activities were measured on a subsample of sieved soil stored at −20 °C following (German et al. 2011). Briefly, 1 g of soil was homogenized with 125 mL of 100 mM maleate buffer titrated to pH 6.5 (German et al. 2012). Enzyme activity was induced with the addition of methylumbelliferone (MUB)-linked substrates. All plates were incubated for 3 h at 23 °C. At the end of the incubation, 10 µL 1 M NaOH was added and plates were read using a fluorometer after a 3-min development period (360 nm excitation and 460 nm emission; BioTek Instruments, Inc., Winooski, VT, USA). Eight analytical replicates per sample and substrate combination were run and each plate included a MUB standard curve, substrate controls, and homogenate controls. Enzyme activity was calculated as nmol enzyme g^{-1} dry soil h^{-1} based on MUB standard curves and accounting for the quench of each sample (German et al. 2011). Linearity of the reaction was confirmed for the 3-h incubation and all reactions were run at saturating substrate concentrations (400 µM for all) as determined for each enzyme with a subsample of soil used in this experiment. Given the wide pH range of experimental plots (4.5–8.5), we chose to run assays at pH optima, as determined by assessing activity of each enzyme with soils from the field site at increments of 0.5 over a pH range of 4–8.5 (Burns et al. 2013; Turner 2010). By doing so, we were best able quantify the enzyme pools as a response to the experimental treatments. We also calculated specific enzyme activity by dividing absolute enzyme activity by MBC. Specific activity is a metric for understanding whether changes in enzyme pools are a function of the size of the microbial community or a shift in the physiological capacity of the microbial community (e.g. Waldrop et al. 2000).

Microbial respiration and net N mineralization

Potential microbial respiration was measured as C mineralization potential using a short-term incubation method (Robertson 1999). Briefly, 15 g sieved soil was brought to 60 % water-holding capacity and incubated for 8 days in the dark in serum bottles capped with a rubber butyl septum. Carbon dioxide concentration in the headspace was sampled on days 1, 5, and 8 using an infrared gas absorption analyzer (LI-7000 CO_2/H_2O Gas Analyzer, LICOR, Lincoln, NE, USA). After each sampling, flasks were vented to prevent CO_2 build-up and possible inhibition of aerobic respiration. In this way, a measurement of microbial respiration rates provides an index for available mineralizable C concentrations. Potential net N mineralization was measured as the difference between extractable inorganic N at initiation and after a 28-days incubation. Incubations were conducted in the dark, at 22 °C and 60 % water holding capacity (Robertson 1999). In addition, microbial respiration

Table 1 Soil characteristics at three sampling dates from an annual (continuous corn) and perennial (switchgrass) agroecosystem ($n = 9$)

Agroecosystem type	Sampling time	Salt-extractable organic C (μg g^{-1})	NO_3 (μg g^{-1})	NH_4 (μg g^{-1})	pH	Moisture (%)
Annual	June	34 (6.1)a	*19.1 (3.8)a*	5.9 (2.3)a	6.7 (0.4)a	14 (1)a
	July	32.8 (2.6)a	5.5 (1.1)b	2.6 (0.3)a	6.6 (0.4)a	10 (1)b
	August	24.5 (2)a	1.6 (0.5)c	1.1 (0.2)b	6.8 (0.3)a	6 (1)c
Perennial	June	29.4 (2.8)a	*3.3 (0.5)A*	2.1 (0.3)A	6.5 (0.4)a	14 (1)a
	July	35.9 (3.8)a	3.6 (0.7)A	3 (0.3)A	6.7 (0.4)a	10 (1)b
	August	31.2 (4)a	1 (0.4)B	1.3 (0.2)B	6.8 (0.3)a	6 (1)c

Means are given and numbers in *parentheses* are standard errors

Letters denote significant differences between sampling dates within an agroecosystem (Tukey's HSD, $P < 0.05$), with lower-case letters for the annual system and upper-case letters for the perennial system (Tukey's HSD, $P < 0.05$)

Italics denote significant differences between agroecosystems at a sampling date (Tukey's HSD, $P < 0.05$)

and N mineralization potentials were calculated as a specific rate, by dividing the absolute rate by MBC.

