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Multifunctional agriculture: Root and nitrogen dynamics in two alternative systems

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Multifunctional agriculture:

Root and nitrogen dynamics in two alternative systems

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ABSTRACT

The Corn Belt of the Midwestern United States is among the most productive grain-producing regions of the world. Yet the development of the Corn Belt has been accompanied by a suite of environmental concerns. Alternative systems have been proposed that remediate environmental quality while relying on fewer external inputs (e.g., synthetic nitrogen fertilizer) than dominate cropping systems of corn and soybean. Two examples of such alternative systems are diversified crop rotations and perennial bioenergy systems. In diverse and less diverse crop rotations, the supply of nitrogen (N) to crops is mediated by the N flux from external inputs as well as internal soil cycling, although evidence suggests that in diverse rotations internal soil N cycling plays a more prominent role. Chapter 2 explores belowground N cycling and provides evidence that diversifying crop rotations increases organic soil N pools and rates of N release from soil organic matter into labile organic forms. Chapter 3 contrasts perennial and annual bioenergy systems by their standing root biomasses and rates of root decomposition as they vary across landscape positions. Results suggest that root biomass is best predicted by choice of annual or perennial crop, but that within cropping systems root biomass is sensitive to landscape position. In contrast to root biomass, rates of root decay for each crop were constant across landscape positions.

CHAPTER I
GENERAL INTRODUCTION

Multifunctional Agriculture

The Corn Belt of the Midwestern United States is among the most productive grain-producing regions of the world (Guanter et al., 2014). Yet its development has been accompanied by a suite of environmental concerns, largely centered around degradation of soil and water quality, which are closely connected to patterns of agricultural management. Most grain-producing land in the Corn Belt is characterized by relatively short periods of soil cover, i.e., 4-5 months of the year, and cropping systems comprised of summer annuals, which are associated with topsoil erosion and depletion of soil organic matter compared to native cover (David et al., 2009; Montgomery, 2007). A combination of summer annual cropping, tile drainage in the region, and use of nitrogen (N) fertilizer, in conjunction with the highly mobile nature of dissolved nitrogen in the environment, has led to N pollution in fresh and coastal waters (David et al., 2010; McIsaac et al., 2001). Furthermore, the simplification of agricultural landscapes has led to a loss of agricultural, as well as natural, biodiversity (Werling et al., 2014). These environmental concerns are closely linked to the substitution of agrichemical inputs for the services provided by a diverse agricultural landscape that includes patches of perennial cover. For example, nitrogen fertilizers have replaced the role of leguminous forages or cover crops in

crop rotations. Overall, Corn Belt systems have been highly optimized for grain production, in part due to the use of external inputs, and in some cases at the expense of environmental quality. In light of these environmental concerns, an emerging framework for multifunctional agriculture in the Corn Belt Region calls for the joint production of both agricultural commodities and a range of ecological services (Swinton et al., 2007). Implied in these systems is often a reduction in use of external inputs, as nutrient, weed, and pest management are to some extent internalized (Davis et al., 2012).

Multifunctional agricultural landscapes are characterized by longer periods of soil cover and greater diversity of vegetative cover in space and time (Liebman et al., 2013). An example of multifunctional agriculture includes diversified crop rotations, which often imply integration of livestock and leguminous N fixation by annual cover crops or perennial legumes. Leguminous N fixation reduces dependence on N fertilizer, and can also cause increases above baseline periods of vegetative soil cover. Multifunctional agricultural systems are also characterized by the reintegration of perennials. Perennials provide year round soil cover, reduce soil erosion, can increase soil organic matter (SOM) through extensive root networks, and in agricultural systems aboveground perennial biomass can be used as biofuels (Schulte et al., 2006; Zan et al., 2001). Overall, these multifunctional landscapes can remediate ecosystem

services, such as the regulation of water flow and quality, carbon storage, forest production, and preservation of habitats and biodiversity (Foley et al., 2005). This thesis explores root and nitrogen dynamics as they connect to particular ecosystem services of soil organic matter maintenance and nitrogen cycling and retention in two multifunctional agricultural systems.

Thesis Organization

Chapter 2 describes nitrogen cycling as mediated by soil microbial communities in diverse and less diverse crop rotations at the Marsden Farm in Boone County, Iowa. The goal of the Marsden Farm experiment is to test the hypothesis that by diversifying simple corn-soybean cropping systems, substantial reductions in agrichemical inputs can be achieved while still maintaining crop yields. A decade of work at the site has shown that diverse rotations can be managed with lower synthetic N fertilizer inputs while maintaining corn and soybean yields (Davis et al., 2012). Although total soil nitrogen and carbon are not different between cropping systems at the site, the similarity in corn yields across cropping systems suggests that belowground activity of N cycling and N retention may be a key mechanism supporting N supply to crops. Furthermore, soil N concentrations as assessed by suction-cup lysimeters are lower in corn in the more diverse, 4-year rotation compared to soil N concentrations under other crops (Tomer and Liebman, 2014), suggesting soil N may be less prone to leaching under the

same crops of different crop rotations. In order to assess the soil N cycle as it supports lower synthetic N fertilizer applications in more diverse rotations, and to test the potential for lower N loss (as assessed by size of the inorganic nitrogen pool), we studied soil N pools and microbial activity under the corn year of each rotation during the 2013 growing season.

Chapter 3 describes root biomass and root decomposition of two bioenergy cropping systems, a perennial (switchgrass, *Panicum virgatum*) and an annual (corn, *Zea mays*) at the Uthe Farm in Boone County, Iowa. The goal of the Landscape Biomass Project conducted at the Uthe Farm is to test a diverse portfolio of bioenergy cropping systems against the current standard, corn, and furthermore to understand how the placement of these crops on the landscape influences their productivity as well as ecosystem function. The first four years of work at the site has shown that productivity is more sensitive to weather conditions across years than to landscape position (Wilson et al., 2014). Nevertheless, at other sites in Iowa, landscape position's effect on aboveground productivity has been reported (Cambardella et al., 2004), though landscape's effect on root decomposition has not been well documented. Root biomass and root decomposition are crucial for their role in soil organic matter maintenance. To test the hypothesis that root biomass and decomposition rates would be sensitive to cropping system as

well as landscape position, from August 2011 through August 2012 we assessed *in situ* decomposition of corn and switchgrass roots.

CHAPTER II
NITROGEN SYNCHRONY IN DIVERSIFIED CROP ROTATIONS

Introduction

Agriculture in the Midwestern United States relies heavily on manufactured inputs of nitrogen (N) fertilizer to increase yields (Robertson and Vitousek, 2009), primarily in systems of one or two dominant crops. Much of this N is not actually acquired by crop plants, and its loss to the environment has caused degradation of water quality (David et al., 2010; Sobota et al., 2013). Diversifying crop systems through rotation reduces the need for manufactured fertilizers (Davis et al., 2012; Robertson and Swinton, 2005), and overall results in a smaller discrepancy between inputs and harvested exports of N (Blesh and Drinkwater, 2013).

Nitrogen management in intensive cropping systems focuses on fertilizing to create a pool of inorganic N large enough to meet crop demands. A crop with particularly high N demands, corn, can take up 225 kg N ha^{-1} in the six weeks during peak growth, and a high yielding corn crop may take up 308 kg N ha^{-1} total (Sawyer et al., 2006). The recovery of applied N in grain is low, however, at only about 37% (Cassman et al., 2002), and this in combination with N pollution from wayward fertilizer has prompted development of the 'N synchrony framework' (Cassman et al., 2002). This framework attempts to minimize pools of available N in soil by aligning soil N availability with crop N demand, often through pulsed applications of

mineral N or use of chemical nitrification inhibitors. Continued N pollution in surface water has prompted mandates to improve water quality (EPA, 2008), and suggests that current approaches to N synchrony have not been widely effective in reducing N loss; even if enacted over all farmed land in Iowa, a state central to the Corn Belt, projected state-level reductions in riverine nitrate-N loading from this suite of N synchrony practices falls below 10% (ISU, 2013).

In diversified crop rotations, the focus of N management shifts from supplying large pools of N to promoting gradual N input and release, from either biological N fixation or from the decomposition of manure, compost, or crop residues. The ability of these organic fertilizers to supply adequate nitrogen for a crop such as corn with high N demands has been questioned, although research suggests that adding cover crops, green manures and use of compost (all techniques in diversified systems) leads to larger pools of labile soil organic matter (SOM; Blesh and Drinkwater, 2013; Power and Doran, 1984), and faster turnover rate of SOM pools, i.e., more active N cycling, has also been hypothesized (McDaniel et al., 2014). Supplying N to crops in diversified rotations thus may rely less on pinpointing external N applications and more on promoting internal cycling of soil N, and these methods may in themselves promote smaller pools of inorganic N, a safeguard against N loss. While the efficacy of diversified rotations in increasing the efficiency of N use has been shown across seasons or years (Blesh and Drinkwater,

2013), suggesting that the goals of N synchrony are being met in diversified rotations, the mechanisms underpinning N synchrony within a season in diversified rotations have not been well explored.

Microbes govern many of the internal N transformations that release plant-available N from organic matter, and are thus of central importance to N availability in both diverse and less-diverse rotations. The N decomposition pathway, from complex macromolecules into polypeptides and amines, is driven by activity of extracellular enzymes secreted by microbes. Specific classes of enzymes, aminohydrolases and proteases (the latter a broader term), liberate amino acids from soil proteins. Proteolysis is considered to be the rate-limiting step in the release of amino acids and N mineralization, as mineralization to N occurs more quickly (Jan et al., 2009). Not all enzyme activity is associated with N release, however; carbon cycling enzymes facilitate microbial uptake of carbon, although their activity is also sensitive to inputs of high C:N ratio, and may occur simply as an expression of overflow metabolism (Schimel and Weintraub, 2003). Microbial biomass itself is a sink for applied N, and has previously been described as acting as a 'source and sink' for N at seasonal time scales (Garcia and Rice, 1994), however these dynamics in relation to N synchrony have not been rigorously tested to our

knowledge. The microbial habitat, and therefore microbial abundance and activity in relation to N cycling, is highly sensitive to agricultural management.

Management systems influence microbes through the quantity and quality of organic matter inputs, the duration of soil cover they provide, and the frequency and intensity of soil disturbance with tillage. The common, baseline management systems throughout the Midwest, corn and corn-soy with mineral N fertilizer, are associated with decreased microbial abundance and activity relative to more diverse systems, presumably due to the relative paucity of plant inputs and shortened duration of plant cover throughout the year (Drinkwater and Snapp, 2007; McDaniel et al, 2014). Adding manure or compost to a corn-soy rotation, increasing periods of soil cover with a perennial or cover crop, and decreasing tillage, tend to increase microbial biomass and activity (Drinkwater and Snapp, 2007).

We framed our work around the overarching question of whether diversifying crop systems affects pool sizes and fluxes of organic N, and whether this cycling aligns soil N availability with plant N demand in more diversified rotations. Specifically, we hypothesized that throughout a growing season, more diverse rotations (compared to less diverse rotations) would support 1) more microbial biomass and higher rates of enzyme activity, but rely on 2)

smaller pools of dissolved inorganic nitrogen (DIN). We also hypothesized that diversified rotations would 3) exhibit seasonal dynamics in microbial biomass and enzyme activity that would constitute greater N alignment with demand (i.e., greater decrease in microbial biomass nitrogen (MBN) around the time of peak corn N uptake; greater increase in enzyme activity), in contrast to a pulse of N at the beginning of the season in the less diverse rotation. In order to hold constant the effect of crop on soil N cycling, we studied only soils under corn in a group of three crop rotations ranging from less diverse (2-yr corn-soy) to more diverse (3- and 4-yr, which also incorporate a green manure or a perennial legume/forage). This whole-systems site is not designed to isolate particular management practices as they influence soil biological process, but rather to study the impacts of cropping systems as they are likely to appear on farms or across landscapes.

