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Soil microbial biomass and function are altered by 12 years of crop rotation

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Abstract

Agriculture-driven declines in plant biodiversity reduce soil microbial biomass, alter microbial functions, and threaten the provisioning of soil ecosystem services. We examined whether increasing temporal plant biodiversity (by rotating crops) can partially reverse these trends and enhance microbial biomass and function. We quantified seasonal patterns in soil microbial biomass, respiration rates, extracellular enzyme activity, and catabolic potential three times over one growing season in a 12-year crop rotation study at the W.K. Kellogg Biological Station LTER. Rotation treatments varied from one to five crops in a three-year rotation cycle, but all soils were sampled under corn to isolate historical rotation effects from current crop effects. Inorganic N, the stoichiometry of microbial biomass and dissolved organic C and N varied seasonally, likely reflecting fluctuations in soil resources during the growing season. Soils from biodiverse cropping systems increased microbial biomass C by 28-112% and N by 18-58% compared to monoculture corn. Rotations increased potential C mineralization by as much as 64%, and potential N mineralization by 62%, and both were related to substantially higher hydrolase and lower oxidase enzyme activities. The catabolic potential of the microbial community, assessed with community-level physiological profiling, showed that microbial communities in monoculture corn preferentially used simple substrates like carboxylic acids, relative to more diverse cropping systems. By isolating plant biodiversity from differences in fertilization and tillage, our study illustrates that crop biodiversity has overarching effects on soil microbial biomass and function that last throughout the growing season. In simplified agricultural systems, relatively small increases in plant biodiversity have a large impact on microbial community size and function.

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Keywords: crop rotation; agriculture biodiversity; soil carbon; soil nitrogen; nitrogen mining; community-level physiological profile; mineralization; extracellular enzymes; soil microbial biomass

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Introduction

Research manipulating aboveground biodiversity in grasslands has shown a strong link between plant species richness and soil functions (Tilman et al. 1997, Zak et al. 2003, Eisenhauer et al. 2010, Mueller et al. 2013). While this research has contributed to our understanding of aboveground-belowground biodiversity in natural ecosystems, it fails to capture the biodiversity dynamics in agroecosystems, where crop rotations can be used to substitute temporal for spatial biodiversity. Given that species richness at any given time in a rotated cropping system is one, the aboveground-belowground relationships dependent on diversity in agroecosystems and spatially diverse ecosystems (e.g. grasslands) may not be the same.

Crop rotations have been shown to have large positive effects on soil C, N, and microbial biomass (McDaniel et al., 2014a), plant pathogen suppression (Krupinsky et al. 2002), and yields (Ret al. 2008, Riedell et al. 2009). These positive effects of crop production have been colloquially referred to as the "rotation effect." However, the mechanistic processes that link aboveground crop rotational biodiversity and belowground soil processes and contribute to the "rotation effect" remain elusive. One hypothesis explaining the benefits of crop rotations is that greater diversity of plant inputs to soil organic matter (SOM) over time enhances belowground biodiversity and soil ecosystem functioning (Hooper et al., 2000; Waldrop et al., 2006; Grandy and Robertson, 2007). Despite being low in spatial diversity, crop rotations have been shown to increase soil microbial and faunal biodiversity (Ryszkowski et al. 1998, Wu et al. 2008, Tiemann et al. 2015) and increase microbial carbon use efficiency (Kallenbach et al. 2015). One essential function of soil microbial communities is the catabolism of newly added substrates from crops. The range and efficiency of microbial catabolism has great implications for ecosystem services

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such as sequestering C and soil fertility (Carpenter-Boggs 2000; Kallenbach et al. 2015), but also for ecosystem "dis-services" such as emission of soil-to-atmosphere greenhouse gases (McDaniel et al. 2014b). Furthermore, the partitioning of resources used in catabolism of residue and formation of SOM will likely affect long-term soil fertility (Lange et al. 2015; Kallenbach et al. 2015).

Soil microbial catabolic function can be measured using community-level physiological profiles (CLPP), sometimes referred to as catabolic response profiles. The basic method for measuring soil CLPP involves adding a suite of C substrates to soils and measuring the catabolic response as CO_2 production or O_2 consumption with redox indicators (i.e. Biolog; Guckert et al. 1996). These C substrates are typically ecologically-relevant compounds found in soils, and are intended to represent root exudates, microbial or plant cell structures, or other more-processed soil organic molecules. By measuring the respiration response, we can establish a catabolic "fingerprint" to distinguish soil microbial communities from one another by how they catabolize these new C substrates. We can also derive catabolic evenness of that community – a measure of biodiversity. Modern agriculture's use of monocultures could have unknown consequences for soil microbial catabolism and related processes such as SOM mineralization, but to date the effect of rotation practices and crop diversity on soil microbial functioning remains poorly understood.

Considering the lack of a clear understanding of how soil microbial functions are influenced by crop rotations, we sought to examine their effects on soil microbial biomass and function. We measured soil microbial catabolic potential, C and N mineralization, extracellular enzyme activities, and microbial biomass three times over one growing season in a long-term

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crop rotation experiment at the W.K. Kellogg Biological Station (est. 2000). All soils were measured under the same crop, allowing us to separate historical rotation from current crop effects. We hypothesized that soils under more diverse crop rotations would show greater catabolic diversity and have higher measures of soil function (enzyme activities, soil microbial biomass, potentially mineralizable C and N). In addition, we hypothesized that crop rotation effects would vary seasonally, being greatest in the spring and lessen over the growing season with the emerging influence of the current crop. The rationale for this second hypothesis is that early in the season all soils are coming out of different crops from the previous year, but over the growing season under corn the soils will become more functionally similar as the immediate crop has greater influence. Alternatively, significant Rotation by Season interactions on soil microbial functioning that do not converge over the growing season point to historical effects of rotations on differences in soil microbial communities and SOM.

Materials and Methods

This study was conducted in the Cropping Biodiversity Gradient Experiment (CBGE) at the W.K. Kellogg Biological Station Long-term Ecological Research site (42° 24', 85° 24' W). The CBGE was established in 2000 and consists of crop rotations ranging from monocultures to a 5-species rotation (http://lter.kbs.msu.edu/research/long-term-experiments/biodiversity-gradient/). The crop rotations were repeated but with different rotation phases within all four blocks. For example, the corn-soy-wheat rotation is replicated three times within each block, but these replicates are planted to a different crop each year. The plot dimensions were 9.1 x 27.4 m and received the same chisel plow tillage to a depth of approximately 15 cm, and received no inputs (e.g. pesticides or fertilizers) that would have confounded the treatment effects of rotational diversity (Smith et al. 2008). Mean annual temperature and precipitation at the site are

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9.7°C and 890 mm, respectively. The two main soil series located at the site are Kalamazoo, a fine-loamy, mixed, mesic Typic Hapludalf, and Oshtemo, a coarse-loamy, mixed, mesic Typic Hapludalf (KBS, 2012). Soil pH in the top 10 cm ranges from 4.9 to 6.1 (1:1 v of 0.01 M $CaCl_2$).

Soils were collected from the following cropping systems: monoculture corn (*Zea maize L.,* mC), corn-soy (*Glycine max,* CS), corn-soy-wheat (*Triticum aestivum,* CSW), corn-soy-wheat with red clover cover crop (*Trifolium pretense,* CSW1), and corn-soy-wheat with red clover + rye cover crops (*Secale cereale,* CSW2). Soil sampling took place on April 27th, 2012; July 19th, 2012; and November 1st, 2012 – hereafter referred to as spring, summer, and autumn. Corn was planted in all plots on June 11th 2012. Three 5 cm soil cores (0-10 cm deep) were collected within each plot, homogenized in the field, and then put on ice and shipped to the University of New Hampshire. In the lab, field-moist soils were immediately sieved using a 2 mm sieve. A sub-sample was taken from sieved soil and dried at 105 °C to determine gravimetric water content. Water-holding capacity was determined as the water content after soils were saturated and drained for 6 h.

Soil carbon and nitrogen parameters

Five g of field-moist soil were extracted for inorganic N with 40 ml of 0.5 M K_2SO_4. The soil slurries were shaken for 1 h before the extracts were filtered on Whatman GF/C (5) filters and filtrate frozen and stored until analysis. Soil nitrate (NO_3^-) and ammonium (NH_4^+) were measured using the methods detailed in McDaniel et al. (2014c). We also used the same extracts to measure dissolved organic C and N (DOC and DON). The extracts were run on a TOC-TN analyzer (TOC-V-CPN; Shimadzu Scientific Instruments Inc., Columbia, MD, USA). Total C

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and N were analyzed by sieving soils through 2 mm sieve, grinding and analyzing on an ECS 4010 CHNSO Elemental Analyzer (Costech Analytical Technologies, Inc., Valencia, CA).

Potential mineralization rates of C (PMC) and net N (or PMN) estimate the quantity of potentially-mineralizable SOM at an optimal temperature and soil moisture, and reflect both the activity of the microbial community and availability of SOM (Paul et al. 1999; Robertson et al. 2000). These mineralization assays provide a good indicator of the potential for a soil to provide plants with N (Stanford and Smith 1972, Robertson et al. 1999). Both PMC and PMN were measured on air-dried soils that were placed into Wheaton serum vials and brought to 50% water-holding capacity and incubated for 6 months. During this 6-month period CO_2 efflux was measured on a LI-820 infrared gas analyzer (LI-COR, Lincoln, NE). Efflux was measured using the change in headspace CO_2 concentration measured between two time points. Each soil efflux measurement began by aerating jars, capping, and injecting a time-zero sample and then a second sample between 5 hours up to 2 days later. Efflux was calculated as the difference in CO_2 concentration between the two time points divided by time. Measurements of PMC occurred more frequently at the beginning of the experiment (daily) and became less frequent toward the end (once every other week), for a total of 19 sampling events over 120 days. The PMN was assessed by extracting the inorganic N produced at the end of the incubation with the methods described above.