Statistical analysis

When necessary, data were log-transformed to meet assumptions of normality. Potential activity of each enzyme, microbial biomass, microbial respiration, net N mineralization, and soil nutrients were analyzed using a mixed model analysis for repeated measures with agroecosystem type and topographic position as fixed effects, block as a random effect, and sampling date as the repeated effect. Covariance structure of the data was accounted for using compound symmetry and degrees of freedom were adjusted using a Satterthwaite correction. Comparisons among means were analyzed by Tukey's HSD post hoc tests. We also tested the effect at each sampling date using a two-way ANOVA. All ANOVAs were analyzed using SAS statistical software (SAS v. 9.2, SAS Institute, Inc, Cary, NC, USA). Pearson correlations between enzyme activity and soil physical and chemical properties were performed in R (V2.12.2).

Results

Soil characteristics

Soil pH was unaffected by any of the treatments in this study and did not correlate with microbial activity or biomass (Table 1). Salt-extractable organic carbon was unaffected by any of the treatments tested ($P > 0.05$). Soil moisture varied only by sampling date, and became progressively drier from June to August ($P < 0.05$; Table 1). The concentration of nitrate in both perennial and annual agroecosystems was lowest late summer and was significantly higher in the annual system in June ($P < 0.01$; Table 1). In contrast, ammonium concentrations decreased throughout the season in both agroecosystems ($P < 0.01$; Table 1) with no other main or interaction effects.

Microbial biomass

Average MBC was greater in the perennial compared to the annual agroecosystem, but results were highly variable and we were unable to detect a treatment effect irrespective of topographic position and sampling date ($P > 0.05$, Table 2). MBN was 1.3 times higher in the perennial agroecosystem compared to the annual system ($P = 0.02$; Table 2) and was unaffected by topographic position. We detected no significant interaction effects on microbial biomass.

Enzyme activity

All four hydrolase enzymes responded similarly throughout the growing season (Fig. 1). The activity of BG, BX, CB, and NAG increased in the perennial agroecosystem in July and was unaffected by agroecosystem type in June and August (agroecosystem × time interaction, $P < 0.05$). Compared to the annual system and other sampling dates, average activity of BG, BX, CB, and NAG in July was over 150, 180, 200, and 200 % higher in the perennial agroecosystem, respectively.

Table 2 Microbial biomass and specific activity in an annual (continuous corn) and perennial (switchgrass) agroecosystem throughout the growing season in 2011 ($n = 9$)

Agroecosystem type	Sampling time	Microbial biomass carbon ($\mu g\ g^{-1}$)	Microbial biomass nitrogen ($\mu g\ g^{-1}$)	Specific BG (nmol $g^{-1}\ h^{-1}$ MBC^{-1})	Specific BX (nmol $g^{-1}\ h^{-1}$ MBC^{-1})	Specific CB (nmol $g^{-1}\ h^{-1}$ MBC^{-1})	Specific NAG (nmol $g^{-1}\ h^{-1}$ MBC^{-1})	Specific respiration ($\mu g\ CO_2\ g^{-1}\ day^{-1}$ MBC^{-1})	Specific net N mineralization (ng N $g^{-1}\ day^{-1}$ MBC^{-1})
Annual	June	138.3 (16.9)	21.5 (5.3)	2.73 (0.48)a	0.48 (0.08)a	0.32 (0.05)a	1.08 (0.15)a	0.11 (0.024)a	2.97 (0.85)a
	July	140 (10.5)	17.5 (1.5)	*3.86 (0.56)a*	*0.61 (0.08)a*	*0.41 (0.07)a*	*1.71 (0.22)a*	*0.085 (0.005)a*	*1.66 (0.18)a*
	August	148.7 (9.5)	15.8 (1)	2.20 (0.25)a	0.44 (0.05)a	0.31 (0.06)a	1.33 (0.23)a	0.1 (0.01)a	0.76 (0.22)a
Perennial	June	172.5 (7.9)	26.1 (1.7)	1.55 (0.21)B	0.29 (0.03)B	0.17 (0.03)B	0.74 (0.11)B	0.094 (0.006)A	1.08 (0.26)A
	July	166.6 (12.1)	21.7 (1.7)	*5.39 (0.77)A*	*1.07 (0.17)A*	*0.79 (0.16)A*	*4.12 (0.75)A*	*0.125 (0.012)A*	*0.12 (0.14)A*
	August	165.6 (19.7)	21.1 (1.8)	2.56 (0.46)B	0.57(0.12)B	0.32 (0.05)B	1.72 (0.32)B	0.103 (0.01)A	0.19 (0.13)A