Methods

Field Site

Field work was conducted at the Iowa State University Marsden Farm in Boone County, Iowa (42°01’ N; 93°47’ W; 333 m above sea level) during the 2013 growing season (May – October). Soils at the site are predominately Clarion loam (fine-loamy, mixed, superactive, mesic, Typic Hapludolls), Nicollet loam (fine-loamy, mixed, superactive, mesic, Aquic Hapludolls), and

Webster silty clay loam (fine-loamy, mixed, superactive, mesic, Typic Endoaquolls). Weather conditions were measured about 1 km from the site. Fifty year average annual rainfall from May to October at the site is 592 mm and mean temperature is 19°C for the same period. During 2013, total rainfall from May to October was slightly lower than the longer term average, 436 mm, and average air temperature was 19°C. Soil properties measured 0-20cm are as follows: soil organic matter, 51 g kg^{-1}, Bray P, 31 mg kg^{-1} (Liebman et al., 2008). Soil pH across 0-20cm, as measured in May 2013, was 7.5. Prior to establishment of experimental plots, the site had been managed for at least 20 years in corn-soybean rotation receiving conventional fertilizer inputs.

Plots were established in 2002 in a randomized complete block design, with each crop phase of every crop rotation present every year. Plots were 18 m x 85 m. A 2-yr (corn/soybean) rotation was managed with conventional fertilizer inputs. The 3-yr (corn/soybean/small grain + red clover green manure) and 4-yr (corn/soybean/small grain + alfalfa/alfalfa hay) rotations were representative of diversified farming systems in the Midwest. Compared to the 2-yr rotation, the 3- and 4-yr rotations received lower synthetic N fertilizer: averaged across 2002-2011, inorganic N fertilizer application in the 2-yr rotation was 4.8 and 6.9 fold greater than in the 3-yr and 4-yr rotations, respectively (Davis et al., 2012). Synthetic N fertilizer was applied in the 2-yr rotation

at conventional rates based on soil tests. Three- and 4-yr rotations received composted cattle manure and reduced rates of synthetic fertilizers. Calculated application rates of total N, P, and K in composted manure, and a more complete site description, can be found in Davis et al. (2012) and Liebman et al. (2008). In all rotations, the late spring nitrate test (Blackmer et al., 1997) was used for corn to determine rates for post-emergence side-dress N applications. Mineral N applications during the 2013 study season were as follows: corn in 2-yr rotations received 112 kg ha^{-1} N just before planting on 15 May, whereas no synthetic N was applied to the 3- and 4-yr rotations at planting; side dress N applications on June 28 were 112, 84, and 56 kg N ha^{-1} for the 2-, 3-, and 4-yr rotations, respectively. All N was in form of 32% liquid urea ammonium nitrate (UAN).

In all cropping systems, fall chisel plowing was used after corn harvest and spring field cultivation was used before soybean planting. In the 3-yr system, zero tillage or spring disking was used after soybean harvest to prepare for small grain and red clover planting, followed by fall moldboard plowing after establishment of red clover. Spring disking and field cultivation was then used prior to corn planting. Tillage practices in the 4-yr system were similar to those in the 3-yr system, with the only exception that moldboard plowing was used to incorporate alfalfa rather than red clover in the fall before corn planting. Working depth for moldboard plow used

to incorporate red clover and alfalfa in the diversified rotations was 23 cm, and working depth for chisel plow used for partial incorporation of corn residue in all rotations was 33 cm, however carbon measurements from this site show that most corn residue was incorporated only to the 0-10 cm depth (Lazicki, 2011).

Soil sampling

Soils were collected from the corn phase of each rotation eight times throughout the 2013 growing season (22 May, 5 June, 19 June, 8 July, 28 July, 5 August, 3 September, 7 October). At each sampling date, a set of 10 cores was taken from each plot using a 2.2-cm diameter core, and soil cores were divided into 0-10 cm and 10-20 cm depths before compositing. Sampling locations were randomized but were taken in a ratio of 3 row:1 inter-row to control for strong variability of root and N locations (e.g., Buczko et al., 2008). Soils were sieved to 4 mm and stored at 4°C until subsampling (within 48 hours) for subsequent analyses. Gravimetric moisture content was measured as water mass loss upon drying at 105 °C to a constant weight and was used to determine wet-weight to dry-weight ratios. All response variables are presented on a dry weight basis.

Microbial biomass and inorganic N

Microbial biomass was estimated using direct chloroform-fumigation-extraction (modified from Vance et al., 1987). Briefly, ~15 g fresh soil was extracted with 45 mL 0.5 M K_2SO_4 either immediately or following a 24-hour incubation with chloroform. Three replicates per plot and depth were analyzed for each extraction, giving a total number of 144 samples to analyze at each sampling date. Extracts were analyzed for non-purgeable organic C and total N via combustion catalytic oxidation (Shimadzu TOC-L analyzer, Shimadzu Corporation, Columbia, Maryland, USA). Microbial biomass C and N were calculated as the difference between fumigated and unfumigated extracts, with conversion factors of 0.45 for C (Vance et al., 1987) and 0.54 for N (Brookes et al., 1985) used to convert organic C and N to microbial biomass. Unfumigated extracts were used to measure NO_3^- and NH_4^+ concentrations via spectrophotometry (BioTek Synergy HT plate reader, BioTek Instruments, Inc., Winooski, VT, USA) following Hood-Nowotny et al. (2010). As cropping systems did not significantly affect bulk densities, conversions to kg N ha^{-1} were made using separate bulk densities by depth, with 1.03 and 1.18 g cm^{-3} for 0-10 and 10-20-cm depths, respectively (Lazicki, 2011).

Enzyme activity: proteolysis of native soil substrate

Potential activity of protease was measured with a method modified from Lipson et al. (1999) and Watanabe and Hayano (1995) on subsamples of soil stored at -80°C (Brzostek et al., 2012). Soils were preincubated at 23°C for 12 hours. All soil samples (~3.0-3.5 g) received 10 mL 0.02 M MOPS (3-(N-morpholino)propanesulfonic acid) buffer at pH 7.5. All samples also received 0.4 mL toluene, which inhibits microbial uptake (Skujins, 1967). Initial samples, used to determine standing pool of free primary amines, received 3 mL trichloroacetic acid (TCA) mix immediately in order to halt proteolytic reactions. Incubated samples were shaken lengthwise at 120 rpm for 4 hours at 23°C before addition of 3 mL TCA mix. All samples were centrifuged at 2300 rpm for 5 min and the supernatant filtered through Whatman #42 papers. Three technical replicates per plot and depth combination were run for both initial and incubated samples, giving a total of 144 samples per sampling date. An incubation time of 4 hours is common for proteolytic assays (Brzostek and Finzi, 2011; Brzostek et al., 2012), and preliminary work showed that 4 hours was within the linear range of reaction for these soils. While a buffer of sodium acetate has been used commonly in recent soil protease assays (Brzostek and Finzi, 2011; Hofmockel et al., 2010), we chose MOPS buffer over sodium acetate for its ability to buffer soils at either end of our pH range (~6.5-8.5). Sodium acetate buffers at lower pH (4-6). Extracts were stored at -20°C until analysis.

The concentration of total free primary amine-N (TFPA-N) in soil extracts was evaluated using the o-phthaldialdehyde (OPA) and β-mercaptoethanol method, similar to Darrouzet-Nardi et al. (2013). Concentrations were calculated against an L-leucine standard curve, and total free primary amine-N was calculated following Darrouzet-Nardi et al. (2013). Three analytical replicates per sample and incubation time combination were run on each plate, along with buffer blanks and an OPA standard curve. Note that the OPA reagent used here reacts with amino acids as well as other free primary amines such as peptides and amino sugars (Chen et al., 1979), thus the designation total free primary amine-N rather than amino acid-N. The interference of NH_4^+ with OPA reagent was subtracted following (Darrouzet-Nardi et al., 2013) using NH_4^+ concentrations in MOPS extracts determined via colorimetric analysis (Hood-Nowotny et al., 2010). Native proteolytic rate (ηmol g $soil^{-1}$ hr^{-1}) was found as the difference between incubated and initial samples.

Enzyme activity: aminopeptidase and C-cycling activity under saturating conditions

Potential enzyme activities were measured on subsamples of soil stored at -20°C. A suite of six enzymes was measured, three involved in carbon decomposition as well as three aminohydrolases, which liberate amino acids or tripeptides from polypeptides. Carbon cycling

enzymes were tested with MUB (Methylumbelliferone) -linked substrates: CB, 4-MUB-β-D-cellobioside; BG, 4-MUB β-D-glucopyranoside; BX, 4-MUB β-D-xylosidase. Aminohydrolases were tested with MUC (7-amino-4-methylcoumarin) -linked substrates: LAP, L-leucine-7-amido-4-MUC; Ala, L-alanine-7-amido-4-MUC; AAP, L-alanine-alanine-phenylalanine-7-amido-4- MUC, AAP. Briefly, 1 g of soil was homogenized with 125 mL of 100 mM tris maleate buffer, pH 7.5. MUB- or MUC- linked substrates were added at saturating concentrations of 400μM for all substrates. Plates were incubated at 23° C for 3 hours and read using a fluorometer (360 nm excitation and 460 nm emission; BioTek Instruments, Inc., Winooski, VT, USA) without the addition of NaOH. Eight analytical replicates per sample and substrate combination were run and each plate included a MUB or MUC standard curve, substrate controls, and homogenate controls. Enzyme activity was calculated as ηmol enzyme g^{-1} soil h^{-1} based on MUB or MUC standard curves and accounting for the quench of each sample (Anderson-Teixeira et al., 2013; German et al., 2011). The linearity reaction of the 3-hour incubation was confirmed in preliminary work.

Statistical analyses

All analyses were performed using repeated measures mixed effects ANOVA in JMP Pro 11. When present, replicates from the same plot and depth were averaged before analysis, and

values for the whole soil profile (0-20 cm) were found by averaging values for samples of both depths. Data were transformed where necessary to meet assumptions of normality as judged visually and by the Shapiro-Wilk test. In the ANOVA mixed model, a full factorial model was used, with sampling date, depth, and cropping system as main effects, as well as each of their interactions. An 'ID' term, coded by plot and depth (12 plots x 2 depths; total 24 'ID') was repeated across each sampling date, and served to account for plot-to-plot variability. Residual, AR(1) and unstructured covariance structures were compared, and the 'ID' term was used differently based on covariance structure: in the residual covariance structure, it was used as a random effect; in AR(1) and unstructured, it was used as the subject term under repeated effects. Covariance structures were chosen based on the lowest Akaike Information Criterion (AIC). Subsequent pair-wise comparisons were made using Tukey's Honestly Significant Difference. Significance was determined at $\alpha = 0.05$.

Results

Microbial biomass

The response of both microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) to cropping system was dependent on depth (MBC, $P = 0.006$; MBN, $P = 0.001$), where the 2-yr rotation at the 10-20 cm depth supported significantly lower MBC and MBN than any

other cropping system and depth combination. Other cropping system and depth combinations were not significantly different from each other. Microbial biomass C varied strongly by day (P = 0.0001, Table 2.1), with MBC values peaking in the middle of the season (23 July) and rising again later in the season (through 7 October). Microbial biomass N exhibited a day × depth interaction (P = 0.044), however overall patterns of MBN across the season were similar to those of MBC (Table 2.1), and overall comparisons of depth revealed no significant differences between depths at any sampling day. The peak in MBC and MBN on 23 July corresponded to periods of relatively low gravimetric water content (Fig. 2.4). There was a trend toward the 2-yr rotation at 10-20 cm depth having higher MBC:MBN ratio than the 2-yr rotation at 0-10 cm depth or the 4-yr rotation at 10-20 cm depth (Table 2.5); MBC:MBN ratios also varied by sampling day, with C:N ratios lower at 5 June and 19 June and peaking at 8 July (Table 2.1).

Enzyme activity: proteolysis of native soil

Proteolysis of native soil substrate varied by cropping system and by day (p = 0.005 and 0.0001, respectively), and these effects were independent of depth. The 4-yr rotation was significantly higher in native proteolysis than the 2-yr rotation (p = 0.004), however the 2-yr and 3-yr or 3-yr and 4-yr rotations were not significantly different (p = 0.274 and p = 0.120,

respectively). Proteolysis of native soil substrate fluctuated strongly throughout the season, with highest production at 22 May and 8 July, with lower rates at 23 July and 3 September (Table 2.1).