Soil microbial parameters

Soil microbial biomass C and N were determined using the modified chloroform fumigation and extraction method (Vance et al. 1987), but modified for extraction in individual test tubes (McDaniel et al. 2014c). Briefly, two sets of fresh, sieved soil (5 g) were placed in 50 ml test tubes, and 1 ml of chloroform was added to one set of tubes and capped. The tubes sat

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overnight (24 h) and were then uncapped and exposed to open air in a fume hood to allow chloroform to evaporate. Soils were then extracted in the tubes with 25 ml of 0.5 M K_2SO_4. The chloroform fumigated and non-fumigated extracts were run on a TOC-TN analyzer (TOC-V-CPN; Shimadzu Scientific Instruments Inc., Columbia, MD, USA). We used 0.45 (Joergensen 1996) and 0.54 (Brookes et al. 1985) for the C and N extraction efficiencies.

Soils were analyzed for 7 extracellular enzyme activities (EEAs): β-1,4-glucosidase (BG), β-D-1,4-cellobiohydrolase (CBH), β-1,4-N-acetyl glucosaminidase (NAG), acid phosphatase (PHOS), Tyrosine aminopeptidase (TAP), Leucine aminopeptidase (LAP), polyphenol oxidase (PO), and peroxidase (PER). Given the large number of samples (60) and variety of measurements made at each of 3 sampling dates, soil EEAs were conducted on frozen samples within 4 weeks of sampling. Extracellular enzyme activity assays were carried out following previously published protocols (Saiya-Cork et al. 2002, German et al. 2011), but with some modifications. Briefly, 1 g of soil was homogenenized with a blender in 80 ml of sodium acetate buffer at pH 5.6 (the average pH at the site). Soil slurries were pipetted into 96-well plates and then analyzed on a Synergy 2 plate reader (BioTek Instruments, Inc., Winooski, VT). For oxidoreductase enzymes, the supernatant from the slurry plates were pipetted into a clean plate to avoid interference with soil particles. Hydrolase assays were read at 360/40 and 460/40 fluorescence and oxidoreductases at 450 nm absorbance. For more details on the extracellular enzyme methods see McDaniel (2014c).

Community-level physiological profiles (CLPP) were conducted using the MicroResp™ system (Chapman et al. 2007, Zhou et al. 2012, McDaniel et al. 2014b). The MicroResp™ system allows for high-throughput measurement of soil catabolic responses to multiple C substrates. Each soil was loaded into 96 deep-well plates using the MicroResp™ soil dispenser,

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and then brought to 50% water-holding capacity. Thirty-one substrates were used at concentrations ranging from 7.5 to 30 mg C per g of soil H_2O, as recommended by the MicroResp™ manual (Table S1). Soil and substrates were combined in analytical triplicates and a CO_2 detection plate (agar containing creosol red) was immediately placed onto the deep-well plate with an air tight seal provided by the MicroResp™ kit. The soil and substrates were incubated in the dark for 6 h at 25 °C. The detector plate absorbencies were read at times 0 and 6 h at 540 nm on a Synergy 2 plate reader (BioTek Instruments, Inc., Winooski, VT). Absorbance data were normalized and converted to a CO_2 efflux rate (μg CO_2-C g $soil^{-1}$ h^{-1}), according to the MicroResp™ procedure (Chapman et al. 2007).

Data analyses

Cumulative potentially mineralizable C and N were calculated in SigmaPlot v12.5 (Systat Software, Inc., San Jose, CA) using the integration macro, *area below curves.* Data not conforming to ANOVA assumptions of homogeneity of variances and normality were transformed before analyses (Zuur et al. 2010). Catabolic evenness (CE), a measure of substrate diversity, was calculated using the Simpson-Yule index, $CE = 1/\Sigma p_i^2$, where p_i is the proportion of a substrate respiration response to the total response induced from all substrates (Degens et al. 2000, Magurran 2004). Metabolic quotient was calculated simply as the basal respiration over 6 h (determined in the MicroResp™ method) divided by the MBC.

Response variables were analyzed using a 2-way analysis of variance (ANOVA), with Season and Rotation as main effects. The ANOVAs were conducted in SAS 9.3 (SAS Institute, Cary, NC) using the *proc mixed* function and post-hoc *t* tests were used to determine significant differences among means using *ls means*. Block was assigned as a random effect variable within

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the model. Correlations between variables were made using *proc corr*, and Pearson's correlation coefficients are reported. Model effects were deemed significant if $\alpha < 0.05$.

All multi-variate data analyses were performed with R software (The R Foundation for Statistical Computing, Vienna, Austria). CLPP data were checked to ensure they conformed to principal components analysis assumptions. The *prcomp* function in the *vegan* package (Oksanen et al. 2016) was used for PCA of CLPP data. In order to correlate environmental variables with the multi-variate CLPP data we used the *envirfit* function.

Results

It was a relatively dry year at the KBS-LTER in 2012, which had an annual precipitation of 742 mm, compared to the historical mean of 870 mm (Hamilton et al. 2015). There was also an anomalous warm spell in mid- to late-March (Fig. S1). After harvest, the corn yield (kg ha^{-1} ± SE) in each treatment was as follows: mC = 2846 ± 152, CS = 4208 ± 575, CSW = 4107± 220, CSW1 = 4015 ± 187, CSW2 = 5219 ± 1180 (KBS-LTER 2015).

Soil C and N biogeochemistry

There were no significant Rotation or Season effects on total soil C and N, although both soil C and N tended to increase with the number of crops in rotation (Table1). Soil NO_3^--N was the only variable that showed a significant Season X Rotation interaction ($P < 0.001$). Seasonal soil NO_3^--N concentrations were highest in summer (10.33) followed by spring (2.98), and autumn (1.28 mg kg^{-1}). Soil NH_4^+-N was generally low, but summer had more than twice the concentrations of spring and autumn. Dissolved organic C (DOC) and N (DON) was very dynamic over the year. The DOC was highest in the autumn, while DON was over six times greater in the summer than the other seasons (Ps < 0.001). The mean DOC:DON in autumn was

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22.5, twice that of spring and 13 times that of the summer. There were significant crop rotation effects on NO_3^--N, DOC, and DON. During the summer the two cover crop treatments had NO_3^- concentrations 67% greater than CSW and CS treatments, and 158% greater than mC. The CSW1 treatment had 112% greater DOC concentrations than mC ($P < 0.001$), and two cover crop treatments had 107% greater DON than non-cover crop treatments and 211% more than the mC treatment. The potentially mineralizable pools of C and N showed significant main effects of both Season and Rotation ($P < 0.03$), but no interactions. Generally, both PMC and PMN increased with increasing number of crops in rotation (Fig. 1), and PMC was highest during the autumn, while PMN was highest during the summer.

Soil microbial dynamics

The mean soil microbial biomass C (MBC) was 332 µg C g $soil^{-1}$ across all seasons and crop rotations, but both Season ($P < 0.001$) and Rotation ($P = 0.008$) had significant effects on MBC (Fig. 2). Soils collected in autumn had more than twice the MBC than those collected in spring and summer. Microbial biomass C was increased by increasing crop diversity across all seasons (Fig. 2). Increasing the number of crops in rotation increased MBC on average by 28, 113, 112, and 72% in the CS, CSW, CSW1, CSW2 rotations, respectively, compared to mC (across all seasons). Microbial biomass N (MBN) also showed both Season ($P < 0.001$) and Rotation ($P = 0.005$) effects, but no interaction. These effects were strongest in the spring and summer (Fig. 2), but also showed an increase with increasing number of crops. Increasing the number of crops in rotation increased MBN on average by 18, 58, 54, and 50% in the CS, CSW, CSW1, and CSW2 compared to mC (across all seasons). Microbial biomass C:N showed a significant interaction ($P = 0.013$), with more diverse cropping systems having greater MBC:MBN in summer and autumn, but not spring. The metabolic quotient (qCO_2), is often used

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as a proxy for microbial respiration efficiency (Anderson & Domsch 1989, 2010; Wardle & Ghani 1995). Season ($P < 0.001$) and Rotation ($P = 0.006$) both influenced qCO_2, with increased crop diversity decreasing the qCO_2 by 16, 40, and 28 % in CSW, CSW1, and CSW2 compared to mC. However, the CS rotation increased qCO_2 by +15 % qCO_2 compared to mC (Fig. 2).

Soil extracellular enzymes were very dynamic over the three seasons, as evidenced by radar plots in which the area and shape for each treatment change quite drastically over the growing season (Fig. 3). A MANOVA with all eight EEAs showed significant Season ($P < 0.001$) and Rotation ($P < 0.001$) main effects, but no interaction. Most individual enzymes showed only significant Rotation effects except for PO, which also showed a significant Season effect with autumn greater than the other seasons (Table 2). The soil enzyme responsible for cleaving a glucosamine from chitin (NAG) and the lignin-reducing enzyme that uses peroxide (PER) were the only enzymes that showed a significant Season X Rotation interaction ($P < 0.001$). Spring had the greatest activities of LAP, 175% greater than the average of the other seasons (Fig. 3, Table 2). In summer, we see a shift to the highest PHOS activity – 25% greater than spring and 99% greater than autumn. Season had no effect on BG or CBH but showed significant main effects of rotation, with the CSW1 treatment having an average of 42 and 50 % higher BG and CBH activity than CS and mC soils, respectively. The majority of the hydrolase enzymes were higher in the cover crop treatments compared to that of the non-cover crop treatments, especially mC (Table 2, Fig. 3). The two oxidoreductase enzymes (PO and PER) decreased with crop diversity. There were no significant main effects on the enzyme ratio used to assess C-versus-N demand (BG to NAG+LAP).

The community-level physiological profile (CLPP), a catabolic profile of the soil microbial communities, showed both significant Season ($P < 0.001$) and Rotation ($P = 0.003$)

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main effects (Figs. 4, S2; Table 3). A principal components analysis of the CLPP data showed that the summer soils corresponded with highest carboxylic acid utilization (Fig. 4), as Season was the strongest discriminating factor along principal component 1 (PC1, Table 3). However, when rotating and examining PC2 and PC3, there was a strong treatment gradient from the bottom right to upper-left quadrants of the graph (Fig. 4, right panel). The lower-diversity treatments corresponded with greater use of carboxylic acid substrates. Across seasons, summer exhibited the lowest catabolic evenness (12.9), but there was no crop rotation effect on catabolic evenness using all substrates (Table 4).