Means are given and numbers in *parentheses* are standard errors

Letters denote significant differences between sampling dates within an agroecosystem, with lower-case letters for the annual system and upper-case letters for the perennial system (Tukey's HSD, $P < 0.05$)

Italics denote significant differences between agroecosystems at a sampling date (Tukey's HSD, $P < 0.05$)

BG ß-glucosidase, *BX* ß-xylosidase, *CB* cellobiohydrolase, *NAG* N-acetyl glucosaminidase, *MBC* microbial biomass carbon

Similarly, the biomass-specific activity of the hydrolase enzymes was over 200 % higher in the perennial agroecosystem in July compared to the annual system and compared to the other sampling dates in both systems ($P < 0.01$ for all; Table 2). We detected no significant main effects of topographic position or interaction effects of topographic position and agroecosystem type on absolute or specific enzyme activities. Of the soil properties examined, total soil N was positively correlated with C degrading enzymes, explaining 27 % of the variation in BG, 29 % in BX, and 29 % in CB (Supplemental Table 1). CB was positively correlated with soil silt + clay content ($r = 0.28$, $P < 0.05$; Supplemental Table 1).

Microbial respiration and net N mineralization

Average potential microbial respiration in the perennial agroecosystem was higher than in the annual system at all topographic positions ($P < 0.01$; Fig. 2). Compared to the annual agroecosystem, microbial respiration in the perennial system was stimulated by over 120 % in June, 160 % in July, and 120 % in August. Specific respiration was higher in the perennial agroecosystem only in July (agroecosystem × time, $P = 0.05$; Table 2), with no significant main effects of agroecosystem, sampling date, or topographic position ($P > 0.05$ for all). Among sampling dates, specific respiration in July was highest for the perennial agroecosystem and lowest for the annual system, but these trends were not statistically significant (time, $P = 0.16$).

Greater microbial respiration coincided with reduced rates of net N mineralization in the perennial compared to the annual agroecosystem ($P < 0.01$, Fig. 3). Consistent with microbial respiration, the effects of agroecosystems were most pronounced in July and August, when net N mineralization in the perennial agroecosystem was less than 10 and 20 % that of the annual system, respectively. In contrast, average net N mineralization in June was 52 % lower in the annual compared to the perennial agroecosystem but this effect was not significant ($P = 0.62$). Net N mineralization was positively correlated with soil moisture ($r = 0.30$, Supplemental Table 1) and negatively correlated with salt-extractable organic C ($r = -0.31$, Supplemental Table 1). Consistent with the pattern of net N mineralization, specific net N mineralization in the perennial system was lower than