Enzyme activity: aminopeptidase and C-cycling activity under saturating conditions

Potential activity of all enzymes varied by day ($P < 0.0001$ for all), although seasonal trends for C-cycling (BG, BX, and CB) and aminohydrolase (LAP, Ala, and AAP) were not consistent with each other (Table 2.2, Figures 2.1 and 2.2). Aminohydrolase enzymes generally peaked in the middle of the season (8 July) while BG, BX and CB shared an increase late in the season (7 October). Averaging across the season, stratification of the 2-yr rotation system was apparent for all enzymes, with higher potential activity in the 0-10 cm depth compared to the10-20 cm depth (Table 2.7). In contrast to the 2-yr rotation, the 3-yr and 4-yr treatments lacked consistent stratification (Table 2.7).

Specific enzyme activity

Potential activity of all enzymes was scaled to MBC to determine biomass-specific activity. For all enzymes, potential activity showed a pronounced stratification in the 2-yr system, with 0-10 cm having higher specific enzyme activity compared to the 10-20 cm depth (Table 2.7). In contrast to the 2-yr rotation, 3-yr and 4-yr treatments lacked consistent stratification (Table

2.7). In most pair-wise comparisons of the 3-yr and 4-yr rotations at the 0-20 cm depth, the 3-yr rotation had higher specific activity than the 4-yr rotation, the exceptions being specific activity of LAP and Ala. Specific activity in 2-year and 3-yr rotations were not statistically different for any enzyme. Consistent across enzymes was a numerical trend for the 4-yr rotation to be lower in specific activity than the 2-yr rotation, although this was statistically significant only for CB (Table 2.7).

Inorganic nitrogen

The temporal response of DIN varied by depth ($P = 0.021$, Fig. 2.3). Across all cropping systems and sampling dates, DIN was greater in 0-10 cm than 10-20 cm depths by 1.5-fold (0-10 cm = 31.8 ± 1.1 μg N g-1 soil; 10-20 cm = 21.3 ± 1.1 μg N g-1 soil, $p = 0.024$), however high DIN concentration in 0-10 cm depth was most evident later in the season (28 July and after). When separated by N species, ammonium and nitrate concentrations showed a similar pattern across the season, with an increase later in the season, after side dress N, which was more pronounced in the 0-10 cm depth (data not shown; total DIN, Fig. 2.3). Ammonium concentrations were higher in the 0-10 cm than 10-20 cm depths by a factor of 1.6 ($P = 0.088$), and also showed an interaction of depth and day ($P = 0.012$), with the 0-10 cm depth higher later in the season, after fertilizer N sidedressing, compared to the 10-20 cm depth. Nitrate

concentrations exhibited a depth by treatment interaction, with the 2-yr rotation at 0-10 cm depth higher in NO_3- concentrations than the 10-20 cm depth. Concentrations of total K_2SO_4 - extractable inorganic nitrogen (DIN) varied by 5.6-fold across sampling dates ('Day'; $p < 0.0001$). DIN concentrations were generally lower early in the season and higher later in the season, after side dress N on 28 June. Across the season and both depths, cropping systems did not differ from each other ($P = 0.546$).

Standing pool of total free primary amine-N (TFPA-N) was sensitive to day and a day by depth interaction ($P < 0.0001$ and 0.003, data not shown), however because of the rapid turnover time of TFPA (Jones et al., 2009; Hobbie and Hobbie, 2013) this form of nitrogen comprised a small pool compared K_2SO_4- extractable inorganic nitrogen and so was not included in subsequent analyses. Total free primary amine-N, averaged across all observations, was three orders of magnitude lower than average DIN (0.076 and 26 μg N g^{-1} soil for TFPA-N and DIN, respectively).

Discussion

All measured variables (microbial biomass, native protease activity, potential enzyme activity, and DIN) were highly variable throughout the season ($P < 0.0001$, Table 2.4). For only

a few variables did the effect of rotation depend on the day; for some, the effect of depth varied by sampling date. Trends in nutrient cycling between rotations are discussed below without regard to day, followed by an examination of whether variability between sampling dates supports seasonal expression of biological activity that underpins N synchrony.

Does diversification support more microbial biomass?

Consistent with our hypothesis, diversifying crop rotations was accompanied by an increase in microbial biomass. Microbial biomass is well-know to responds to inputs of organic matter in the context of crop rotations (Gunapala and Scow, 1998), and sources of organic matter in diversified rotations included composted manure, red clover as green manure, or root input of alfalfa. A particularly consistent pattern in the distribution of microbial biomass between rotations and depths was the depletion in microbial biomass at 10-20 cm in the less diverse, but not in the more diverse rotations at this site (Table 2.5). Less disruptive tillage is a likely driver of this stratification of microbial biomass in the less diverse rotation, and is consistent with others who have found that chisel tilled (Karlen et al., 2013) or reduced tillage (Kandeler et al., 1999) concentrates MBC in surface soil compared to deeper tillage, while soils at depth are relatively depleted of MBC. Cropping systems with a heavy representation of corn (compared to deep rooted perennials) may be especially prone to this kind of stratification with reduced tillage

because corn supplies fewer deep root inputs compared to perennials but an abundance of aboveground input (Anderson-Teixeira et al., 2013). In this study, the effects of tillage and diversification (with either cover crop or perennial crop) were confounded. More diversified rotations were moldboard plowed, a more intensive form of soil disturbance than the chisel tilling and disking in the less diverse rotation. Although tillage is generally reported to reduce microbial biomass (Pandey et al., 2014), such that deep tillage like moldboard plowing is discouraged for the sake of conserving soil organic matter stocks (Karlen et al., 2013), our findings suggest that, given relatively flat land as at the Marsden Farm, perennial forages and cover crops may remediate soil microbial biomass beyond the detrimental effects of the intensive tillage they often require. Indeed, losses in soil C during conversion of native grasslands to agriculture may be due more to annual cropping and attenuated periods of soil cover, in the place of perennial cover, rather than tillage per se (DuPont et al., 2010).

Does diversification support faster rates of N cycling?

Enzyme activity liberates N from soil organic matter, and proteolysis is an important step in the decomposition pathway from polymers to free amines, preceding mineralization to NH_4^+. Few studies have tested proteolysis with only native soil as a substrate, and to our knowledge those have used forests, alpine systems, native grasslands, or other unmanaged systems

(Hofmockel et al., 2010; Raab et al., 1999; Weintraub and Schimel, 2005). In these systems DIN concentrations are orders of magnitude lower than in corn systems, and likely display different patterns of microbial resource allocation related to N cycling. Most studies in agricultural systems test enzyme activity under saturating substrate conditions, which estimates potential activity, or the enzyme pool. In order to estimate pools of aminohydrolase and carbon-degrading enzymes we assessed potential enzyme activity. In order to understand potential limitations of substrate on liberation of N from SOM, we assessed proteolysis of native soil. These assays revealed different patterns in the responses of native proteolysis and potential aminohydrolase activity to crop rotation (Tables 2.5 and 2.7).

With native soil as a substrate, the liberation of free primary amines from soil organic matter was higher in more diverse rotations compared to less diverse rotations (Table 2.5). In studies of native proteolysis, albeit from unmanaged systems, it is often substrate that controls enzyme activity (Rejsek et al., 2007; Vranova et al., 2013). For example, when comparing native proteolysis with soil amended with proteinaceous substrate in alpine, subalpine fen, and short grass steppe soils, Raab et al. (1999) found that NaOH-extractable soil protein explained 81.6% of the variability in proteolysis of native soil. Increases in proteolysis in diversified rotations are consistent with those of Fauci and Dick (1994), who found that manure-treated plots had higher

protease activity relative to inorganic N fertilized plots, as well as laboratory incubations in which added substrate increased rates of proteolysis (Geisseler and Horwath, 2008). At the Marsden Farm, proteinaceous substrate in the more diverse rotations could have originated from composted manure, red clover green manure (3-yr rotation only) or alfalfa root inputs (4-yr rotation only).

Estimates for leguminous nitrogen fixation from clover and alfalfa vary widely. In red clover, nitrogenous inputs have been estimated as ranging between 69 and 373 kg N ha^{-1} annually (Hogh-Jensen and Schoerring, 2001; Peoples et al., 1995). In alfalfa, N fixation has been estimated as ranging between 90 and 386 kg N ha^{-1} annually (Peoples et al., 1995; Russelle and Birr, 2004). While previous work at this site has estimated total external N inputs to the 4-yr rotation to be about 60% of N inputs in the 2-yr rotation (Lazicki, 2011), it may be that a more tightly constrained accounting of biological N fixation would reveal a greater total flux of N into more diverse cropping systems. Regardless of relative N inputs, however, the coupled C and N inputs from biological N fixation are likely a key factor both driving and limiting rates of native proteolysis in all rotations.

With saturating conditions, there was a trend for enzyme activity of aminohydrolases to be lower in the 4-yr rotation compared to the 2-yr or 3-yr rotations, and strongly sensitive to depth in the 2-yr rotation. In studies of potential activity, enzymatic response to diversifying crop rotation are generally report an increase in enzyme activities (Ekenler and M, 2002; Klose and Tabatabai, 2000) as a response to greater substrate availability. However, enzyme production may also be an expression of resource allocation toward nutrient demand. Whether enzyme production is driven by microbial demand or triggered by substrate availability (two seemingly opposing circumstances) is an ongoing debate in the literature (Weintraub and Schimel, 2005). Taken together, our results suggest that substrate availability limited N release from SOM in all rotations, and that diversified rotations were higher in substrate than less diverse rotations. The fact that potential activity gives mixed signals regarding N release in different rotations highlights the need to consider use of native substrate assays, or other methods, such as potentially mineralizable C and N, when estimating *in situ* rates of nutrient transformation.

Potential activity of C-cycling enzymes responds positively to both mineral N fertilization and additions of carbon substrate (Grandy et al., 2013; Hargreaves and Hofmockel, 2013). In identifying mineral N availability as a driver of C-cycling enzyme activity we should

be cautious, however, because in all systems concentrations of inorganic N were relatively high throughout the season, and thus did not align with divergences in C-cycling enzyme activity between treatments and depths. Increases in C-cycling activity were therefore more likely driven by inputs of corn residue. The similarity between potential aminohydrolase activity and potential activity of the C-cycling enzymes BG, BX, and CB (Table 2.7), when considered across the season, suggests that at this site aminohydrolase synthesis may be more attuned to supply and demand of C than of N. While proteases are sometimes described as N acquisition enzymes (Geisseler et al., 2010), neither the patterns of potential aminohydrolase nor native protease appeared to be suppressed by increases in DIN concentration throughout the season, as would be expected were protease enzymes production stimulated by N demand. Indeed, laboratory incubations have often failed to find protease suppression with the addition of inorganic N (Geisseler and Horwath, 2008; Jan et al., 2009), and a review of field studies found that protease activity was insensitive to additions of inorganic N (relative to unfertilized controls, (Geisseler and Scow, 2014)). While there is evidence that proteases may serve as an N acquisition enzymes when N is limiting (Geisseler and Horwath, 2009), DIN concentrations at this site were high throughout the season (Table 2.6). In agricultural systems, where N is especially abundant, protease and aminohydrolase activity may be viewed more appropriately not as a signal of N demand but for their by-products of N release.

Does biomass-specific enzyme activity differ between cropping systems?

Both microbial biomass and potential enzyme activity are considered measures of soil health (Bandick and Dick, 1999; Stockdale and Watson, 2009). However, microbial biomass and potential activity do not always increase or decrease in concord with one another; increases in potential enzyme activity without subsequent increases in microbial biomass can be a sign of resource allocation toward enzyme production and respiration rather than biomass, which in turn leads to reduced substrate use efficiency.

We found an overall increase in biomass-specific BG activity in the 3-yr system compared to the 4-yr system, a strong depth response in the 2-yr system, but overall trends suggested that the 4-yr rotation reduced specific activity compared to the 2-yr rotation (Table 2.8). Specific activity is often interpreted as a measure of microbial stress (Dilly and Munch, 1998; Rietz and Haynes, 2003), and agricultural use may induce a stress response. Trasar-Cepeda et al. (2008) found that agricultural use lowered SOM and microbial biomass but increased specific activity of hydrolytic enzymes compared to forested soils. In a comparison of conventional and organic systems, where crops and tillage were held constant but organic management used cover crops and manure rather than mineral fertilizer, organic management was reported to increase MBC but decrease metabolic quotient and specific activity of the

enzyme BG (Lagomarsino et al., 2009). The author attributed the effects to a decrease in metabolic requirements of the microbial community.