Due to the overwhelming influence of carboxylic acids in the PCA variation, and their possible role in abiotic reactions leading to CO_2 emissions (Maire et al. 2012, Pietravalle and Aspray 2013), we split the 31 substrates into two sets to analyze separately: 1) Non-carboxylic acid substrates – a total of 21 substrates, and 2) carboxylic acids by themselves – 10 substrates. Season, again, was a dominant significant effect on the MANOVAs in both groups of substrates (P values < 0.001, Fig. S3). The non-carboxylic acid CLPP showed a significant treatment effect with PC1 and PC2, and clear separation between low and high diversity cropping systems (P = 0.012, Fig. S3). The monoculture corn, and lower diversity treatments, associated with more complex substrates. In the carboxylic acid CLPP there was also a significant treatment effect, but with PC2 and PC3, and clear separation between low and high diversity cropping systems along PC3 (P = 0.035, Fig. S3). Interestingly, the lower diversity (especially monoculture corn), were more associated with simple carboxylic acids (Cit, Mlo, and Mli) on the positive half of PC3. When carboxylic acids were split from the substrates, crop rotation had a significant effect on catabolic evenness – decreasing the catabolic evenness both within non-carboxylic acids and carboxylic acids by as much as 4 and 13% respectively (Table 4).

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We used the soil microbial responses of EEA and the CLPP because we assumed they would be complimentary. Indeed, this was the case. Measuring NAG enzyme and adding the Nag amine to the soils showed a somewhat tight relationship, but this relationship was not constant over the seasons. More specifically, the NAG enzyme was quite higher in the autumn compared to summer and spring, and showed a steeper linear relationship with the CO_2 response after adding the Nag amine to soils (Fig. S4). Additionally, when the CLPP substrates were grouped by guild and correlated with EEA there were strong relationships (Fig. S5). For example, total amino acid catabolic response positively correlated well with LAP+TAP enzymes ($r^2 = 0.35$, $P < 0.001$) meaning that high activity of these enzymes in soils corresponded with high relative use of these substrates when added to soils, compared to other substrates added to the soil. This suggests that the LAP and TAP enzymes strongly reflect demand for N-bearing amino acids in soils. However, the catabolic response of the 'Complex' guild was negatively correlated with PO ($r^2 = 0.29$, $P < 0.001$).

Relationships between soil biogeochemical factors, microbial functioning and yield

Over the three seasons many soil biogeochemical factors correlated with microbial catabolic potential, both with individual C substrate guilds and catabolic evenness (Table 5). Abiotic factors such as pH and sand content correlated with the specific use of particular substrates. Soil pH positively correlated with N-containing and complex substrates, but strongly negative with carboxylic acids. Sand content negatively correlated with amino acids and carbohydrates, but positively with carboxylic acids. The microbial response to amino acids and amines correlated best with NO_3^--N and many of the specific enzyme activities, showing negative relationships which indicated a linkage between demand for N and usage of N-bearing substrates (i.e. when supply is high, demand and usage of N substrates is low). Soil NO_3^--N was

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296 also significantly negatively correlated with catabolic evenness. We used the soil microbial
297 responses of EEA and the CLPP because we assumed they would be complimentary. For
298 example, adding N-acetyl glucosamine in the CLPP should be related to ß-1,4-N-acetyl
299 glucosamindase (NAG) enzyme activity. Indeed, this was the case. Measuring NAG enzyme
300 and adding the Nag amine to the soils showed a somewhat tight relationship (Fig. S4).
301 Additionally, when the CLPP substrates were grouped by guild and correlated with EEA there
302 were strong relationships (Fig. S5). For example, total amino acid catabolic response positively
303 correlated well with LAP+TAP enzymes ($r^2 = 0.35$, $P < 0.001$) meaning that high activity of
304 these enzymes in soils corresponded with high relative use of these substrates when added to
305 soils, compared to other substrates added to the soil. This suggests that the LAP and TAP
306 enzymes strongly reflect demand for N-bearing amino acids in soils. However, the catabolic
307 response of the 'Complex' guild was negatively correlated with PO ($r^2 = 0.29$, $P < 0.001$). Soil
308 PMN was better correlated with crop yields ($r^2 = 0.61$) than NO_3^- in early spring (Fig. S6).

309 **Discussion**

310 Increasing biodiversity in this long-term crop rotation experiment has altered the soil
311 microbial dynamics across an entire growing season. This is despite the fact that the soils in our
312 study were all under the same crop (corn) for the season, indicating that observed differences
313 among soils reflect long-term rotation effects rather than the current crop. Microbial biomass C,
314 N, potential mineralization, and catabolic potential were all altered by crop rotations, although
315 the rotation effect for some of these indicators of microbial functioning also depends upon the
316 season. Soil microbial biomass and activity are now widely recognized as pillars of soil health
317 (Doran and Zeiss 2000). Our results clearly indicate that practices like diversifying
318 agroecosystems (through crop rotations) enhances this aspect of soil health, and this is also likely

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linked to changes in SOM dynamics (Tiemann et al. 2015) as well as the observed differences in yield among crop rotations (Fig. S6).

Seasonal dynamics and N limitation

Season strongly influenced the measured pools of labile C and N (Table 1), as well as the microbial biomass size and functioning within this agroecosystem (Figs. 1-4). We showed that the greatest microbial biomass and activity occurred in autumn, but that potentially mineralizable N peaked in summer. In perennial and annual cropping systems in Iowa, potentially mineralizable N declined from spring to late summer (Hargreaves and Hofmockel 2013); in addition, extracellular enzyme activities peaked in July but there was little effect of the cropping system. Season was shown to affect microbial biomass and potentially mineralizable C and N pools in a wheat-sorghum-soybean rotation in south-central Texas (Franzluebbers et al. 1994, 1995, Franzluebbers 2002), but timing for peak values differed depending on the study and cropping systems, likely reflecting different climates and soil types. The frequently observed late-summer spike in microbial biomass and activity may be related to higher temperatures during this time period; however, even within agroecosystems, the timing for maximal microbial biomass varies substantially, although few microbial maxima are reported in winter (Wardle, 1992). Our findings highlight the dynamic nature of soil microbial biomass and activity, especially with regards to the supply and demand of N (e.g. microbial C:N, substrate utilization, and extracellular enzyme activities), which is likely a limiting nutrient in these agroecosystems that are receiving no exogenous N inputs.

The summer warrants discussion because the sample was collected after a prolonged period of hot and dry days, but right after a large rainfall event. This rainfall event (> 18 mm d^{-1},

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341 Fig. S1) increased the volumetric water content in the 0-10 cm of a nearby soil by over 54%
342 from the lowest value of the year (0.1, data shared from Hamilton et al. 2015), and we know
343 from previous research that drying-wetting cycles are important soil biogeochemical drivers
344 (Borken and Matzner, 2009) and can alter microbial structure and functioning (Fierer et al. 2003,
345 Schimel et al. 2007, Tiemann and Billings 2011, McDaniel et al. 2014b). Indeed, the summer
346 showed several signs of the soil microbial community being impacted by a rapid dry-wet event:
347 lower overall microbial biomass C, extremely high NO_3^--N concentrations (Table 2), high
348 potential N mineralization (Fig. 1), high extracellular enzyme activities per unit of microbial
349 biomass (Fig. S2, presumably a result of lysed intracellular enzymes, Burns et al. 2013), and the
350 particularly strong response of the summer soils to carboxylic acids (a highly-labile class of
351 compounds used by fast-growing, opportunistic microbes, that would be found after a
352 disturbance such as a dry-wet event, Figs. 4 and S3). Dry-wet cycles may drive microbial C and
353 N to be reallocated to stress-response compounds instead of growth or reproduction, making C
354 and N more vulnerable to loss from soils (Schimel et al. 2007). We captured one of these dry-
355 wet events during one of the driest summers in the Kellogg Biological Station LTER's history
356 and we show high soil inorganic N concentrations and altered microbial dynamics relative to the
357 other dates. Climate change may increase the frequency and magnitude of these rapid dry-wet
358 cycles (Groffman et al. 2001, McDaniel et al. 2014d), and thus may have long-term impacts on
359 soil microbial functioning and biogeochemistry.

360 In the autumn we found several lines of evidence that indicate soil microbes are N, rather
361 than C, limited. These lines of evidence include: lowest soil inorganic N concentrations, low
362 potentially mineralizable N, high microbial biomass C:N and DOC:DON ratios, and high TAP
363 and NAG enzymes relative to other enzymes (although interestingly not LAP), and finally strong

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364 respiration response to the addition of amines and amino acids (Fig. 4). The unusually high
365 microbial biomass C:N in autumn was very surprising (mean of 18.2 versus 7.5 and 9.9 in spring
366 and summer), but microbial biomass C:N has been known to reach as high as 30 in laboratory
367 conditions (Schimel et al. 1989). Additionally, the few days before and after the collection of the
368 autumn sample were unusually cold (Fig. S1), and cold temperatures and freezing can cause
369 accumulation of carbohydrates in fungi (Tibbits et al. 2002), which could widen microbial C:N
370 ratio. Regardless of environmental conditions widening microbial biomass C:N, it is likely that
371 N limitation is a major factor considering this experiment receives no fertilizer N for 12 years.

372 *Crop biodiversity and soil microbial functioning*

373 Both soil microbial biomass and functioning were strongly affected by increased crop
374 diversity through rotation. This rotation effect on soil microbial biomass and functioning were
375 largely independent of the season, as indicated by the limited number of observed Season X Crop
376 Rotation interactions. The exception to this was microbial biomass C:N and two extracellular
377 enzyme activities (NAG and PER), which are likely indicative of the enhanced ability of soil
378 microbes under diverse rotations to process, provision, and retain N. These findings are
379 consistent with plant biodiversity studies which find increased aboveground diversity enhances
380 soil microbial biomass and functioning in natural (Stephan et al. 2000, Zak et al. 2003, Lange
381 2015) and agricultural ecosystems (Lupwayi et al. 1998; Xuan et al. 2012; Tiemann et al. 2015).

382 In our study, the strong response of soil microbial functioning to crop diversity is
383 particularly interesting given that all treatments were under the same crop (corn) over the entire
384 growing season. Some studies have shown that the current plant species identity often trumps
385 biodiversity legacy in controlling belowground microbial structure and functioning (Stephan et

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al. 2000, Wardle et al. 2003, Bartelt-Ryser et al. 2005). Conversely, several studies have pointed to weak or no influence of current plant species on soil microbial structure and functioning (Costa et al. 2006, Kielak et al. 2008). The question of whether plant species identity versus spatial and temporal diversity has a stronger control on soil biota remains a critical question in terrestrial ecology. Here we show strong evidence for a biodiversity "carry-over" effect (Bartelt-Ryser et al. 2005), where the legacy of crop rotation affects soil microbial biomass and functioning of the current year, even though the soils are all planted under the same crop species.