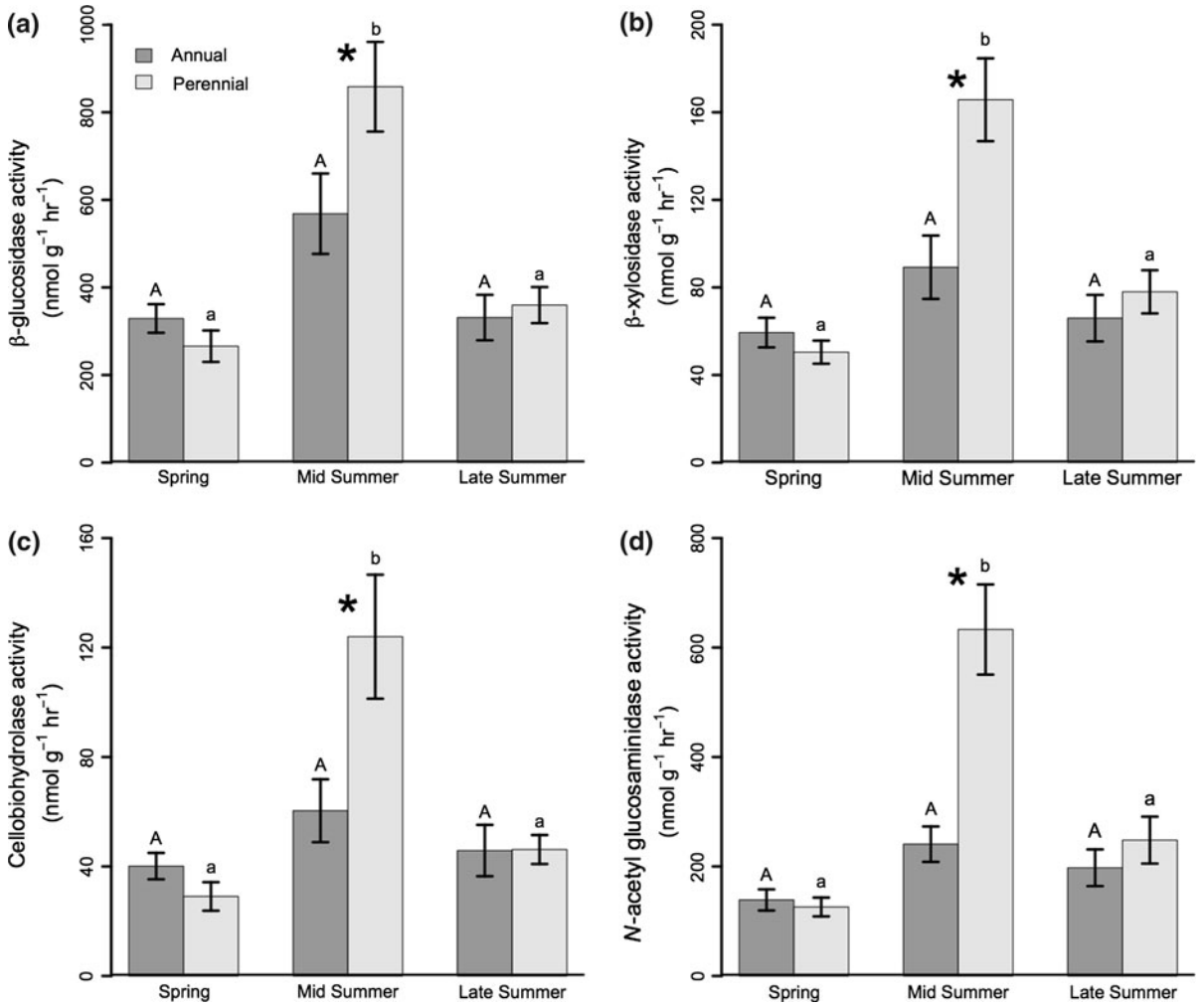

Fig. 1 Activity of **a** ß-glucosidase, **b** ß-xylosidase, **c** cellobiohydrolase, and **d** *N*-acetyl-glucosaminidase showing the effects of agroecosystem type at three sampling dates. Mean ± SE are shown. *Letters (lower-case, annual; upper-case, perennial)* denote significant differences between sampling dates within an agroecosystem and *asterisks* denote significant differences between agroecosystems at a sampling date (Tukey's HSD, $P < 0.05$)

in the annual system by 36 % in June, 7 % in July, and 25 % in August ($P < 0.01$; Table 2). Both net N mineralization and specific net N mineralization were higher in June and than in August ($P < 0.05$).

Discussion

Carbon storage and nitrogen retention in agricultural ecosystems may be enhanced if perennial species are used as cellulosic biofuel crops (Robertson et al. 2011). Most studies examining the biogeochemical benefits of perennial plants have focused on direct effects of perennial roots on C storage and N uptake (Glover et al. 2010; Robertson et al. 2011; Robertson and Vitousek 2009). Apart from these direct impacts, plants indirectly influence C and N cycling through linkages with microbes, which are important to understand in order to model the biogeochemistry of these ecosystems (Treseder et al. 2011). To our knowledge, this study is the first to identify plant–microbial linkages in a fertilized perennial monoculture cultivated for bioenergy production.