The bacterial:fungal biomass ratio may be predictive of maintenance requirements. Fungi are thought to require lower specific maintenance than bacteria (Sakamoto, 1994). While we did not assay microbial community, microbial C:N may reflect bacterial:fungal ratio (Fierer et al., 2009; Joergensen and Emmerling, 2006). In the less diverse 2-yr rotation, stratification by depth is consistent with this hypothesis, as the 10-20 cm depth had higher C:N ratios than the 0-10 cm depth (Table 2.5) and also lower specific activity compared to other treatment by depth combinations (Table 2.8), however trends across other rotations are not evident. These interpretations of specific activity should be taken in light of recent research finding that the C:N ratios between fungi and bacteria may overlap (Strickland and Rousk, 2010).

Does diversification supply crop N demand while relying on smaller pools of DIN?

Some evidence shows that diversified crop rotations may rely on attenuated DIN pools (Power and Doran, 1984), a safeguard against N loss. During our study period, however, DIN pools were high in all crop rotations (Table 2.5). Interestingly, these similarities in DIN concentrations across crop rotations do not align with the disparity in side dress N rates (112, 84,

and 56 kg N ha^{-1} for the 2-, 3-, and 4-yr rotations, respectively). Relatively high DIN pools in 3-yr and 4-yr rotations suggest that net N mineralization may play a larger role in available N in these rotations. In support of this interpretation, Lazicki (2011) found larger pools of potentially mineralizable N in more diversified rotations at this site, similar to Pang and Letey (2000), and we found higher rates of native proteolysis at this site as well. Furthermore, diversified crop rotations surpassed N supply to crops compared to the less diverse 2-yr rotation; N uptake from corn was higher in the 4-yr rotation compared to the 2-yr rotation (W. Osterholz, forthcoming work), suggesting faster rates of replenishment of DIN pools in the 4-yr system compared to the 2-yr system. These results from the 2013 growing season are, however, limited in their breadth: rates of side dress N fertilizer application are informed in part by spring weather patterns, which are variable across growing seasons. Taken together with differences in the side dress rate in 2013, however, our DIN results indicate a greater supply of N from SOM in diverse rotations than in short rotations, which in itself may be a mechanism explaining reduced reliance on mineral N fertilizers in more diverse rotations.

Do seasonal dynamics support N synchrony more in one rotation than another?

Every soil indicator measured fluctuated strongly throughout the sampling season (Table 2.4), although few of these variations were attributable to definite causes from management or

sampling day. An exception was the sharp increase in DIN pools in July (Fig. 2.3), which corresponded to side dress N fertilizer application, and the gradual decline of DIN throughout the season, which corresponded with corn uptake of N. An increase in aminohydrolase activities after sidedress, on 8 July (Fig. 2.3), may also be a response to sidedress N, which was applied as 32% UAN and contained 35% urea. While urea behaves as an inorganic fertilizer in soil, it possesses the same chemical group that characterizes a peptide bond, thus protease assays could detect urease enzymes produced in response to sidedress N. Enzyme activity is also sensitive to plant inputs to soil. Late in the corn life cycle, after the blister kernel stage (close to 5 August for this study), roots begin to senesce as the plant redirects nutrients to developing kernels (Sawyer et al., 2006), potentially causing an influx of labile root C. The increase in C-cycling activity later in the season may be attributable to this root turnover (Table 2.2). In general, biological activity in cropland is highly variable across time (Jones et al., 2002; Lee and Schmidt, 2014), consistent with the fluctuations found here.

Taken together, the similarities in DIN concentrations between rotations, and thus comparable vulnerability of N to loss, suggest that N efficiency demonstrated elsewhere in diversified rotations may be a product of differences in N storage and cycling enacted over time scales longer than one season. Diversified rotations typically have longer periods of soil cover

throughout the year, especially in systems where perennials can reduce N loss via leaching (Toth and Fox, 1998), which may be a stronger driver of N retention than soil N dynamics under corn.

Conclusions

Diversifying crop systems shifts reliance to organic N forms and promotes larger pools of microbial biomass. We found that crop system diversification also increased rates of native soil proteolysis, but that the response of potential aminohydrolase to crop system diversification was not consistent with that of native proteolysis. Our contrasting results between native and potential enzymatic activity highlight the need to address what kinds of microbial activity are considered "desirable" in agricultural systems. Overall, our data support the hypothesis that crop systems diversification can promote larger pools of microbial biomass N and release of labile organic N.

Figures

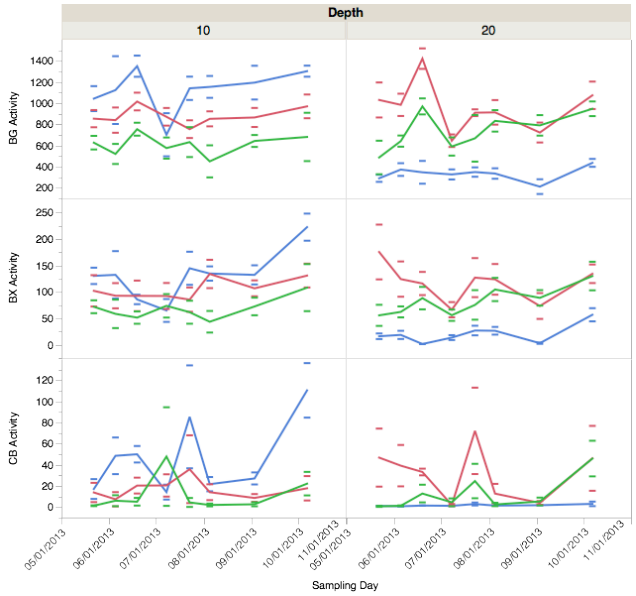

Figure 2.1. Activity of BG, BX, and CB by sampling date, crop system, and depth. Two-yr (blue), 3-yr (red), and 4-yr (green) crop rotations; nmol g^{-1} soil hr^{-1}. Tick marks represent one standard error of the mean (n = 4 per treatment and depth).

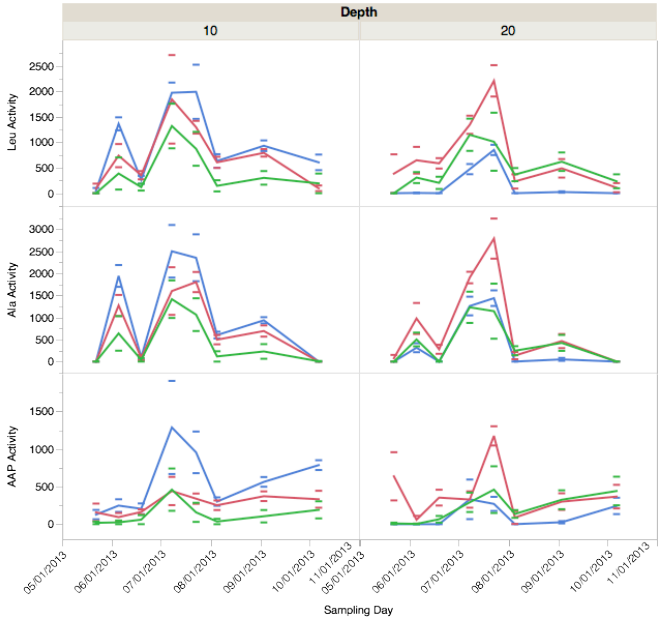

Figure 2.2. Activity of LAP (i.e. Leu), Ala, and AAP by sampling date, crop system, and depth. Two-yr (blue), 3-yr (red), and 4-yr (green) crop rotations; nmol g^{-1} soil hr^{-1}. Tick marks represent one standard error of the mean (n = 4 per treatment and depth).

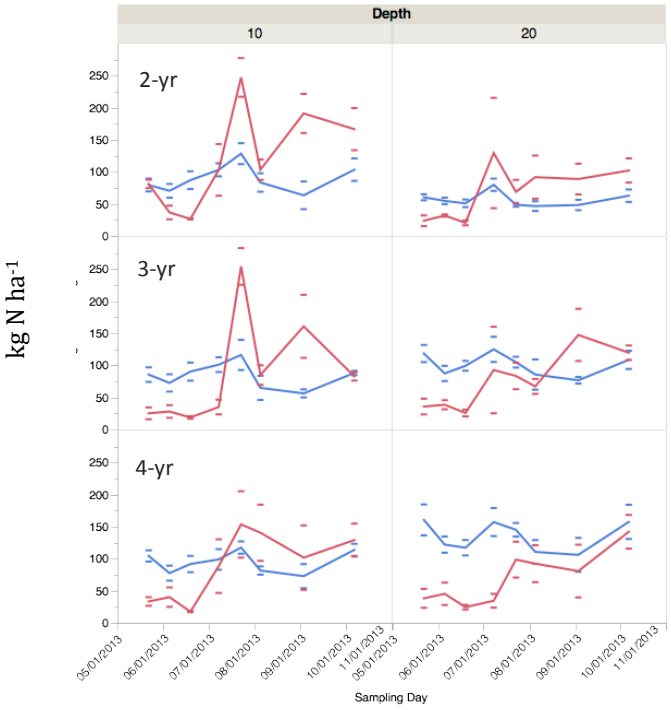

Figure 2.3. Pool sizes of DIN (red) and MBN (blue) by sampling date and depth. Tick marks represent one standard error of the mean (n = 4 per treatment and depth). Inorganic N applied during the 2013 growing season differed among rotations: 2-yr rotations received 112 kg ha^{-1} N just before planting on 15 May, whereas no synthetic N was applied to the 3- and 4-yr rotations at planting. Side dress N applications on June 28 were 112, 84, and 56 kg N ha^{-1} for the 2-, 3-, and 4-yr rotations, respectively.

A B

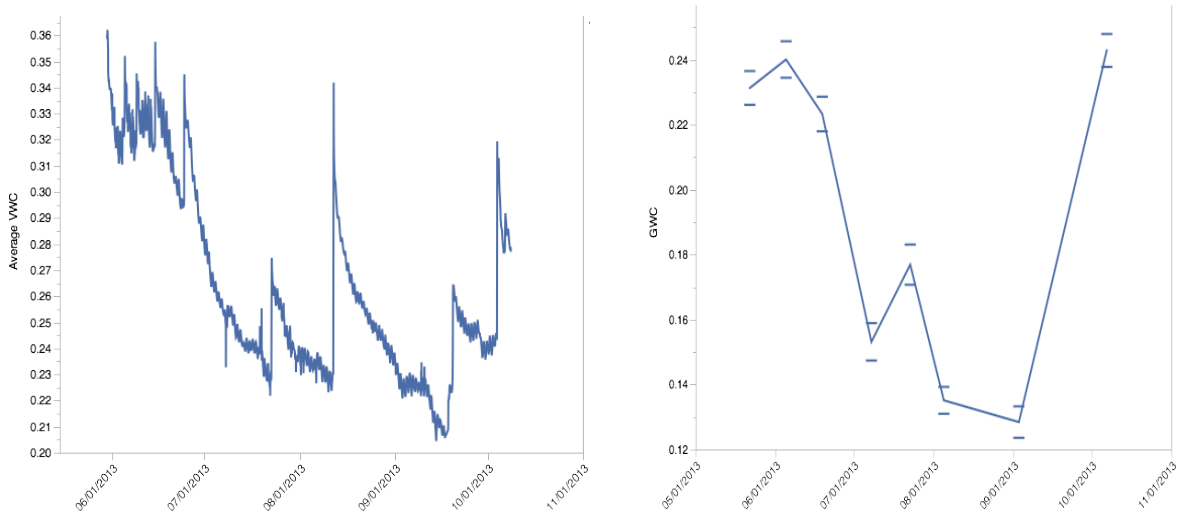

Figure 2.4. (A) Mean volumetric water content (m^3 m^{-3}) across the season. One probe installed in each plot at 10cm depth (ECH2O TE and 5TE probes; Decagon Devices, Pullman, WA). (B) Mean gravimetric water content at each sampling date (g water g^{-1} dry soil), 0-20cm. Tick marks represent standard errors.

Tables

Table 2.1. Variation across sampling dates in microbial biomass, native protease activity, and native ammonification. Pluses (+) and minuses (-) represent significant positive and negative deviations from the overall mean of a column, respectively. Means and standard errors; standard errors calculated with 22-24 observations per cell (12 plots x 2 depths).