We hypothesized that increasing biodiversity in agroecosystems through rotation would result in soil microbial communities that are more diverse, and thus would more evenly use added C substrates (i.e. increase catabolic evenness, or decrease the variation in use among substrates). This hypothesis stems from the "plant diversity hypothesis", which posits that soil community and functional biodiversity is linked to plant biodiversity mostly through the diversity of plant inputs to SOM (Lodge, 1997; Hooper et al., 2000; Waldrop et al., 2006; Korboulewsky et al., 2016). However, in our study, we found no evidence that crop rotational diversity increased overall soil catabolic evenness. There is some evidence that crop rotations can alter soil bacterial catabolic diversity, or the ability to use different C substrates (Lupwayi et al. 1998, Larkin 2003, Govaerts et al. 2007), however all of these studies used Biolog which has several limitations (Preston-Mafham et al. 2002). The MicroResp™ system's main benefit is that it adds C substrates directly to the soil instead of tranferring an inocullum from a soil slurry. The discrepancy between our study and these studies other studies may be due to methodological differences between Biolog and MicroResp™. Our lack of evidence for an aboveground-belowground link to catabolic potential aligns with findings from other studies that have found

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functional diversity measures of soil microbes are not related to plant diversity (Bartelt-Ryser et al. 2005, Jiang et al. 2012), nor plant species in general (McIntosh et al. 2013).

However, it is important to note that in our study when a subset of the C substrates were analyzed (all non-carboxylic), we found that increased crop diversity actually decreased catabolic evenness. This is unusual considering soils from this same study, but collected a year prior, showed increases of soil biodiversity (Shannon-Weiner index or H') with increased crop diversity when measuring phospholipid fatty acids (Tiemann et al. 2015); and diversity has been found to be strongly, positively related to evenness in plants and animals (Stirling & Wilsey 2001). Our finding of no change in (or lower) catabolic evenness with increasing crop biodiversity is also contradictory to the findings of Degens et al. (2000), whom showed that management practices that decrease soil C are associated with low catabolic evenness since we show a general trend of increasing soil C.

Perhaps the incongruity between the positive effect of crop rotation on H', but slightly negative effect on catabolic evenness, could lie in the difference between who is targeted by the catabolic response profile. Most bacteria are thought to be generalists with regard to the use of C substrates (Mou et al. 2008; Goldfarb et al. 2011), whereas fungi may show more specialization (Hanson et al. 2008; Treseder et al. 2015). Not to mention the catabolic response method used here is probably more favorable to detecting response of bacteria rather than fungi, because fungi are more sensitive to the disturbance of preparing the soils (Frey et al., 1999) and bacteria have faster growth and reproduction (i.e. respiration was measured for only 6 h). Thus a high catabolic evenness may not be a good indicator of soil biodiversity in soils with high relative bacterial biomass and activity, which is typical of many agroecosystem soils (Strickland & Rousk 2010). Instead, a low catabolic evenness may actually be better suited to detect C-use

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specialization in bacterial-dominated microbial communities. In support of this idea, we have evidence from these same soil samples that crop diversity significantly decreased H' for bacterial 16S rRNA by as much as 5 % compared to monoculture corn (Peralta et al. *in preparation*). These inconsistencies, especially between methods of measuring soil microbial diversity, are highlighted in a recent quantitative review (Venter et al. 2016) but overall crop rotations tend to increase soil biodiversity by 3 % and richness by 15 %. Regardless of belowground diversity trends, crop rotations did create functionally distinct microbial communities in our study (Fig. 4). We still do not have a complete understanding of how crop rotations alter soil microbial diversity, nor (arguably more importantly) how these changes in belowground diversity might provide beneficial soil ecosystem services like increasing soil C or mineralizing more N to increase crop yields.

One trend that emerges across the suite of 31 C substrates is that crop rotations altered the preference for substrates of differing quality (i.e. complex versus simple C substrates). The soils from monoculture corn corresponded to greater use of simple C substrates (especially carboxylic acids), and showed less response to the suite of N-containing and complex substrates. This finding corroborates a previous study we conducted using whole-plant residues, in which we showed diverse crop rotations resulted in greater decomposition of low quality crop residues (e.g. corn and wheat, McDaniel et al. 2014c). Further, when looking only within the relatively labile carboxylic acid substrates, microbial communities in the less diverse crop rotations (mC, and CS to a lesser extent) responded to more labile, low-molecular weight carboxylic acids (e.g. citric, malonic, and malic acid), while soil microbes from more biodiverse crop rotations responded more to complex, higher-molecular weight carboxylic acids (e.g. caffeic, tartaric, and vanillic acids - Fig. S4). The strong effects of crop biodiversity on both overall usage of and the

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454 types of catabolized carboxylic acids is not surprising due to the small, yet dynamic pool of these
455 compounds in soil (Strobel 2001). Since soil microbial function (as measured by CLPPs) is an
456 aggregate measure of both the community composition and available resources, it is impossible
457 to tease out which (or both) have changed due to increased crop biodiversity. However, our
458 overall findings indicate that increased aboveground biodiversity through crop rotations and
459 cover crops appears to facilitate soil microbial communities' use of complex C substrates relative
460 to simple ones.

461 *Conclusions*

462 As the growing population is increasingly reliant on soils for food, fiber, and fuel we will
463 either need to consume less, put more land into production, or better use the land we already
464 have in production. Putting more land in production will likely result in declines in local and
465 global biodiversity. Thus, it is critical to incorporate biodiversity through any means possible
466 into the existing managed ecosystems – even including biodiversity through time as with crop
467 rotations. Here we show that both microbial biomass and function are strongly influenced by
468 cropping diversity. In fact, the influence of crop rotations on soil microbes and functioning lasts
469 over an entire growing season and even when all soils are under the same crop. Crop rotations
470 clearly enhance soil microbial biomass and activity, which are now considered a pillar of soil
471 health. Furthermore, this rotation effect on soils also appears to facilitate microbes in supplying
472 more N to crops (Fig. S6). Overall, our study highlights the importance of incorporating
473 biodiversity into agroecosystems by including more crops in rotation, especially cover crops, to
474 enhance beneficial soil processes controlled by soil microbes.

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References

Adviento-Borbe, M. A. A., Kaye, J. P., Bruns, M. A., McDaniel, M. D., McCoy, M., and Harkcom, S. 2010. Soil greenhouse gas and ammonia emissions in long-term maize-based cropping systems. Soil Sci. Soc. Am. J. 74:1623–1634.

Anderson, T.H., and Domsch, K.H. 1985. Maintenance carbon requirements of actively-metabolizing microbial populations under *in situ* conditions. Soil Biol. & Biochem. 17: 197-203.

Anderson, T.H., and Domsch, K.H. 2010. Soil microbial biomass: The eco-physiological approach. Soil Biol. & Biochem. 42: 2039-2043.

Bartelt-Ryser, J., Joshi, J., Schmid, B., Brandl, H., and Balser, T. 2005. Soil feedbacks of plant diversity on soil microbial communities and subsequent plant growth. Perspect. Plant Ecol. 7:27–49.

Behnke, G., David, M. B., and Voigt, T. 2012. Greenhouse gas emissions, nitrate leaching, and biomass yields from production of *Miscanthus* × *giganteus* in Illinois, USA. BioEnerg. Res. 5:801–813.

Bérard, A., Capowiez, L., Mombo, S., Schreck, E., Dumat, C., Deola, F., and Capowiez, Y. 2015. Soil microbial respiration and PICT responses to an industrial and historic lead pollution: a field study. Environ. Sci. & Poll. Res. 1-11.

SOIL Discuss., doi:10.5194/soil-2016-39, 2016
Manuscript under review for journal SOIL
Published: 4 July 2016

504 Borken, W., and Matzner, E. 2009. Reappraisal of drying and wetting effects on C and N
505 mineralization and fluxes in soils. Global Change Biol. 15:808–824.

506 Brookes, P. C., Landman, A., Pruden, G., and Jenkinson, D. S. 1985. Chloroform fumigation and
507 the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass
508 nitrogen in soil. Soil Biol. and Biochem. 17:837–842.

509 Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E, Wallenstein,
510 M.D., Weintraub, M.N., and Zoppini, A. 2013. Soil enzymes in a changing environment:
511 Current knowledge and future directions. Soil Biol. & Biochem. 58:216-234.

512 Cambardella, C. A., Moorman, T. B., Jaynes, D. B., Hatfield, J. L., Parkin, T. B., Simpkins, W.
513 W., and Karlen, D. L. 1999. Water Quality in Walnut Creek Watershed: Nitrate-Nitrogen in
514 Soils, Subsurface Drainage Water, and Shallow Groundwater. J. Environ. Qual. 28:25–34.

515 Chapman, S., Campbell, C., and Artz, R. 2007. Assessing CLPPs using MicroResp^{TM}. J. Soils
516 Sediments 7:406–410.

517 Carpenter-Boggs, L., Picul Jr., J.L., Vigil, M.F., and Riedell, W.E. 2000. Soil nitrogen
518 mineralization influenced by crop rotation and nitrogen fertilization. Soil Sci. Soc. Am. J.
519 64:2038-2045.

520 Costa, R., Götz, M., Mrotzek, N., Lottmann, J., Berg, G., and Smalla, K. 2006. Effects of site
521 and plant species on rhizosphere community structure as revealed by molecular analysis of
522 microbial guilds. FEMS Microbiol. Ecol. 56:236–249.

523 Degens, B. P., Schipper, L. A., Sparling, G. P., and Vojvodic-Vukovic, M. 2000. Decreases in
524 organic C reserves in soils can reduce the catabolic diversity of soil microbial communities.
525 Soil Biol. & Biochem. 32:189–196.

526 Doran, J.W., and Zeiss, M.R. 2000. Soil health and sustainability: managing the biotic omponent
527 of soil quality. Appl. Soil Ecol. 15:3-11.