Contrary to our hypothesis, the perennial cropping system did not affect microbial biomass. This was surprising, considering the 3 years old switchgrass had

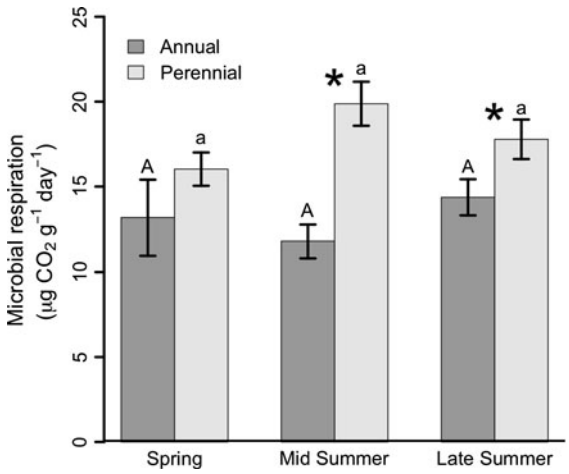

Fig. 2 Potential microbial respiration showing the effects of agroecosystem type at three sampling dates in 2011. Mean ± SE are shown. *Letters (lower-case, annual; upper-case, perennial)* denote significant differences between sampling dates within an agroecosystem and *asterisks* denote significant differences between agroecosystems at a sampling date (Tukey's HSD, $P < 0.05$)

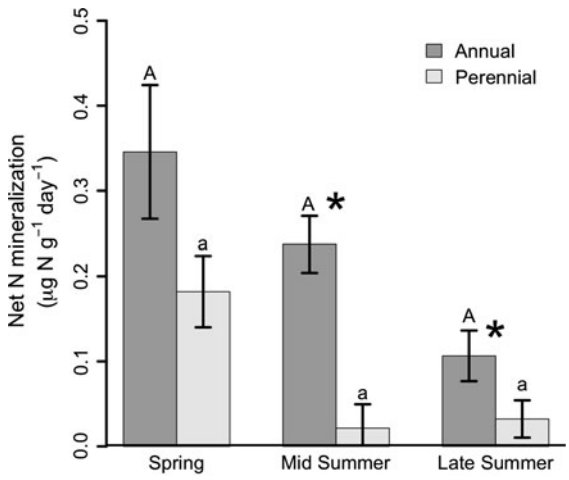

Fig. 3 Potential net N mineralization showing the effects of agroecosystem type at three sampling dates in 2011. Mean ± SE are shown. *Letters (lower-case, annual; upper-case, perennial)* denote significant differences between sampling dates within an agroecosystem and *asterisks* denote significant differences between agroecosystems at a sampling date (Tukey's HSD, $P < 0.05$)

more than twice as much annual root production (Ontl et al. 2013) and increases in MBC in response to changes in cropping systems have been documented on similar time scales (1–3 years) (Kallenbach and Grandy 2011). The lack of effect of the perennial cropping system on microbial biomass may be attributable to chronic disturbance associated with intensively managed ecosystems (Allison and Martiny 2008; Dethlefsen and Relman 2011; Meier et al. 2007) or the lack of coupling of C and N additions in these systems (Kallenbach and Grandy 2011; Lagomarsino et al. 2006). Primary inputs of C (root inputs with high C:N) and N (inorganic fertilizer) were from different sources, which can result in a temporal disconnect in resource availability and subsequent reduction in microbial energy and enzyme efficiency relative to high quality (low C:N) organic sources (Hobbie 2005). A legacy of N fertilization can also change the soil nutrient and ion status and the physiological capacity of the microbial community, leading to a suppression of microbial growth (Treseder 2008; Ramirez et al. 2012). Therefore, our results suggest that microbial growth may be delayed in perennial agroecosystems fertilized with inorganic N, such that increases in microbial biomass may only become detectable as the crop ages (i.e. decadal time scale) and a new equilibrium SOC is established (Baer et al. 2002; Debosz et al. 1999; Powlson et al. 1987).