Day	MBC			MBN			MBC: MBN			Native protease activity			Native ammonification		
	ug g^{-1} soil									*nmol g^{-1} soil hr^{-1}*					
22-May	424.08	(1.08)		44.05	(3.09)		9.77	(2.77)		9.07	(4e-10)	+	10.36	(4.4e-5)	
5-Jun	255.61	(1.06)	-	35.31	(2.91)		7.37	(2.76)	-	4.96	(3.4e-9)		19.46	(1.1e-4)	
19-Jun	323.63	(1.08)	-	39.35	(2.96)		8.30	(2.79)	-	3.59	(9e-10)		12.63	(5.3e-5)	
8-Jul	754.53	(1.06)	+	48.69	(2.94)	+	15.69	(2.75)	+	7.05	(1e-10)	+	6.98	(8e-5)	-
23-Jul	433.40	(1.06)		48.08	(3.33)	+	9.54	(2.78)		1.17	(3.3e-9)	-	120.09	(4.7e-5)	+
5-Aug	279.25	(1.09)	-	33.82	(3.27)	-	8.81	(2.78)		4.46	(3e-10)		18.04	(7.5e-5)	
3-Sep	324.67	(1.07)	-	30.08	(3.23)	-	10.65	(2.78)		1.37	(7.1e-9)	-	46.95	(5.1e-5)	
7-Oct	471.05	(1.06)	+	46.08	(3.15)	+	10.45	(2.78)		4.39	(1.7e-9)		59.39	(7.7e-5)	

Table 2.2. Variation across sampling dates in potential enzyme activity. Pluses (+) and minuses (-) represent significant positive and negative deviations from the overall mean of a column, respectively. Means and standard errors; standard errors calculated with 22-24 observations per cell (12 plots x 2 depths).

Day	BG activity			BX activity			CB activity			LAP activity			Ala activity			AAP Activity		
	nmol g^{-1} soil hr^{-1}																	
22-May	721.48	(71.3)		76.39	(0.67)		2.67	(1.5e-3)		6.67	(0.15)	-	1.06	(1e-5)	-	26.52	(4.7e-4)	-
5-Jun	746.77	(78.02)		66.41	(0.65)		2.66	(4.3e-3)		283.10	(0.58)		620.49	(2.5e-4)	+	11.71	(2.4e-4)	-
19-Jun	975.89	(81.97)	+	59.79	(0.55)		7.65	(1e-3)		133.69	(0.23)	+	14.15	(2.8e-4)	-	50.16	(2.7e-4)	
8-Jul	618.19	(52.1)	-	52.74	(0.36)	-	2.18	(3.2e-3)		1090.64	(0.26)	+	1320.49	(1.5e-4)	+	195.82	(1.1e-3)	+
23-Jul	742.09	(67.63)		73.88	(0.56)		5.51	(1e-2)		1109.15	(0.28)		1355.00	(2.3-4)	+	305.88	(3.7e-4)	+
5-Aug	755.90	(69.35)		82.70	(0.51)		2.72	(9.7e-4)		167.90	(0.31)		74.42	(9.4e-4)		39.63	(3.7e-4)	
3-Sep	737.41	(70.9)		66.64	(0.55)		3.20	(4e-4)		337.54	(0.31)		210.01	(7.2e-4)		123.97	(4.5e-4)	
7-Oct	927.41	(74.44)	+	118.60	(0.51)	+	15.65	(1.7e-3)	+	56.88	(0.35)	-	1.03	(3e-7)	-	203.72	(4e-4)	-

Table 2.3. Variation across sampling dates biomass-specific potential enzyme activity. Pluses (+) and minuses (-) represent significant positive and negative deviations from the overall mean of a column, respectively. Means and standard errors; standard errors calculated with 22-24 observations per cell (12 plots x 2 depths).

Day	Specific BG Activity			Specific BX Activity			Specific CB Activity			Specific LAP Activity			Specific Ala Activity			Specific AAP Activity		
	nmol ug^{-1} MBC g^{-1} soil hr^{-1}																	
22-May	1.60	(0.007)		0.18	(0.002)		0.008	(2e-11)		0.01	(5e-7)	-	0.003	(2e-8)	-	0.06	(1e-6)	-
5-Jun	2.61	(0.007)	+	0.26	(0.002)	+	0.015	(9e-11)		0.78	(6e-6)		2.460	(1e-6)	+	0.05	(9e-7)	-
19-Jun	2.81	(0.009)	+	0.17	(0.002)		0.029	(2e-11)		0.30	(1e-6)		0.050	(9e-7)	-	0.15	(7e-7)	
8-Jul	0.78	(0.002)	-	0.07	(0.0004)	-	0.004	(2e-11)	-	1.37	(3e-7)	+	1.800	(3e-7)	+	0.27	(2e-6)	
23-Jul	1.60	(0.004)		0.17	(0.001)		0.019	(1e-10)		2.37	(6e-7)	+	3.180	(7e-7)	+	0.72	(1e-6)	+
5-Aug	2.59	(0.009)	+	0.29	(0.002)	+	0.012	(9e-12)		0.45	(3e-6)		0.280	(4e-6)		0.15	(2e-6)	
3-Sep	2.30	(0.007)		0.20	(0.002)		0.012	(6e-12)		0.83	(2e-6)		0.640	(2e-6)	+	0.38	(1e-6)	
7-Oct	1.89	(0.006)		0.25	(0.001)		0.043	(7e-11)	+	0.07	(2e-6)	-	0.002	(7e-10)	-	0.44	(9e-7)	+

Table 2.4. P-values from repeated measures ANOVA.

	MBC	MBN	MBC: MBN	NO_3^--N	NH_4^+-N	Total DIN	Native protease	Native Ammonification	BG activity	BX activity	CB activity	LAP activity	Ala activity	AAP activity
Day	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**0.000**	**<.0001**	**<.0001**	**<.0001**
Depth	0.130	0.283	0.694	**0.002**	0.098	**0.026**	0.842	0.315	**0.005**	**0.021**	0.054	**0.014**	**0.075**	0.141
Crop system	**<.0001**	**<.0001**	0.953	0.495	0.408	0.536	**0.008**	0.237	**0.013**	**0.016**	**0.015**	0.082	0.116	**0.049**
Day*Depth	0.445	0.076	0.432	**0.001**	**0.010**	**0.029**	0.472	0.996	0.667	0.571	0.300	0.152	**0.029**	0.240
Day*Crop system	0.962	0.965	0.722	0.447	0.272	0.493	0.131	0.487	0.403	0.108	0.555	0.197	0.403	0.062
Depth*Crop system	**0.006**	**0.001**	**0.014**	**0.037**	0.612	0.363	0.795	0.952	**<.0001**	**0.000**	**0.003**	**0.002**	0.057	**0.006**
Day*Depth*Crop system	0.998	0.991	0.735	0.750	0.288	0.906	1.000	0.396	**0.003**	0.092	0.091	0.069	**0.000**	0.147

Table 2.4 (continued). P-values from repeated measures ANOVA.

	Specific BG activity	Specific BX activity	Specific CB activity	Specific LAP activity	Specific Ala activity	Specific AAP activity
Day	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**
Depth	0.079	**0.027**	0.070	**0.020**	0.132	0.166
Crop system	**0.006**	**0.015**	**0.007**	0.069	0.068	**0.029**
Day*Depth	0.515	0.200	0.351	**0.038**	**0.008**	**0.048**
Day* Crop system	0.274	**0.021**	0.584	0.126	0.304	**0.046**
Depth*Crop system	**0.009**	**0.001**	**0.005**	**0.007**	0.198	**0.016**
Day*Depth* Crop System	0.060	0.053	0.052	**0.012**	**0.000**	0.065

Table 2.5. Mean microbial biomass, native protease activity, and native ammonification by crop system and depth, averaged across sampling dates. Error terms represent standard errors. Standard errors were calculated with observations of either 62-64 (for 0-20cm; 4 plots x 2 depths x 8 sampling dates) or 30-32 (for 0-10 cm and 10-20 cm; 4 plots x 8 sampling dates).

Crop System	Depth	MBC			MBN			MBC:MBN			Native protease activity			Native ammonification		
		ug g^{-1} soil									*nmol g^{-1} hr*					
2	0-10 cm	385.35	(1.07)	a[a]	41.84	(2.96)	a	8.95	(2.79)	a	1.63	(1e-10)	a[a]	33.72	(1e-4)	a
	10-20 cm	253.64	(1.07)	b	23.46	(2.38)	b	10.83	(2.78)	a	1.08	(1e-10)	a	26.05	(6e-5)	a
3	0-10 cm	383.82	(1.09)	a	39.34	(2.89)	a	9.86	(2.78)	a	2.89	(6e-10)	a	34.85	(4e-5)	a
	10-20 cm	393.66	(1.07)	a	41.55	(2.68)	a	9.48	(2.78)	a	4.14	(2e-10)	a	29.12	(4e-5)	a
4	0-10 cm	449.25	(1.08)	a	44.86	(2.69)	a	10.27	(2.78)	a	9.24	(6e-10)	a	22.56	(3e-5)	a
	10-20 cm	496.37	(1.08)	a	55.29	(2.87)	a	9.27	(2.77)	a	11.86	(5e-10)	a	14.76	(6e-5)	a
2	0-20cm	312.64	(1.06)	C[b]	31.90	(2.62)	B	9.82	(2.77)	A	1.33	(8.7e-12)	B[b]	29.69	(1e-5)	A
3		388.71	(1.06)	B	40.44	(2.44)	A	9.67	(2.76)	A	3.47	(3.3e-11)	AB	31.88	(1e-5)	A
4		472.22	(1.05)	A	49.91	(2.48)	A	9.75	(2.76)	A	10.48	(4.5e-11)	A	18.33	(1e-5)	A

a - Entries in a column that share a lowercase letter are not significantly different at P = 0.05 (Tukey's HSD)
b - Entries in a column that share an uppercase letter are not significantly different at P = 0.05 (Tukey's HSD)

Table 2.6. Mean inorganic N concentration by crop system and depth, averaged across sampling dates. Error terms represent standard errors. Standard errors were calculated with observations of either 62-64 (for 0-20cm; 4 plots x 2 depths x 8 sampling dates) or 30-32 (for 0-10 cm and 10-20 cm; 4 plots x 8 sampling dates).

Crop System	Depth	NO_3^--N			NH_4^+-N			Total DIN		
		ug g^{-1} soil								
2	0-10 cm	27.92	(1.9e-5)	a[a]	9.94	(1e-4)	a	42.60	(1.16)	a
	10-20 cm	11.32	(1.7e-5)	b	4.48	(1e-4)	a	20.68	(1.17)	a
3	0-10 cm	16.21	(4.2e-5)	ab	6.02	(2e-4)	a	26.14	(1.2)	a
	10-20 cm	14.26	(9e-6)	b	5.22	(1e-4)	a	22.55	(1.18)	a
4	0-10 cm	19.84	(1.9e-5)	ab	5.46	(1e-4)	a	28.74	(1.18)	a
	10-20 cm	14.51	(2e-5)	b	3.23	(1e-4)	a	20.79	(1.17)	a
2	0-20cm	18.24	(9e-6)	A[a]	6.79	(2.9e-5)	A	29.64	(1.13)	A
3		15.21	(6e-6)	A	5.61	(3e-5)	A	24.36	(1.13)	A
4		17.02	(5e-6)	A	4.23	(2.5e-5)	A	24.44	(1.12)	A

a - Entries in a column that share a lowercase letter are not significantly different at P = 0.05 (Tukey's HSD)
b - Entries in a column that share an uppercase letter are not significantly different at P = 0.05 (Tukey's HSD)

Table 2.7. Mean potential enzyme activity by crop system and depth, averaged across sampling dates. Error terms represent standard errors. Standard errors were calculated with observations of either 62-64 (for 0-20 cm; 4 plots x 2 depths x 8 sampling dates) or 30-32 (for 0-10 cm and 10-20 cm; 4 plots x 8 sampling dates).