528 Eisenhauer, N., Beßler, H., Engels, C., Gleixner, G., Habekost, M., Milcu, A., Partsch, S.,
529 Sabais, A. C. W., Scherber, C., Steinbeiss, S., Weigelt, A., Weisser, W. W., and Scheu, S.
530 2010. Plant diversity effects on soil microorganisms support the singular hypothesis.
531 Ecology 91:485–496.

532 Fierer, N., Schimel, J. P., and Holden, P. A. 2003. Influence of drying-rewetting frequency on
533 soil bacterial community structure. Microb. Ecology 45:63–71.

534 Franzluebbers, A. J. 2002. Soil organic matter stratification ratio as an indicator of soil quality.
535 Soil Till. Res. 66:95–106.

SOIL Discuss., doi:10.5194/soil-2016-39, 2016
Manuscript under review for journal SOIL
Published: 4 July 2016

Franzluebbers, A. J., Hons, F. M., and Zuberer, D. A. 1994. Seasonal changes in soil microbial biomass and mineralizable c and n in wheat management systems. Soil Biol. & Biochem. 26:1469–1475.

Franzluebbers, A. J., Hons, F. M., and Zuberer, D. A. 1995. Soil organic carbon, microbial niomass, and mineralizable carbon and nitrogen in sorghum. Soil Sci. Soc. Am. J. 59:460–466.

German, D. P., Weintraub, M. N., Grandy, A. S., Lauber, C. L., Rinkes, Z. L., and Allison, S. D. 2011. Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biol. & Biochem. 43:1387–1397.

Goldfarb, K. C., Karaoz, U., Hanson, C. A., Santee, C. A., Bradford, M. A., Treseder, K. K., Wallenstein, M. D., and Brodie, E. L. 2011. Differential growth responses of soil bacterial taxa to carbon substrates of varying chemical recalcitrance. Front. Microbiol. 2.

Govaerts, B., Mezzalama, M., Unno, Y., Sayre, K. D., Luna-Guido, M., Vanherck, K., Dendooven, L., and Deckers, J. 2007. Influence of tillage, residue management, and crop rotation on soil microbial biomass and catabolic diversity. Appl. Soil Ecol. 37:18–30.

Grandy, A.S., and Robertson, G.P. 2007. Land-use intensity effects on soil organic carbon accumulation rates and mechanisms. Ecosystems 10:58-73.

Groffman, P., Driscoll, C., Fahey, T., Hardy, J., Fitzhugh, R., and Tierney, G. 2001. Effects of mild winter freezing on soil nitrogen and carbon dynamics in a northern hardwood forest. Biogeochem. 56:191–213.

Hanson, C., Allison, S. D., Bradford, M. A., Wallenstein, M. D., and Treseder, K. K. 2008. Fungal taxa target different carbon sources in forest soil. Ecosystems 11:1157–1167.

Hargreaves, S.K., and Hofmockel, K.S. 2013. Physiological shifts in the microbial community drive changes in enzyme activity in a perennial agroecosystem. Biogeochem. 117:67–79.

Hamilton, S.K., Hussain, M.Z., Bhardwaj, A.K., Basso, B., and Robertson, G.P. 2015. Comparative water use by maize, errennial crops, restored prairie, and poplar trees in the US Midwest. Environ. Res. Lett. 10:064015.

Hooper, D. U., Bignell, D. E., Brown, V. K., Brussard, L., Dangerfield, M. J., Wall, D. H., Wardle, D. A., Coleman, D. C., Giller, K. E. N. E., Lavelle, P., Van Der Putten, W. I. M. H., De Ruiter, P. C., Rusek, J., Silver, W. L., Tiedje, J. M., and Wolters, V. 2000. Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: Patterns, mechanisms, and feedbacks. BioSci. 50:1049–1061.

Jiang, Y., Chen, C., Xu, Z., and Liu, Y. 2012. Effects of single and mixed species forest ecosystems on diversity and function of soil microbial community in subtropical China. J. Soils Sediments 12:228–240.

571 Joergensen, R. G. 1996. The fumigation-extraction method to estimate soil microbial biomass:
572 Calibration of the kEC value. Soil Biol. & Biochem. 28:25–31.

573 Kallenbach, C.M., Grandy, A.S., Frey, S.D., and Diefendorf, A.F. 2015. Microbial physiology
574 and necromass regulate agricultural soil carbon accumulation. Soil Biol. & Biochem.
575 91:279-290.

576 [KBS] Kellogg Biological Station Long-term Ecological Research. http://lter.kbs.msu.edu/.
577 Verified May 2015. http://lter.kbs.msu.edu/datatables/75

578 Kielak, A., Pijl, A. S., Van Veen, J. A., and Kowalchuk, G. A. 2008. Differences in vegetation
579 composition and plant species identity lead to only minor changes in soil-borne microbial
580 communities in a former arable field. FEMS Microb. Ecol. 63:372–382.

581 Korboulewsky, N., Perez, G., and Chauvat, M. 2016. How tree diversity affects soil fauna
582 diversity: A review. Soil Biol. & Biochem. 94:94-106.

583 Krupinsky, J. M., Bailey, K. L., McMullen, M. P., Gossen, B. D., and Turkington, T. K. 2002.
584 Managing plant disease risk in diversified cropping systems. Agron. J. 94:198-209.

585 Lange, M., Eisenhauer, N., Sierra, C.A., Bessler, H., Engels, C., Griffiths, R.I., Mellado-
586 Vázquez, P.G., Malik, A.A., Roy, J., Scheu, S., and Steinbeiss, S., 2015. Plant diversity
587 increases soil microbial activity and soil carbon storage. *Nature Communications*, *6*.

588 Larkin, R. P. 2003. Characterization of soil microbial communities under different potato
589 cropping systems by microbial population dynamics, substrate utilization, and fatty acid
590 profiles. Soil Biol. & Biochem 35:1451–1466.

591 Lodge, D. J. 1997. Factors related to diversity of decomposer fungi in tropical forests. Biodivers.
592 Conserv. 6:681–688.

593 Lupwayi, N. Z., Rice, W. A., and Clayton, G. W. 1998. Soil microbial diversity and community
594 structure under wheat as influenced by tillage and crop rotation. Soil Biol. & Biochem.
595 30:1733–1741.

596 Magurran, A. E. Measuring Biological Diversity. Blackwell Publishing. Malden, MA. 2004

597 Maire, V., Alvarez, G., Colombet, J.,Comby, A., Despinasse, R., Dubreucq, E., Joly, M.,
598 Lehours, A.-C., Perrier, V., and Shahzad, T. 2012. An unknown respiration pathway
599 substantially contributes to soil CO_2 emissions. Biogeosci. Discuss. 9:8663–8691.

600 McDaniel, M. D., Grandy, A. S., Tiemann, L. K., and Weintraub, M. N. 2014a. Crop rotation
601 complexity regulates the decomposition of high and low quality residues. Soil Biol. &
602 Biochem. 78:243–254.

603 McDaniel, M. D., Kaye, J. P., Kaye, M. W., and Bruns, M. A. 2014b. Climate change
604 interactions affect soil carbon dioxide efflux and microbial functioning in a post-harvest
605 forest. Oecologia 174:1437–1448.

606 McDaniel, M. D., Tiemann, L. K., and Grandy, A. S. 2014c. Does agricultural crop diversity
607 enhance soil microbial biomass and organic matter dynamics? a meta-analysis. Ecol. Appl.
608 24:560–570.

609 McDaniel, M. D., Wagner, R. J., Rollinson, C. R., Kimball, B. A., Kaye, M. W., and Kaye, J. P.
610 2014d. Microclimate and ecological threshold responses in a warming and wetting
611 experiment following whole tree harvest. Theor. Appl. Climatol. 116:287–299.

612 McIntosh, A. C. S., Macdonald, S. E., and Quideau, S. A. 2013. Linkages between the forest
613 floor microbial community and resource heterogeneity within mature lodgepole pine
614 forests. Soil Biol. & Biochem. 63:61–72.

615 Morillas, L., Duran, J., Rodriguez, A., Roales, J., Gallardo, A., Lovett, G.M., and Groffman,
616 P.M. 2015. Nitrogen supply modulates the effect of changes in drying-rewetting frequency
617 on soil C and N cycling and greenhouse gas exchange. Global Change Biol. 21:3854-3863

618 Mueller, K. E., Hobbie, S. E., Tilman, D., and Reich, P. B. 2013. Effects of plant diversity, N
619 fertilization, and elevated carbon dioxide on grassland soil N cycling in a long-term
620 experiment. Global Change Biol. 19:1249–1261.

621 Oksanen, J, Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'hara, R.B., Simpson, G.
622 L., Solymos, P. M., Stevens, H., and Wagner, H. 2016. Vegan: Community Ecology
623 Package. R package version 2.3-3. https://CRAN.R-project.org/package=vegan

624 Pietravalle, S., and Aspray, T. J. 2013. CO_2 and O_2 respiration kinetics in hydrocarbon
625 contaminated soils amended with organic carbon sources used to determine catabolic
626 diversity. Environ. Poll. 176:42–47.

627 Plante, A. F., Fernández, J. M., Haddix, M. L., Steinweg, J. M., and Conant, R. T. 2011.
628 Biological, chemical and thermal indices of soil organic matter stability in four grassland
629 soils. Soil Biol. & Biochem. 43:1051–1058.

630 Preston-Mafham, J., Boddy, L., and Randerson, P.F. 2002. Analysis of microbial community
631 functional diversity using sole-carbon-source utilisation profiles – a critique. FEMS
632 Microbiol. Ecol. 42:1-14.

633 Riedell, W. E., Pikul, J. L., Jaradat, A. A., and Schumacher, T. E. 2009. Crop rotation and
634 nitrogen input effects on soil fertility, maize mineral nutrition, yield, and seed composition.
635 Agron. J. 101:870-879.

636 Robertson, G. P., Coleman, D. C., Bledsoe, C. S., and Sollins, P. 1999. Standard Soil Methods
637 for Long-Term Ecological Research. Oxford University Press, New York.

638 Ryszkowski, L., Szajdak, L., and Karg, J. 1998. Effects of continuous cropping of rye on soil
639 biota and biochemistry. CRC CR Rev. Plant Sci.17:225–244.

640 Saiya-Cork, K. R., Sinsabaugh, R. L., and Zak, D. R. 2002. The effects of long term nitrogen
641 deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. Soil Biol. &
642 Biochem. 34:1309–1315.