Consistent with our hypothesis, the perennial cropping system stimulated the activity of enzymes involved in organic matter decomposition. Increases in BG, BX, CB, and NAG activity occurred only mid summer and, therefore, may reflect phenological patterns of relatively labile root inputs (Franzluebbers et al. 1994). Given the almost threefold difference in root inputs in the switchgrass compared to corn (Ontl et al. 2013), greater amounts of unprotected fresh root litter from fine root turnover probably induced enzyme activity. Of the three sampling dates in this study, July corresponded to the time of greatest C inputs from particulate organic matter associated with root turnover and rhizodeposition, as it was closest to tillering of switchgrass (Gill et al. 2002; Swinnen et al. 1995) and maturity of corn (Russell et al. 2009). Consistent with a stimulation of microbial activity from relatively labile sources, potential microbial respiration was higher in the perennial system compared to the annual system and specific respiration was highest in July in the perennial system (Bradford et al. 2008b; Fontaine et al. 2004a). Apart from plant effects, enzyme activity was not significantly correlated to any of the edaphic factors measured. However, low levels of soil moisture in late summer in both cropping systems could have contributed to low levels of enzyme activity at that

time, as suggested by the positive correlation between net N mineralization and soil moisture (Henry 2012; Sardans and Peñuelas 2005; Steinweg et al. 2012).

While an induction of C cycling enzymes in response to increased availability of soluble C has been demonstrated in field and laboratory studies (Burns 1982; Chróst 1991; Reboreda and Caçador 2008; Shackle et al. 2000), other studies have reported no response of C cycling enzymes in the presence of soluble C (Allison and Vitousek 2005; Weintraub et al. 2007). Reasons for the lack of response of C enzymes include allocation of energy towards acquisition of other resources, such as N, in order to balance the elevated concentrations of available C (Kuzyakov et al. 2000). The elevated activity of BG, BX, and CB observed in this study suggests that microbial communities remained constrained by enzyme-mediated C acquisition despite stimulation from fresh root inputs, and is consistent with the lack of increase of MBC that we observed.

When enzyme activities were expressed per unit microbial biomass (i.e. mass specific activity), activity in the perennial agroecosystem was higher than in the annual agroecosystem. Assuming enzyme activity was representative of currently or recently active members of the microbial community, these results suggest that changes in physiological capacity of the microbial community and not microbial biomass explained patterns in enzyme production (Bradford et al. 2008a; Waldrop et al. 2000). Shifts in mass-specific enzyme activity indicate that microbial communities allocated a greater amount of energy to enzyme production rather than growth and is indicative of lower carbon use efficiency (CUE) (Schimel and Schaeffer 2012). Carbon use efficiency is the ratio of the amount of C assimilated into biomass relative to the amount of C that is lost to maintenance, like to respiration and enzyme production (del Giorgio and Cole 1998; Schimel and Weintraub 2003). At a community level, physiological changes associated with CUE might be a consequence of changes in the activity of individual microorganisms and/or shifts in the relative abundances of specific microbial taxa. Inefficient resource use in response to labile C substrates is consistent with findings from N fertilized ecosystems (Fierer et al. 2007). Indeed, previous field and laboratory studies have shown that N fertilization select for copiotrophic microorganisms, which are taxa with high turnover rates, relatively high N demands and specialize on more labile C pools (Campbell et al. 2010; Fontaine and Barot 2005; Ramirez et al. 2012). While few studies have reported simultaneous measurements of microbial enzyme activity and biomass, increases in mass specific enzyme activity have been previously documented in relation to changes in community composition in response to land management (Waldrop et al. 2000).

Mass specific changes in microbial activity stand in contrast to how most biogeochemical models represent microbe-enzyme dynamics. Models generally assume that enzyme activity is controlled by the size of the microbial community (Manzoni and Porporato 2009; Todd-Brown et al. 2011). However, changes in microbial efficiency are important to recognize because they have consequences for the fate of C in agroecosystems. Lower efficiency implies relatively larger C losses via enzyme production and respiration, ultimately affecting the long-term storage of root C in the soil and rates of greenhouse gas production (CO_2) relative to the annual system (Manzoni et al. 2012; Schimel and Weintraub 2003). For example, models have demonstrated that changes in microbial CUE as a function of temperature can impact the sensitivity of soil C pools (Wang et al. 2012). Along with environmental conditions (i.e. precipitation, temperature), our results demonstrate that incorporating temporally dynamic changes in microbial energy allocation as a function of plant inputs may improve predictions of biogeochemical models.