Crop System	Depth	BG activity			BX activity			CB activity			LAP activity			Ala activity			AAP activity		
		nmol g^{-1} hr																	
2	0-10 cm	1145.34	(64.62)	a[a]	119.21	(0.38)	a	21.86	(2e-4)	a	678.77	(0.26)	a	316.85	(2e-3)	a	389.34	(3e-5)	a
	10-20 cm	333.06	(21.73)	d	14.82	(0.18)	b	1.32	(2e-7)	b	30.61	(0.2)	b	47.70	(9e-4)	b	15.17	(2e-4)	b
3	0-10 cm	877.48	(31.63)	abe	98.91	(0.18)	a	6.19	(5e-4)	ab	421.95	(0.32)	a	196.85	(2e-3)	ab	147.52	(1e-4)	ab
	10-20 cm	962.44	(51.83)	ab	109.34	(0.26)	a	8.51	(1e-3)	ab	384.47	(0.44)	a	183.86	(2e-3)	ab	170.22	(3e-4)	ab
4	0-10 cm	610.03	(42.01)	cde	58.61	(0.29)	ab	1.61	(7e-4)	b	146.68	(0.36)	ab	73.91	(9e-4)	ab	18.63	(2e-4)	b
	10-20 cm	740.52	(47.11)	bc	74.71	(0.25)	ab	2.11	(1e-3)	b	209.25	(0.37)	ab	77.09	(1e-3)	ab	52.10	(3e-4)	ab
2	0-20cm	739.20	(59.86)	AB[b]	54.53	(0.34)	B	5.37	(1.27)	AB	211.52	(0.17)	A	134.40	(3e-4)	A	99.55	(6e-5)	AB
3		919.96	(30.59)	A	104.06	(0.11)	A	7.26	(1.29)	A	402.92	(0.13)	A	190.26	(3e-4)	A	158.55	(4e-5)	A
4		675.27	(32.37)	B	66.41	(0.14)	AB	1.84	(1.3)	B	176.12	(0.13)	A	75.48	(2e-4)	A	31.99	(5e-5)	B

a - Entries in a column that share a lowercase letter are not significantly different at P = 0.05 (Tukey's HSD)

b - Entries in a column that share an uppercase letter are not significantly different at P = 0.05 (Tukey's HSD)

Table 2.8. Mean biomass-specific potential enzyme activity by crop system and depth, averaged across sampling dates. Error terms represent standard errors. Standard errors were calculated with observations of either 62-64 (for 0-20cm; 4 plots x 2 depths x 8 sampling dates) or 30-32 (for 0-10 cm and 10-20 cm; 4 plots x 8 sampling dates).

Crop System	Depth	Specific BG activity			Specific BX activity			Specific CB activity			Specific LAP activity			Specific Ala activity			Specific AAP activity		
		nmol ug^{-1} MBC g^{-1} soil hr^{-1}																	
2	0-10 cm	2.96	(0.008)	a[a]	0.33	(1e-3)	a	0.07	(7e-12)	a	1.49	(6e-7)	a	0.83	(4e-6)	a	0.98	(3e-8)	a
	10-20 cm	1.37	(0.003)	b	0.06	(8e-4)	b	0.01	(4e-15)	b	0.06	(8e-7)	b	0.17	(3e-6)	a	0.05	(4e-7)	b
3	0-10 cm	2.36	(0.004)	ab	0.26	(7e-4)	a	0.02	(1e-11)	ab	0.91	(1e-6)	a	0.56	(5e-6)	a	0.40	(3e-7)	ab
	10-20 cm	2.50	(0.005)	ab	0.29	(9e-4)	a	0.03	(2e-11)	ab	0.72	(2e-6)	ab	0.47	(5e-6)	a	0.42	(8e-7)	ab
4	0-10 cm	1.36	(0.005)	b	0.14	(9e-4)	ab	0.01	(7e-12)	b	0.21	(1e-6)	ab	0.16	(2e-6)	a	0.04	(5e-7)	b
	10-20 cm	1.51	(0.005)	b	0.16	(8e-4)	ab	0.01	(9e-12)	b	0.29	(2e-6)	ab	0.16	(2e-6)	a	0.10	(7e-7)	ab
2	0-20cm	2.09	(0.004)	AB[b]	0.17	(1e-3)	AB	0.02	(8e-12)	A	0.38	(4e-7)	A	0.40	(7e-7)	A	0.29	(1e-7)	AB
3		2.43	(0.002)	A	0.28	(4e-4)	A	0.03	(2e-12)	A	0.81	(3e-7)	A	0.51	(8e-7)	A	0.41	(9e-8)	A
4		1.43	(0.002)	B	0.15	(4e-4)	B	0.01	(7e-12)	B	0.25	(3e-7)	A	0.16	(4e-7)	A	0.07	(1e-7)	B

a - Entries in a column that share a lowercase letter are not significantly different at P = 0.05 (Tukey's HSD)

b - Entries in a column that share an uppercase letter are not significantly different at P = 0.05 (Tukey's HSD)

CHAPTER III
ROOT BIOMASS AND DECOMPOSITION OF BIOENERGY CROPS ACROSS A LANDSCAPE GRADIENT

Introduction

Fine roots are a primary source of soil organic matter in agroecosystems (SOM; Puget and Drinkwater, 2001; Rasse et al., 2005), and root decomposition represents a major pathway of root input into SOM as well as nutrient release for crop uptake. In agronomic systems, root tissues contribute ten times more to protected SOM than surface residues (Gale and Cambardella, 2000). In bioenergy systems, where aboveground biomass is aggressively harvested, and whose success is measured in part relative to a mandate for providing an environmentally preferable substitute for fossil fuels, root inputs are equally crucial to SOM maintenance and environmental benefits.

Bioenergy cropping systems, e.g., annual or perennial, differ widely in the ecosystem services they provide. Annual crops, typically corn, are the current standard for bioenergy production and excel at yielding high quantities of grain and stover. As the dominant landscape cover in many agricultural regions, annual cropping systems are accompanied by environmental costs such as soil erosion, nutrient loss, and depletion of SOM over time (Lal, 2004; Pimentel et al., 1995; Raymond et al., 2012). Integrating perennial bioenergy crops such as poplar,

switchgrass, miscanthus, and native plant mixtures such as prairie into agricultural landscapes has been proposed for perennials' ability to provide ecosystem services beyond production (Schulte et al., 2006). Relative to annual crops, perennials reduce soil erosion and nutrient exports (Tilman et al 2006, Glover et al 2010). Perennials also support higher concentrations of SOM than do annuals (Anderson-Teixeira et al., 2013; Buyanovsky et al., 1987). The mechanisms underlying maintenance of a higher SOM pool under perennials are attributed to belowground activity by roots.

Plant tissue chemistry is an important predictor of decomposition rates (Silver and Miya, 2001), however it is not clear whether annuals and perennial crops are easily categorized into non-overlapping categories of labile and recalcitrant root biomass. Following resource acquisition strategies, it has been hypothesized that annuals should produce nutrient-rich, quickly decomposing tissues that favor fast nutrient cycling, whereas perennials should favor more recalcitrant tissues (Wardle 2004). While this hypothesis has been supported for leaf tissues (Wardle et al., 1998), less work has been done regarding whether this hypothesis can be extended to root tissues. Some studies have found that, compared to annuals, perennial roots have lower nitrogen (N) concentrations (Birouste et al., 2012; Roumet et al., 2006), higher carbon to nitrogen (C:N) ratios (Birouste et al., 2012; Mapfumo et al., 2002; Shi et al., 2012), lower

soluble C concentrations (Shi et al., 2012), and higher hemicellulose concentrations (Birouste et al., 2012), which overall corroborates the idea that root tissues of perennial species are slower to decompose than those of annuals. In the limited number of direct comparisons between annual and perennial root decomposition, however, perennial root decomposition has been reported as variable by perennial species (Koteen et al., 2011; Shi et al., 2012). There has been even less direct comparison of decomposition rates between annual and perennial grasses in the context of managed systems.

The strategic integration of bioenergy systems into the landscape requires understanding of how their performance varies across the landscape. Landscape positions, defined according to their slope and elevation along a hillside, create variable conditions for production of aboveground biomass. In an Iowa watershed, long-term corn yields on the backslope were lower than yields on the footslope and toeslope (Cambardella et al., 2004). In a similar study at the Landscape Biomass experiment, switchgrass stand establishment on the backslope was lower than other landscape positions (Ontl et al., 2013). These differences in crop performance may track differences in soil nutrient retention, soil depth, or soil moisture across landscape positions. Steeper, more erosive landscape features, such as the backslope, have less potentially mineralizable N compared to other landscape positions, while depositional areas,

such as the footslope have more POM-C and POM-N (Cambardella et al., 2004). This suggests there is higher soil quality in depositional landscape positions compared to erosive landscape positions. Additionally, water is more likely to pool in depositional areas of the landscape, creating wetter but cooler soil conditions (Moorman et al., 2004), which may decrease rates of decomposition. These suites of soil properties characterize the landscape positions of agricultural regions, yet their effect on root biomass and decomposition has not been well studied.

In order to address root biomass and decomposition between annual and perennial bioenergy crops, and to describe their variability by landscape position, we used a toposequence of corn and switchgrass and measured *in situ* root decomposition across one year. We hypothesized that switchgrass, compared to corn, would have 1) greater standing root biomass, 2) higher C:N, and 3) slower rates of decomposition, and that these effects would override any influence of landscape in determining quantity of root input decomposed. We also hypothesized that 4) root biomass of each crop would be lower on more erosive elements, such as the backslope or summit, compared to the footslope and that 5) decomposition rates would be slower on wetter landscape positions, ie, the footslope, than backslope and summit. Overall, we sought to describe the quantity of root biomass input to soil through decomposition processes over 1

year in annual and perennial bioenergy cropping systems, and the influence of landscape on these processes.

Methods

Field Site

This study was conducted as part of the Landscape Biomass Project, located at Iowa State University's Uthe Research and Demonstration Farm in Boone County, Iowa (41°55' N; 93°45' W). The experiment consists of a randomized complete block design with bioenergy cropping systems replicated three times at each of five landscape positions. We studied cropping systems on three landscape positions (summit, backslope, and toeslope) situated across a topographic gradient from 325-m to 305-m elevation. Plots measuring 0.05 ha were established in fall 2008 on land previously in corn-soybean rotation. Soils at the site are classified as fine-loamy Hapludoll Mollisols. Among the three landscape positions studied here, soil N, SOC, depth of A horizon, and POM were statistically equivalent (Ontl et al., 2013). There was a trend toward higher POM on the toeslope (0.226 ± 0.018 vs 0.230 ± 0.017 and 0.199 ± 0.003 g kg^{-1} for toeslope, summit, and backslope, respectively) and a trend toward deeper A horizon on the toeslope (46.5 ± 9.7 vs 33.0 ± 6.2 and 37.8 ± 6.5 cm for toeslope, summit and backslope, respectively) (Ontl et al., 2013). Soil drainage classes varied among landscape positions. All

three landscape positions included Clarion soils, which are classified as moderately-well drained, and were the only soil class present on the backslope. Soils on the summit were Nicollet (somewhat poorly drained) and Zenor (excessively drained). Soils on the toeslope were Spillville (somewhat poorly drained) (Ontl et al., 2013). For a complete site description, see Wilson et al. (2014).

Two bioenergy cropping systems were evaluated in this study: switchgrass (*Panicum virgatum* L., cv: 'Cave-In-Rock') and continuous corn (*Zea mays* L.), which were sampled between August 2011 and August 2012. Nitrogen fertilization rates were based on nutrient demands of crops (Vogel et al., 2002); in 2011 corn and switchgrass were fertilized at rates 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}, and were managed without tillage. Weather conditions were measured approximately 15 km from the site. Fifty-year average annual precipitation is 844 mm, and mean annual air temperature is 9°C. For the year approximating the study period, 1 August 2011 – 31 July, 2012, total precipitation was 626 mm and mean air temperature was 12°C.