643 Schimel, J., Balser, T. C., and Wallenstein, M. D. 2007. Microbial stress-response physiology
644 and its implications for ecosystem function. Ecology 88:1386–94.

645 Smith, R. G., Gross, K. L., and Robertson, G. P. 2008. Effects of crop diversity on
646 agroecosystem function: Crop yield response. Ecosystems 11:355–366.

647 Stanford, G., and Smith, S. J. 1972. Nitrogen mineralization potentials of soils. Soil Sci. Soc.
648 Am. J. 36:465–472.

649 Stephan, A., Meyer, A. H., and Schmid, B. 2000. Plant diversity affects culturable soil bacteria
650 in experimental grassland communities. J. Ecology 88:988–998.

651 Stirling, G., and Wilsey, B. 2001. Empirical relationships between species richness, evenness,
652 and proportional diversity. Am. Nat. 158:286-299.

653 Strobel, B. W. 2001. Influence of vegetation on low-molecular-weight carboxylic acids in soil
654 solution—a review. Geoderma 99:169–198.

655 Strickland, M.S., and Rousk, J. 2010. Considering the fungal:bacterial dominance in soils –
656 Methods controls, and ecosystem implications. Soil Biol. & Biochem. 42:1385-1395.

657 Thoms, C., Gattinger, A., Jacob, M., Thomas, F. M., and Gleixner, G. 2010. Direct and indirect
658 effects of tree diversity drive soil microbial diversity in temperate deciduous forest. Soil
659 Biol. & Biochem. 42:1558–1565.

660 Tiemann, L. K., and S. A. Billings. 2011. Changes in variability of soil moisture alter microbial
661 community C and N resource use. Soil Biol. & Biochem. 43:1837–1847.

662 Tiemann, L. K., Grandy, A. S., Atkinson, E. E., Marin-Spiotta, E., and McDaniel, M. D. 2015.
663 Crop rotational diversity enhances belowground communities and functions in an
664 agroecosystem. Ecol. Lett. 18:761-771.

665 Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M., and Siemann, E. 1997. The influence of
666 functional diversity and composition on ecosystem processes . Science 277 :1300–1302.

667 Treseder, K. K., Marusenko, Y., Romero-Olivares, A.L., and Maltz, M.R. 2016. Experimental
668 warming alters potential function of the fungal community in boreal forest. Global Change
669 Biol. - doi:10.1111/gcb.13238

SOIL Discuss., doi:10.5194/soil-2016-39, 2016
Manuscript under review for journal SOIL
Published: 4 July 2016

Trivedi, P., Rochester, I. J., Trivedi, C., Van Nostrand, J. D., Zhou, J., Karunaratne, S., Anderson, I. C., and Singh, B. K. 2015. Soil aggregate size mediates the impacts of cropping regimes on soil carbon and microbial communities. Soil Biol. & Biochem. 91:169-181

Vance, E. D., Brookes, P. C., and Jenkinson, D. S. 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. & Biochem. 19:703–707.

Venter, Z.S., Jacobs, K., Hawkins, H.-J. 2016. The impact of crop rotation on soil microbial diversity: A meta-analysis. Pedobiologia xxx-xxx.

Waldrop, M. P., Zak, D. R., Blackwood, C. B., Curtis, C. D., and Tilman, D. 2006. Resource availability controls fungal diversity across a plant diversity gradient. Ecol. Lett. 9:1127–1135.

Wardle, D.A. 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biol. Rev. 67:321–358.

Wardle, D.A. and Ghani, A. 1995. A critique of the microbial metabolic quotient (qCO_2) as a bioindicator of disturbance and ecosystem development. Soil Biol. & Biochem. 12: 1601-1610.

Wardle, D. A., Yeates, G. W., Williamson, W., and Bonner, K. I. 2003. The response of a three trophic level soil food web to the identity and diversity of plant species and functional groups. Oikos 102:45–56.

Wu, T., Chellemi, D., Graham, J., Martin, K., and Rosskopf, E. 2008. Comparison of soil bacterial communities under diverse agricultural land management and crop production practices. Microb. Ecol. 55:293–310.

Xuan, D., Guong, V., Rosling, A., Alström, S., Chai, B., and Högberg, N. 2012. Different crop rotation systems as drivers of change in soil bacterial community structure and yield of rice, *Oryza sativa*. Biol. Fert. Soils 48:217–225.

Zak, D. R., Holmes, W. E., White, D. C., Peacock, A. D., and Tilman, D. 2003. Plant diversity, soil microbial communities, and ecosystem function: Are there any links? Ecology 84:2042–2050.

Zhou, X., Wu, H., Koetz, E., Xu, Z., and Chen, C. 2012. Soil labile carbon and nitrogen pools and microbial metabolic diversity under winter crops in an arid environment. Appl. Soil Ecol. 53:49–55.

Zuur, A. F., Ieno, E. N., and Elphick, C. S. 2010. A protocol for data exploration to avoid common statistical problems. Method Ecol. Evol. 1:3–14.

SOIL Discuss., doi:10.5194/soil-2016-39, 2016
Manuscript under review for journal SOIL
Published: 4 July 2016

Table 1. Soil carbon (C) and nitrogen (N) pools by season and crop rotation

Season	Crop Rotation	Total Organic C g kg^{-1}	Total N	NO_3^--N mg kg^{-1}	NH_4^+-N	DOC	DON	C:N	DOC:DON
Spring									
	mC	8.1 (0.8)	0.8 (0.1)ab	2.66 (0.79)	0.06 (0.01)B	14 (4)bB	5 (1)bB	9.8 (0.3)	2.8 (0.2)B
	CS	7.8 (1.2)	0.8 (0.1)ab	2.97 (1.13)	0.06 (0.01)B	11 (1)abB	5 (1)bB	10.3 (0.4)	2.1 (0.2)B
	CSW	7.0 (0.6)	0.7 (0.1)b	2.67 (0.39)	0.10 (0.02)B	21 (8)abB	6 (1)abB	10.4 (0.4)	4.2 (1.9)B
	CSW1	8.7 (0.4)	0.9 (0.1)a	3.10 (0.66)	0.10 (0.02)B	44 (18)aB	8 (1)aB	9.6 (0.2)	5.4 (2.6)B
	CSW2	8.2 (1.4)	0.8 (0.1)ab	3.49 (0.62)	0.12 (0.03)B	26 (7)abB	8 (2)aB	10.2 (0.2)	3.3 (0.4)B
Summer									
	mC	7.9 (0.8)	0.8 (0.1)ab	5.58 (0.67)c	0.08 (0.02)A	35 (4)bB	18 (1)bA	10.2 (0.4)	2.0 (0.1)C
	CS	7.6 (0.9)	0.8 (0.1)ab	9.47 (1.96)b	0.08 (0.01)A	32 (4)abB	33 (7)bA	9.8 (0.1)	1.0 (0.1)C
	CSW	7.6 (0.7)	0.8 (0.0)b	7.76 (0.75)b	0.08 (0.01)A	43 (7)abB	28 (4)abA	9.7 (0.3)	1.6 (0.3)C
	CSW1	8.1 (0.8)	0.9 (0.1)a	16.68 (0.87)a	0.37 (0.22)A	88 (32)aB	76 (8)aA	9.0 (0.2)	1.2 (0.4)C
	CSW2	8.7 (1.1)	0.9 (0.1)ab	12.14 (4.03)ab	0.34 (0.12)A	54 (7)abB	68 (13)aA	9.5 (0.1)	0.8 (0.1)C
Autumn									
	mC	8.1 (0.6)	0.7 (0.1)ab	1.31 (0.15)	0.07 (0.02)B	58 (21)bA	5 (1)bB	11.4 (0.3)	14.3 (7.3)A
	CS	7.7 (1.1)	0.7 (0.1)ab	1.44 (0.28)	0.06 (0.01)B	46 (15)abA	5 (1)bB	10.9 (1.0)	9.6 (3.2)A
	CSW	7.4 (0.8)	0.7 (0.1)b	1.28 (0.30)	0.08 (0.02)B	117(77)abA	6 (2)abB	10.6 (0.6)	15.6 (5.2)A
	CSW1	9.6 (0.6)	0.9 (0.0)a	1.41 (0.06)	0.05 (0.01)B	102 (27)aA	7 (1)aB	10.6 (0.5)	17.1 (7.2)A
	CSW2	8.9 (0.9)	0.9 (0.1)ab	0.96 (0.15)	0.05 (0.01)B	190 (42)abA	6 (1)aB	10.4 (0.4)	30.4 (4.0)A
ANOVA Factor					*P* values				
Season		0.756	0.769	**< 0.001**	**0.004**	**< 0.001**	**< 0.001**	0.213	**< 0.001**
Rotation		0.298	**0.040**	**< 0.001**	0.084	**0.038**	**< 0.001**	0.223	0.947
Season*Rotation		0.994	0.928	**< 0.001**	0.071	0.965	0.221	0.746	0.192

Note: Significant comparisons are shown among Rotations (lowercase) and Season (capital) with letters.

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Table 2. Soil extracellular enzyme activities (EEA) expressed as nano-moles of product per hour per gram of dry soil.