In addition to changes in C cycling, our results indicate that the perennial agroecosystem stimulated changes in N cycling at this site. The activity of NAG—representing the enzymes associated with the breakdown of chitin peptidoglycan, and other N-rich organic macromolecules—was nearly threefold higher in the switchgrass compared to the corn cropping system in July, indicating moderately faster turnover of N (Phillips et al. 2010; Tabatabai et al. 2010). Consistent with N turnover and acquisition, we observed greater MBN in response to the perennial cropping system. These results were unexpected considering the perennial cropping system was fertilized. However, high leaching rates from tile drains mean that fertilization events are ephemeral, as evidenced from the low inorganic N concentrations mid summer. Therefore, despite fertilization in the spring, these results reflect a physiological shift in the perennial compared to the annual agroecosystem towards a microbial community with high N demands.

Changes in N cycling appear to be linked to changes in C availability. It is well documented that plant roots influence N mineralization rates and changes in net N mineralization are often linked to changes in labile C (Kuzyakov et al. 2000; Parkin et al. 2002). Consistent with this, we observed a significant negative correlation between net N mineralization and microbial respiration. Despite no treatment effect on salt-extractable organic C pools, we also observed a significant correlation between net N mineralization and salt-extractable organic C concentration, suggesting a rapid uptake of root C inputs by microorganisms (McLauchlan and Hobbie 2004; Melillo et al. 2002). Some studies have reported a stimulation of net N mineralization by plants attributed to increased microbial activity and subsequent release of inorganic N from grazing on bacteria by protozoa (Clarholm 1985; Franzluebbers et al. 1994) or decreased microbial immobilization due to competition for N with plants (Griffiths and Robinson 1992; Jingguo and Bakken 1997). Other studies have documented no effect of plants on net N mineralization (Breland and Bakken 1991; Parkin et al. 2002). In contrast, our results suggest that greater quantity of labile C from the switchgrass resulted in a decline in net N mineralization due to greater microbial immobilization, as evidenced by higher MBN in the perennial agroecosystem (Bremer and Kuikman 1997; Clarholm 1985). Therefore, while other factors cannot be ruled out as contributors to the patterns in N cycling, these data provide further support to our assertion that fresh plant inputs from the perennial crop support a microbial community that cycles and retains more N, independently of its size.

Linking changes in C availability to microbial N cycling also has important implications for the biogeochemical predictions associated with these ecosystems. High N demand and turnover of microbial communities in the perennial agroecosystem may extend the amount of time N is available to plants through cyclic pulses of re-mineralized N (Badalucco and Kuikman 2007; Manzoni and Porporato 2009; Marinari et al. 2010), thus promoting N retention by reducing fertilizer-associated N losses (de Vries and Bardgett 2012). However, mining of organic matter for N in the perennial agroecosystem may increase soil organic matter decomposition relative to the annual agroecosystem (Craine et al. 2007). This balance between N-retention and rates of decomposition as a function of microbial physiology denotes a new modeling challenge for bioenergy production, where attempts to predict cropping system effects on the environment may require seasonal flexibility in C and N demands as a driver of decomposition.

Conclusions

Understanding the microbial mechanisms regulating C and N cycling in biofuel cropping systems is important for modeling their biogeochemical impact. Compared to an annual cropping system, our results provide evidence that short-term effects of perennial cropping systems can promote plant–microbial linkages by changing the physiological capacity of the microbial community, but not by increasing microbial biomass. Increased microbial activity associated with soil organic matter formation and the depolymerization and immobilization of N may lead to faster rates of decomposition and greater N retention. Further, seasonal changes in enzyme activity illustrate the dependence of C and N dynamics on short-term substrate availability from crop roots. Data provided here represent highly accessible parameters for integrating soil microorganisms into models, yet they are rarely generated and interpreted together. As such, this work is an important step in guiding models to include microbial physiology related enzyme production for a more accurate representation of temporal dynamics of C and N cycling. This type of modeling may be especially important for ecosystems that experience persistent disturbances or uncoupled sources of C and N, such as intensively managed agricultural ecosystems.

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