Root decomposition cores

To estimate fine root decay rates, we used the intact core method, following Dornbush et al. (2002). Root distribution in row crops is highly heterogeneous, and to obtain a representative estimate we used a nonrandom sampling scheme: for every core adjacent to a corn plant, three were placed midway between corn rows, a ratio which protects from overestimating root density at the plot scale, as would a 1:1 ratio of row:interrow sampling (Buczko et al., 2008). Switchgrass cores were placed randomly in plots. A set of 216 cores was taken in August 2011, and one third of cores (72 cores) was used to estimate initial biomass of fine roots (12 cores per crop/landscape position). While corn root cores captured only the roots produced in the 2011 growing season, switchgrass root cores included roots produced in previous growing seasons, possibly dating to switchgrass seeding in May 2009. Two thirds of cores were left in the field for collection 1 month and 1 year after collection of 'initial' cores, however due to soil movement, only 33 out of 72 cores were recovered for the one-year time point. Loss of cores did not prevent statistical analysis because all plots were represented by at least one core. While in the field, soil cores were placed in 25 cm plastic sleeves, which accommodated a 20 cm soil core above 5 cm of sand, used for drainage. Both ends of the tube were fitted with 160 μm polyethylene mesh to allow passage of water and gas while discouraging root ingrowth. After removal from the field, soil cores were stored at 4°C and processed within one week by washing

over 250 μm mesh for 3 hours. Remaining material was oven-dried at 65°C and then hand-sorted in deionized water to remove sand and debris from roots. Crown nodes were also removed, if present. Roots were again oven-dried at 65°C, weighed for total mass, and ground in a ball-mill for chemical analyses.

Chemical Analyses

Total fine root C and N were measured using a Carlo Erba NA 1500 Elemental Analyzer (CE Instruments, Milan, Italy). Root mass on a g m^{-2} basis was calculated as (root biomass g / 883 cm^{-3} per decomposition core) x (20 cm depth) x (10,000 cm^{-2} / m^{-2}). Mass of C and N in fine root was calculated as total root mass multiplied by the concentration of C or N.

Statistical Analyses

All analyses were run in R v. 3.0.1 (R Core Team, 2013). Replicate cores within plots were averaged before analysis, and all data were transformed to fit assumptions of normality. We tested for differences in root biomass, root C, root N, and root C:N using analysis of variance (ANOVA) with main effects for sampling day, landscape position, and cropping system, as well as each of their interactions. Models were initially tested with block as an error term in a mixed effects linear model; however, these models were qualitatively the same as ANOVAs without a

random term. Therefore, we used the simpler model and Tukey's Honestly Significant Difference for pairwise comparisons after observing significant factors within each ANOVA. To test decay rates of fine roots, nonlinear least-squares estimates for decay parameters in exponential decay functions were produced using the nls function in R. Root variables from 1 month and 1 year samplings were first normalized by initial root variables, and then root decay was estimated as

$$M(t) = M(0)e^{\beta t} \quad (1)$$

where *M(t)* is litter mass at time t, and M(0) is initial litter mass, and ß is litter decomposition rate. Differences in ß were tested with ANOVA, with crop and landscape as main effects as well as their interaction. Significance was determined at $\alpha = 0.05$.

Results

On a land area basis, root biomass, root C, root N, and root C:N ratios at all sampling dates were higher under switchgrass compared to corn ($P<0.0001$; Table 3.1). Averaging over time, both corn and switchgrass varied by landscape position. Corn at the summit had significantly lower root biomass ($P<0.0006$), root C ($P<0.0106$), and root N ($P<0.001$) when

compared to corn at other landscape positions (Fig. 3.1A). Switchgrass root biomass and root C were significantly lower on the backslope than on other landscape positions (P=0.003 and 0.039 respectively). Switchgrass but not corn root C:N was sensitive to landscape position (Fig. 3.1A), and was lower on the backslope than other landscape positions. Corn root C:N was stable during decomposition, while switchgrass root C:N decreased between one month and one year (P<0.007).

Quantity of root biomass decomposed after 1 year differed by crop and landscape position. Averaging across landscape positions, switchgrass decomposed twice the root biomass of corn (98.84 vs 47.76 g m^{-2}), however N released from root biomass was similar between crops (0.67 and 0.7 g N m^{-2} for corn and switchgrass, respectively), owing to separation in initial C:N ratios (24.5 vs 57 for corn and switchgrass, respectively). At the summit, corn root biomass decomposed was approximately half of that on backslope and toeslope (30 vs 53.1 and 60.2 g m^{-2} on summit, backslope, and toeslope, respectively). Switchgrass root decomposed at the backslope was lower than on the summit or toeslope (84.4 vs 91.4 and 120. 7 g m^{-2} for backslope, summit, and toeslope, respectively). Decay functions for root variables show that corn root biomass, root C, and root N decomposed more quickly than switchgrass (Fig. 3.2, Table 3.3). Decay rates were not sensitive to landscape position (P >0.6).

Discussion

Fine roots are an important source of soil organic matter (Gale and Cambardella, 2000; Gale et al., 2000), especially in bioenergy cropping systems where aboveground biomass is exported for feedstock rather than returned to soil. By estimating root decomposition, this study addresses potential root input to SOM and nutrient release from decomposition from annual and perennial bioenergy crops. We found that, compared to corn, switchgrass produced greater standing root biomass, which decomposed more slowly. Nevertheless, switchgrass root biomass inputs to soil were greater than those of corn, while nitrogen release was on par with that of corn.

Research suggests that for perennials to persist across seasons, and to be relatively conservative of nutrients, they have greater investment in root biomass of lower quality compared to annuals (Mapfumo et al., 2002; Roumet et al., 2006). Our findings, although perhaps novel for a bioenergy system of highly managed crops, are consistent with these ecological paradigms. The faster decomposition rates of the annual crop, corn, corresponded to its lower C:N ratio. It should be noted, however, that C:N ratio, while an good predictor of root decomposition rates across broad taxonomic groups (i.e., conifer, graminoid, broad leaf, Silver and Miya, (2001)), has not often been found to be a strong predictor of decomposition rates

among species as closely related as perennial grasses (Gorissen and Cotrufo, 2000; Vivanco and Austin, 2006). Between and among crop species, specific controls over decomposition rates of fine roots may also include root architecture (de Graaff et al., 2013), or parameters of root quality not measured here such as concentrations of lignin or other phenolic compounds, but in general are yet to be elucidated. Our findings suggest that annual and perennial bioenergy crops may be divergent enough in root tissue quality for C:N ratio to serve as a useful predictor of their relative decomposition rates.

Despite slower rates of decomposition, over a year switchgrass decomposed more root biomass than did corn, and, at then end of a year, 13 times more switchgrass root biomass remained yet to decompose. These patterns in belowground biomass allocation are consistent with those observed under other perennial bioenergy systems compared to corn (Anderson-Teixeira et al., 2013), and provide a mechanism for SOC accrual typically witnessed under perennial systems (Frank et al 2004). It is important to note though that root biomass and root decomposition may not necessarily reflect soil SOM accumulation, as the mechanisms of SOM protection are complex and depend on many factors (e.g., stabilization efficiency, soil temperature, and soil carbon saturation). Perhaps most of interest in comparing crop choice at field scale is the concept of stabilization efficiency, which holds that assimilation of organic

input by microbes biomass is highly dependent on its C:N ratio. If these principles hold true for roots, switchgrass may move into protected SOM with less efficiency, as more biomass is channeled to overflow metabolism and respiration compared to corn. Nevertheless, root biomass has been found to predict SOM accumulation (Frank et al., 2004; Zan et al., 2001), and studies showing SOC increases under perennial cropping systems relative to annual systems suggest that even though stabilization efficiency per unit root mass may be higher with lower C:N ratio (as in annuals), differences in root biomass between perennial and annual cropping systems outweigh potential variability in stabilization efficiency.

For both crops, landscape position drove variability in root biomass, and in general variability supported the idea that drier, more erosive features, such as the summit and backslope, sustain less root biomass, while wetter, more depositional features promote more root biomass. An alternative framework for predicting root biomass would hold that drier soils on the summit would encourage root growth, however at this site moisture may have stunted overall corn productivity. Root biomass data across multiple growing seasons would be needed to test either of these hypotheses. Overall, root biomass at this site appears to be more sensitive to landscape position than aboveground yield; across years 2009-2011, aboveground yield of switchgrass and corn at this site were relatively stable across landscape positions (Wilson 2014). The significant

influences of landscape position on root biomass suggest that crop plants' belowground productivity is more adaptive to environmental cues than is their aboveground productivity. It should be noted, however, that aboveground yields at this site are more sensitive to year and associated weather than to landscape positions, and in some but not all years the effect of crop varies by landscape (Wilson, 2014). Data presented here should thus be interpreted with the understanding that similar patterns may arise in root biomass – i.e., the interaction of crop and landscape may not be consistent across growing seasons.

In contrast to root biomass, and contrary to our hypotheses, rates of root decomposition (expressed as a percentage of initial biomass), were stable across landscape position. As root biomass differed across the landscape, while rates root decomposition were unaffected, root inputs via decomposition corresponded to root biomass as it varied by landscape position. Altered root input by landscape position may point to a feedback mechanism in which poorer quality soils on erosive features maintain relatively low root biomass, which in turn maintains less SOM, whereas depositional areas with deeper, richer soils and higher soil moisture support greater plant input and thus may accrue SOM more rapidly. Stable root decomposition rates across the landscape counter our hypothesis, but are supported by the fact that potential factors governing decomposition, such as microbial biomass, enzyme activity, and respiration at this site

were stable across landscape positions in 2011 (Hargreaves and Hofmockel, 2013). Overall, our findings suggest that landscape position affects root growth, but that plant traits govern decomposition rate. A further implication of these findings is that at different landscape positions within the same cropping system, the turnover time of roots is similar (Larreguy et al., 2011). However, differences in root biomass and root input between crops overwhelmed the effect of landscape position in determining root biomass and inputs; even the least productive switchgrass position, the backslope, had 2.3 times more root biomass and 1.4 times more root input than most productive corn position, the toeslope. Heterogeneity across landscapes may tip the scales of performance for more closely related annual crops, however for crops as widely divergent as corn and switchgrass, our data show that landscape position is secondary to crop in determining root biomass and root input.

Conclusions

This study provides a side-by-side comparison of decomposition rates of annual and perennial bioenergy systems across a landscape gradient. Irrespective of landscape position, switchgrass produced more root biomass, and switchgrass roots decomposed more slowly than did corn roots. With crops, root production varied across landscape positions, however decay

rates were constant. Overall, root decomposition rates were more sensitive to crop and associated differences in root tissue quality (C:N ratio) than to landscape position.

Figures

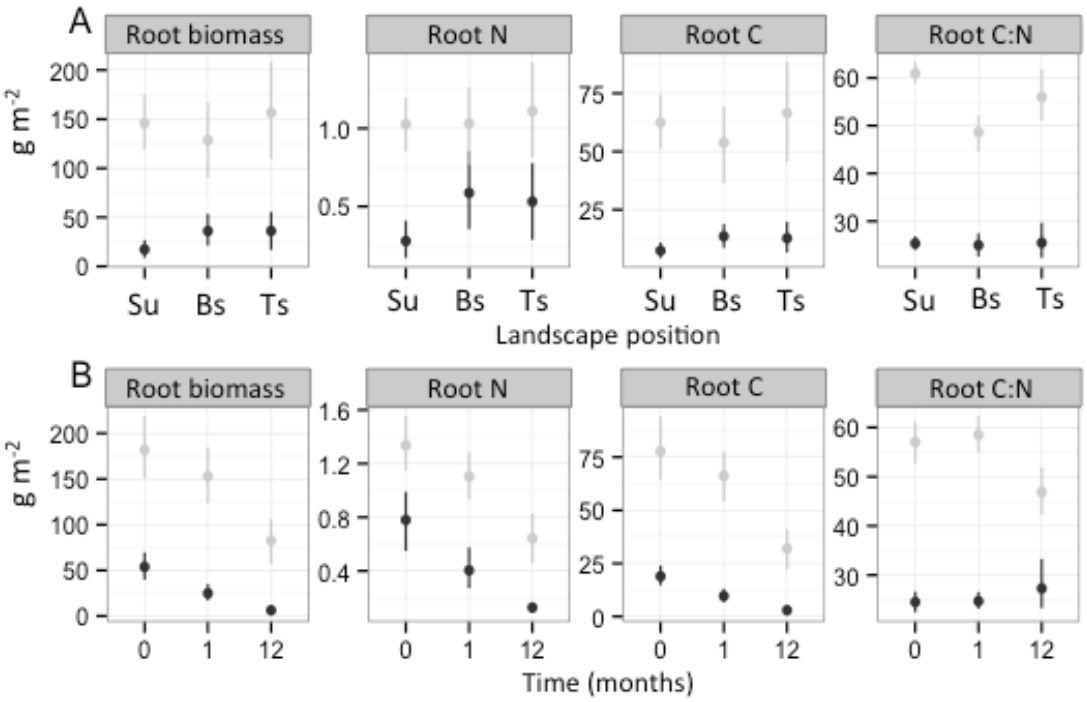

Figure 3.1. Effects of crop by landscape position (A) and by sampling date (B) on root biomass, root N, root C, and root C:N ratio. Points on each plot represent mean values for corn (black) and switchgrass (gray), and bars represent bootstrapped 95% confidence intervals. Landscape positions: Su = summit, Bs = backslope, Ts = toeslope.