Season	Rotation	Soil Extracellular Enzyme Activity (nmol hr^{-1} g^{-1} soil)							
		BGase	CBHase	LAPase	NAGase	PHOSase	TAPase	PPOase	PERase
Spring									
	mC	94 (8)b	27 (2)b	24 (4)bA	27 (2)ab	133 (19)bC	10 (1)abA	140 (47)B	614 (12)a
	CS	107 (18)b	28 (5)b	28 (4)abA	20 (2)b	129 (20)bC	11 (0)abA	100 (30)B	634 (53)a
	CSW	118 (12)ab	31 (4)ab	26 (8)abA	33 (2)ab	152 (7)abC	12 (2)bA	92 (27)B	602 (59)ab
	CSW1	148 (5)a	50 (5)a	43 (5)abA	47 (3)a	188 (17)aC	16 (1)aA	87 (13)B	516 (24)b
	CSW2	153(13)ab	56 (12)ab	33 (5)aA	48 (5)a	208 (8)aC	16 (1)aA	137 (61)B	562 (24)b
Summer									
	mC	100 (5)b	37 (3)b	7 (2)bB	43 (4)	270 (42)bA	9 (2)abB	174 (67)B	676 (88)a
	CS	111 (17)b	43 (10)b	14 (3)abB	44 (7)	291 (25)bA	9 (1)abB	140 (50)B	580 (124)b
	CSW	102 (7)ab	47 (12)ab	14 (2)abB	47 (3)	280 (13)abA	7 (2)bB	96 (29)B	578 (68)b
	CSW1	146 (12)a	61 (10)a	20 (3)abB	69 (10)	370 (45)aA	14 (1)aB	236 (91)B	317 (144)bc
	CSW2	132 (17)ab	62 (14)ab	13 (4)aB	59 (9)	400 (56)aA	12 (1)aB	126 (73)B	392 (97)c
Autum									
	mC	111 (9)b	44 (6)b	5 (3)bB	67 (13)	238 (57)bB	14 (3)abA	330 (77)A	543 (113)a
	CS	110 (17)b	42 (8)b	8 (1)abB	55 (7)	209 (36)bB	11 (2)abA	234 (64)A	461 (103)bc
	CSW	115 (19)ab	49 (15)ab	9 (2)abB	54 (9)	245 (34)abB	14 (2)bA	176 (18)A	517 (150)b
	CSW1	138 (10)a	59 (6)a	8 (1)abB	63 (13)	277 (42)aB	18 (2)aA	300 (30)A	396 (76)c
	CSW2	117 (15)ab	46 (8)ab	17 (3)aB	63 (2)	308 (24)aB	18 (2)aA	202 (51)A	336 (49)c
ANOVA Factor		*P* values							
Season		0.775	0.063	**<0.0001**	**<0.0001**	**<0.0001**	**0.003**	**<0.0001**	**<0.0001**
Rotation		**0.017**	**0.006**	**0.007**	**<0.0001**	**0.0003**	**0.002**	0.224	**<0.0001**
Season*Rotation		0.852	0.839	0.314	**<0.0001**	0.967	0.647	0.837	**<0.0001**

Note: Significant comparisons are shown among Rotations (lowercase) and Season (capital) with letters.

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Table 3. Analysis of variance of results from the principal components analysis of community-level physiological profile (Fig. 4).

ANOVA§ Parameter	PC1		PC2		PC3		PC4		PC5		MANOVA (Total)	
Proportion of variance	38.7		17.7		14.5		9		3.8		83.7	
ANOVA Factor	F	*P* value	F	*P* value	F	*P* value	F	*P* value	F	*P* value	F	*P* value
Season	**64.02**	**< 0.001**	**22.57**	**< 0.001**	**5.4**	**0.008**	0.68	0.510	**10.33**	**< 0.001**	**33.28**	**< 0.001**
Crop Rotation	0.69	0.605	**3.03**	**0.028**	**12.82**	**< 0.001**	0.36	0.834	1.81	0.146	**2.19**	**0.003**
Season*Rotation	0.16	0.995	1.22	0.311	0.55	0.81	0.88	0.544	0.27	0.973	0.65	0.949
Significant comparisons¥	1=3≠2		1=2≠3, CS ≠ CSW1		1=2≠3, mC=CS≠CSW= CSW2				1≠2=3,			

§ Degrees of freedom: Season = 2, Crop Rotation = 4, Season*Rotation = 8.

¥ Significant comparison abbreviations: 1 = spring, 2 = summer, 3 = autumn

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Table 4. Catabolic evenness by season and crop rotation (showing full suite of C substrates, without carboxylic acids, and carboxylic acids only).

Season	Rotation	Catabolic Evenness		
		Full	No Carboxylic Acids	Carboxylic Acids Only
Spring				
	mC	24.37 (0.79)A	20.20 (0.05)aA	7.60 (0.23)aB
	CS	23.79 (0.91)A	19.80 (0.15)aA	7.21 (0.13)abB
	CSW	22.98 (0.63)A	19.65 (0.15)bA	6.56 (0.35)bB
	CSW1	24.28 (0.44)A	18.95 (0.19)abA	6.91 (0.12)abB
	CSW2	24.52 (0.72)A	19.75 (0.24)bA	6.90 (0.31)bB
Summer				
	mC	14.99 (1.61)B	18.95 (0.59)aA	4.91 (0.54)aC
	CS	12.86 (1.77)B	20.20 (0.18)aA	4.32 (0.38)abC
	CSW	12.10 (1.02)B	19.82 (0.54)bA	3.93 (0.20)bC
	CSW1	13.83 (1.65)B	18.59 (0.83)abA	4.34 (0.50)abC
	CSW2	12.78 (0.92)B	19.24 (0.51)bA	3.75 (0.11)bC
Autumn				
	mC	25.81 (0.79)A	19.62 (0.16)aB	8.47 (0.24)aA
	CS	25.82 (0.55)A	19.11 (0.22)aB	8.41 (0.22)abA
	CSW	25.71 (0.74)A	18.98 (0.28)bB	8.12 (0.61)bA
	CSW1	27.41 (0.63)A	18.63 (0.12)abB	8.90 (0.24)abA
	CSW2	26.08 (0.67)A	18.17 (0.28)bB	8.11 (0.08)bA

ANOVA Factor			
Season	**< 0.001**	**0.002**	**< 0.001**
Treatment	0.357	**0.035**	**0.028**
Season*Treatment	0.928	0.058	0.807

Note: Significant comparisons are shown among Rotations (lowercase) and Season (capital) with letters.

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Table 5. Pearson correlation coefficients between soil properties and community-level physiological profile (CLPP) parameters.

Soil Variable	Substrate Guilds					Catabolic Evenness		
	Amino acids	Amine	Carboxylic Acids	Carbohydrates	Complex	Full	No Carboxylic Acids	Only Carboxylic Acids
Water content	ns	ns	ns	ns	ns	0.40	ns	**0.52**
pH	0.27	**0.43**	**-0.41**	ns	**0.53**	**0.68**	ns	**0.74**
Sand	**-0.36**	ns	0.28	-0.27	ns	ns	ns	ns
Silt	0.30	ns	ns	ns	ns	ns	ns	ns
Clay	ns	ns	ns	ns	ns	ns	-0.33	ns
Total C	ns	ns	ns	ns	ns	ns	**-0.40**	ns
Total N	ns	ns	ns	ns	ns	ns	**-0.40**	ns
C-to-N ratio	ns	0.27	ns	ns	0.30	**0.45**	ns	**0.53**
NH_4^+	ns	-0.31	0.33	ns	**-0.37**	**-0.40**	ns	**-0.38**
NO_3^-	**-0.58**	**-0.55**	**0.66**	-0.30	**-0.72**	**-0.74**	ns	**-0.70**
PMC	ns	0.29	ns	ns	ns	ns	**-0.63**	ns
PMN	ns	-0.27	0.32	ns	**-0.55**	**-0.49**	ns	**-0.52**
MBC	0.31	**0.49**	**-0.37**	ns	ns	**0.41**	**-0.38**	**0.47**
MBN	**0.36**	0.34	**-0.37**	**0.42**	ns	**0.36**	ns	0.31
MBC:MBN	ns	**0.40**	ns	ns	ns	0.31	-0.34	**0.40**
BGase	ns	**-0.43**	**0.30**	ns	ns	-0.29	0.32	-0.28
CBHase	-0.32	**-0.47**	**0.39**	-0.27	ns	-0.33	ns	-0.28
LAPase	ns	-0.29	ns	ns	ns	ns	**0.49**	ns
TAPase	ns	**-0.37**	ns	ns	ns	ns	ns	0.37
NAGase	**-0.35**	**-0.56**	**0.47**	**-0.39**	-0.29	**-0.46**	**0.29**	**-0.41**
PHOSase	**-0.45**	**-0.66**	**0.56**	**-0.46**	-0.34	**-0.63**	**0.34**	**-0.60**
PPOase	-0.38	-0.33	0.37	-.31	ns	ns	ns	ns
PERase	-0.40	-0.54	0.42	-0.37	ns	-0.30	0.43	ns

Note: Only significant correlations are shown (P values < 0.05), ns = non-significant

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FIGURES

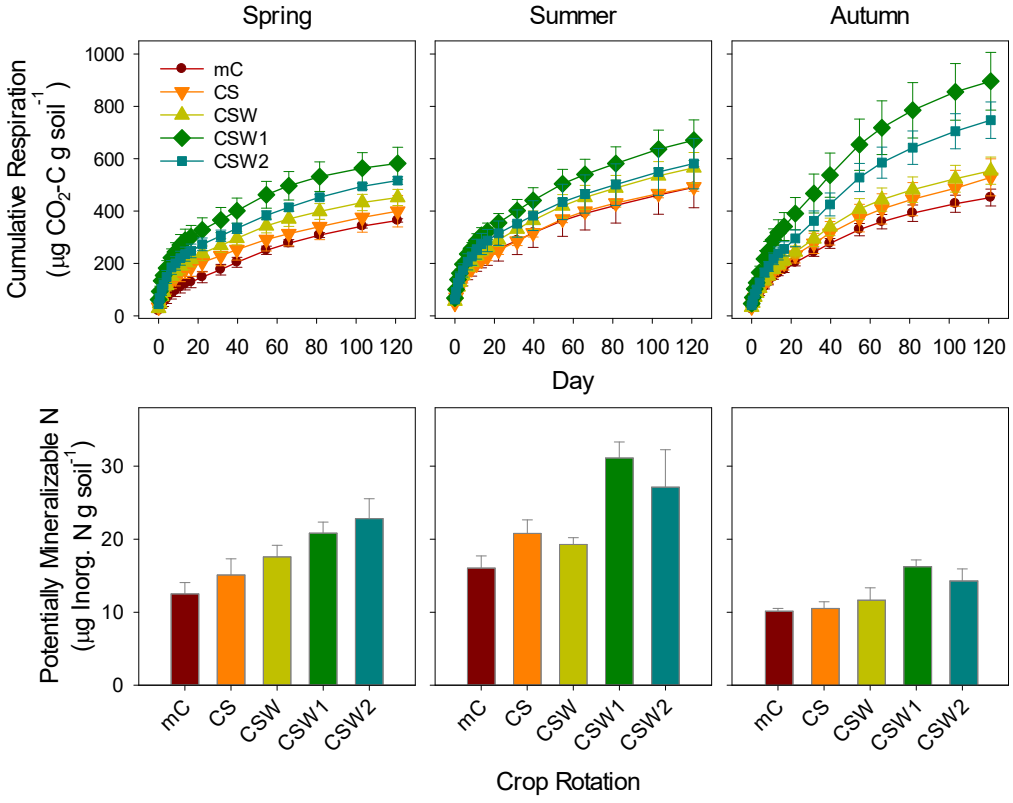

Figure 1. Potentially mineralizable carbon (top row) and potentially mineralizable nitrogen (bottom row). Crop rotation abbreviations are: monoculture corn (mC), corn-soy (CS), corn-soy-wheat (CSW), corn-soy-wheat with red clover cover crop (CSW1), and corn-soy-wheat with red clover + rye cover crops (CSW2). Means are shown and error bars are standard errors (n = 4).

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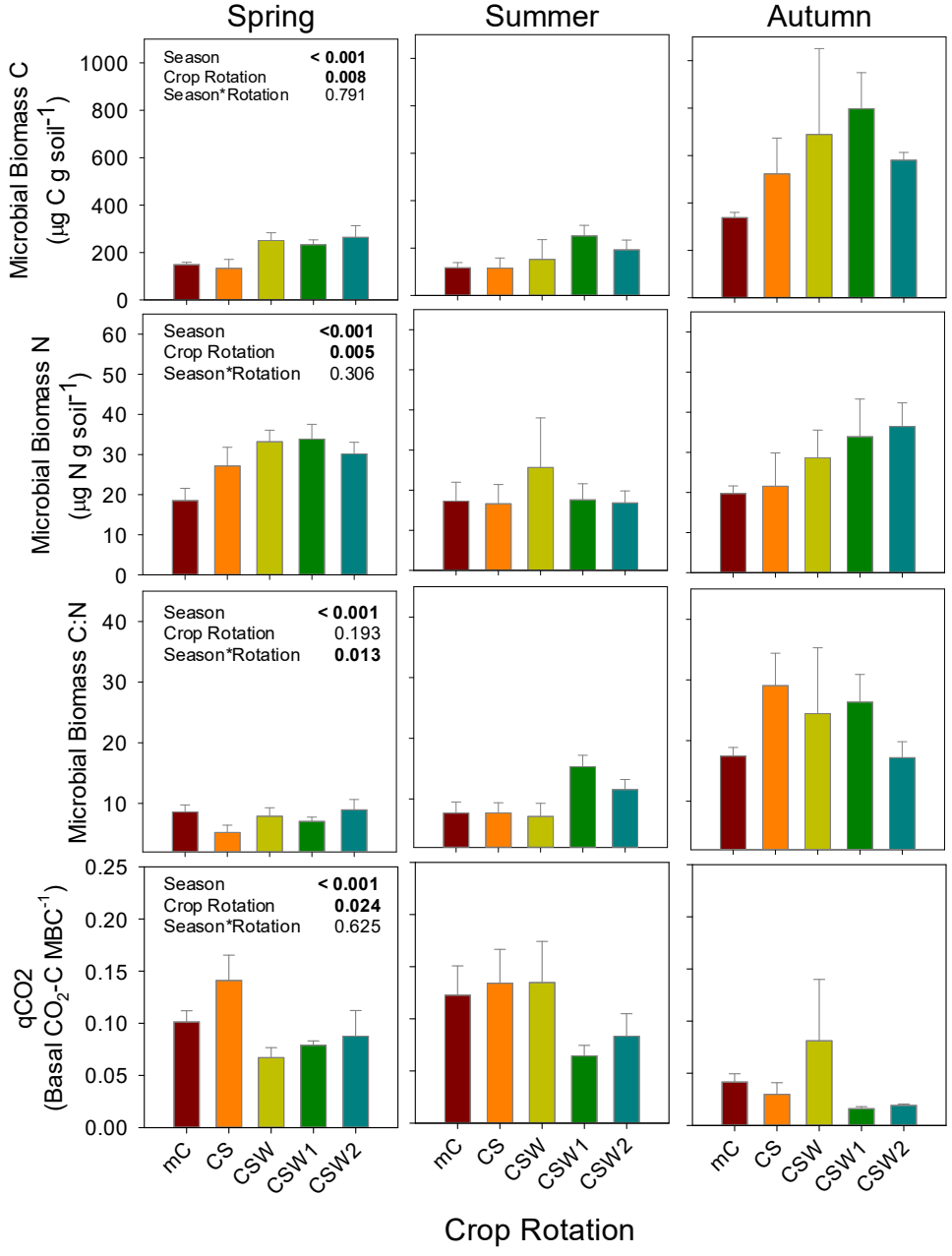

Figure 2. Soil microbial biomass parameters by season and crop rotation. See Fig.1 for crop rotation abbreviations. Means are shown and error bars are standard errors (n = 4).

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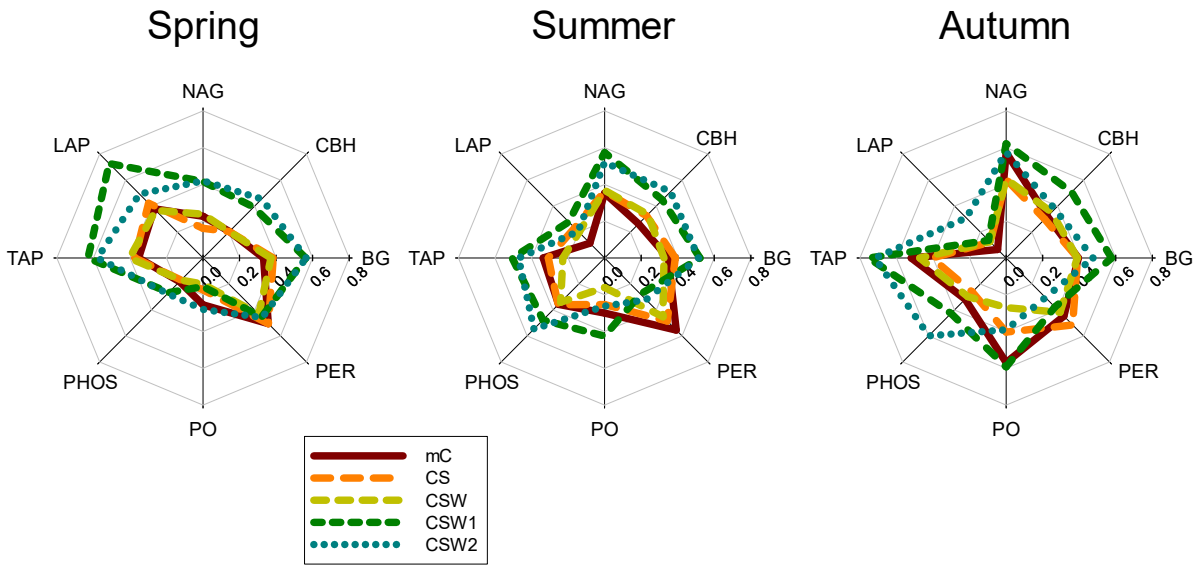

Figure 3. Extracellular enzyme activities (EEA) normalized for the maximum value during each season. EEA abbreviations are: β-1,4,-glucosidase (BG), β-D-1,4-cellobiohydrolase (CBH), β-1,4,-N-acetyl glucosaminidase (NAG), acid phosphatase (PHOS), Tyrosine aminopeptidase (TAP), Leucine aminopeptidase (LAP), phenol oxidase (PO), and peroxidase (PER). See Fig.1 for crop rotation abbreviations.

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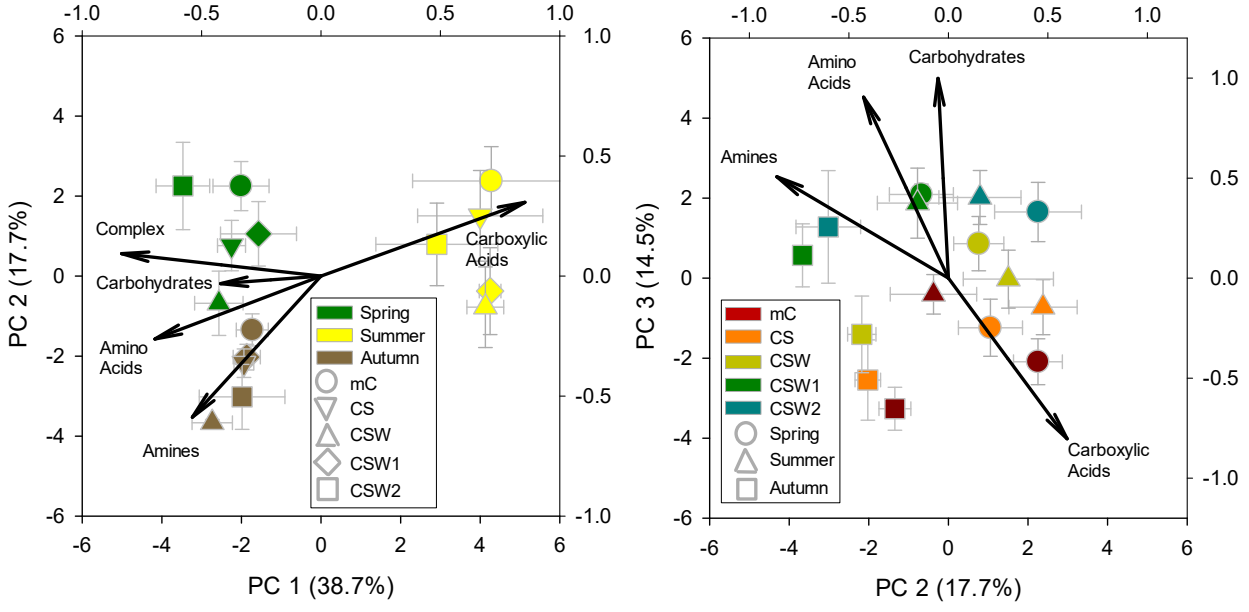

Figure 4. Principal components analysis (PCA) on all 31 substrates. *Left Panel:* Principal components 1 and 2, where Season is dominant discriminating factor ($P < 0.001$) and *Right Panel:* Principal components 2 and 3 where Rotation is highlighted as a dominant discriminating factor. See also Table 5 for PCA and ANOVA results. Means are shown and error bars are standard errors (n = 4). See Fig.1 for crop rotation abbreviations.