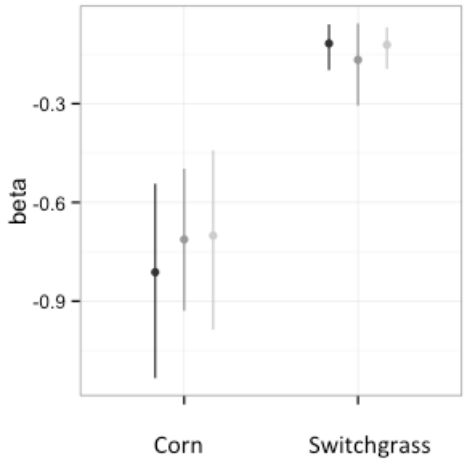

Figure 3.2. Exponential decay constants for root biomass (black), root N (dark gray), and root C (light gray) under each cropping system. Beta values represents decay rates as a percentage of standing root biomass; beta values farther from zero indicate faster decay rates. Points on each plot represent mean values for each variable and bars represent bootstrapped 95% confidence intervals.

Tables

Table 3.1. Mean root variables for each sampling date, landscape position, and cropping system. All root variables represent 0-20cm. Error terms represent standard errors.

Time	**Position**	**Crop**	**Root biomass**		**Root N**		**Root C**		**Root C:N**	
months			*g m $^{-2}$*							
0	Summit	Corn	32.30	(6)	0.40	(0.1)	12.00	(2)	26.4	(0.8)
0	Summit	Switchgrass	176.20	(20.7)	1.20	(0.2)	74.90	(8.1)	62.6	(2.8)
0	Backslope	Corn	61.20	(11.6)	1.00	(0.2)	21.80	(3.3)	23.7	(2.6)
0	Backslope	Switchgrass	157.20	(22.2)	1.30	(0.1)	67.50	(8.8)	51.7	(2.5)
0	Toeslope	Corn	68.00	(17.2)	0.90	(0.1)	23.00	(5.8)	23.4	(2.6)
0	Toeslope	Switchgrass	211.90	(49.3)	1.50	(0.3)	90.70	(22.3)	56.8	(4.7)
1	Summit	Corn	12.20	(1)	0.20	(0)	4.90	(0.5)	24.9	(1.6)
1	Summit	Switchgrass	135.80	(4.4)	1.00	(0)	58.40	(2)	60.1	(0.6)
1	Backslope	Corn	29.50	(1)	0.50	(0.1)	11.80	(0.5)	25.4	(2.6)
1	Backslope	Switchgrass	155.90	(29.7)	1.20	(0.1)	68.20	(12.4)	52.9	(2.4)
1	Toeslope	Corn	32.70	(13.4)	0.50	(0.2)	12.30	(4.6)	23.9	(1)
1	Toeslope	Switchgrass	166.80	(43.4)	1.10	0.3	72.10	(18.8)	62.4	(4.5)
12	Summit	Corn	2.30	(0)	0.00	NA	1.10	NA	23.6	NA
12	Summit	Switchgrass	84.80	NA	0.60	NA	36.90	NA	58.1	NA
12	Backslope	Corn	8.10	(0.7)	0.20	(0)	3.90	(0.4)	26.3	(2.5)
12	Backslope	Switchgrass	72.80	(29.5)	0.60	(0.2)	25.70	(10.2)	41.2	(2.2)
12	Toeslope	Corn	7.80	(0.3)	0.10	(0)	3.00	(0)	29.2	(6.1)
12	Toeslope	Switchgrass	91.20	(15.2)	0.70	(0.1)	36.70	(8.5)	48.8	(2.3)

Table 3.2. ANOVA tables for root variables on a g m^{-2} basis.

Root biomass		**Df**	**Sum Sq**	**Mean Sq**	**F**	**P-value**
	Time	2	18.39	9.20	62.00	**<.0001**
	Position	2	3.81	1.90	12.80	**<.0001**
	Crop	1	43.23	43.23	291.52	**<.0001**
	Time*Position	4	0.46	0.12	0.78	0.547
	Time*Crop	2	2.99	1.50	10.09	**0.0004**
	Position*Crop	2	1.92	0.96	6.46	**0.0044**
	Time*Position*Crop	4	0.25	0.06	0.42	0.796
	Residual	32	4.74	0.15		
Root N						
	Time	2	11.39	5.69	46.78	**<.0001**
	Position	2	2.44	1.22	10.00	**0.00044**
	Crop	1	13.72	13.72	112.73	**<.0001**
	Time*Position	4	0.06	0.02	0.13	0.969
	Time*Crop	2	1.89	0.94	7.76	**0.002**
	Position*Crop	2	1.49	0.75	6.14	**0.006**
	Time*Position*Crop	4	0.20	0.05	0.41	0.801
	Residual	31	3.77	0.12		
Root C						
	Time	2	13.24	6.62	45.57	**<.0001**
	Position	2	1.68	0.84	5.80	**0.007**
	Crop	1	43.78	43.78	301.33	**<.0001**
	Time*Position	4	0.12	0.03	0.20	0.934
	Time*Crop	2	1.58	0.79	5.45	**0.009**
	Position*Crop	2	1.75	0.88	6.03	**0.006**
	Time*Position*Crop	4	0.39	0.10	0.68	0.614
	Residual	31	4.50	0.15		
Root C:N						
	Time	2	114	57	2.17	0.131
	Position	2	216	108	4.11	**0.026**
	Crop	1	10767	10767	409.19	**<.0001**
	Time*Position	4	67	17	0.64	0.64
	Time*Crop	2	403	202	7.66	**0.002**
	Position*Crop	2	198	99	3.76	**0.035**
	Time*Position*Crop	4	87	22	0.83	0.517
	Residual	31	816	26		

Table 3.3. ANOVA tables for decay constants for root biomass, root N, and root C.

Root biomass		**Df**	**Sum Sq**	**Mean Sq**	**F**	**P-value**
	Landscape	2	0.101	0.050	0.351	0.711
	Crop	1	2.174	2.174	15.131	**0.002**
	Landscape*Crop	2	0.019	0.010	0.067	0.935
	Residual	12	1.724	0.144		
Root N						
	Landscape	2	0.065	0.032	0.312	0.737
	Crop	1	1.341	1.341	12.988	**0.004**
	Landscape*Crop	2	0.019	0.010	0.094	0.911
	Residual	12	1.239	0.103		
Root C						
	Landscape	2	0.131	0.066	0.505	0.616
	Crop	1	1.513	1.513	11.636	**0.005**
	Landscape*Crop	2	0.035	0.018	0.136	0.874
	Residual	12	1.560	0.130		

CHAPTER IV

GENERAL CONCLUSIONS

Multifunctional agriculture provides an important framework for working toward the dual goals of production and environmental quality. This thesis explores dynamics of two ecosystem services, nitrogen retention and cycling and SOM maintenance, as they are influenced by two multifunctional agricultural systems. Chapter 2 describes nitrogen cycling in diverse and less diverse crop rotations, showing that that diversification increased microbial biomass and rates of proteolysis of native soil. Contrary to our hypotheses, and previous work in diversified systems, inorganic N pools were not different between crop systems. However, the relatively low inorganic N inputs to more diverse rotations during the study season suggest that soil N mineralization was a more important pathway in maintaining the DIN pool while also supplying crops with N.

Chapter 3 describes root biomass and root decomposition of two bioenergy cropping systems, a perennial (switchgrass) and an annual (corn) across a landscape gradient. Root biomass was more sensitive to crop that to landscape positions. Across all landscape positions, switchgrass root biomass was greater than corn root biomass. Switchgrass C:N ratios were higher than those for corn, which was likely an important factor governing the slower rates of

decay in switchgrass roots. Root decay rates were not influenced by landscape position. Root inputs to SOM, estimated as biomass lost over a year, were greater under switchgrass compared to corn.

APPENDIX

POTENTIALLY MINERALIZABLE SOIL NITROGEN IN A LONG-TERM INCUBATION AS INFLUENCED BY CROPPING SYSTEM DIVERSITY

Background

In order to test potentially mineralizable N from soils under diverse and less diverse crop rotations, an incubation was initiated following the method of Nadelhoffer (1990). Soils were collected from three cropping systems experiments in the Midwestern United States: the Iowa State University Marsden Farm, in Boone County, Iowa; the University of Wisconsin Wisconsin Integrated Cropping Systems Trail (WICST) in Columbia County, Wisconsin; and the University of Minnesota Variable Input Crop Management System (VICMS) in Redwood County, Minnesota. At each site, cropping systems representative of diverse and less diverse crop rotations (i.e., short and long, respectively) were used in a 400-day incubation. During the incubation soils were leached 12 times with a dilute nutrient solution containing no nitrogen (Nadelhoffer, 1990), and the leachate analyzed colorimetrically for NO_3-N concentrations.

Results

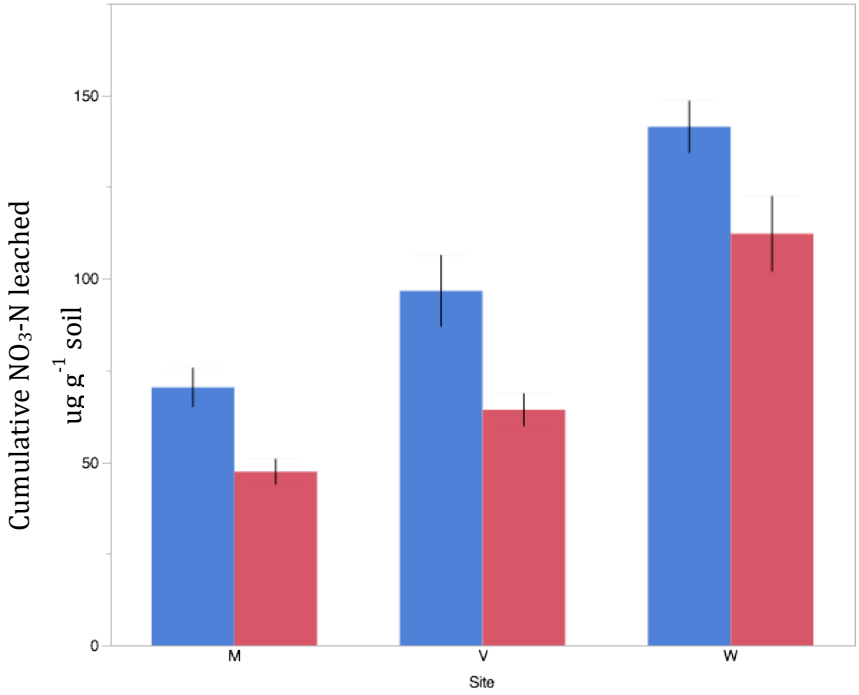

Figure A.1. Mean cumulative NO_3-N leached from short (red) and long (blue) rotations in a 400 day incubation. Soil from Marsden Farm (M), VICMS (V), and WICST (W). Error bars represent standard errors.

Table A.1. ANOVA table for cumulative NO_3-N leached.

	Nparm	df	DfDen	F Ratio	P-value
Site	2	2	16	55.0685	<.0001
Crop System	1	1	16	28.9908	<.0001
Site*Crop System	2	2	16	0.718	0.5028

Table A.2. Estimates of cumulative NO_3-N leached by site and crop system (L = long rotation, S = short rotation). Error terms represent standard errors.

Site	Crop System	Cumulative NO_3-N leached ug g^{-1} soil	
Marsden	L	70.31	(5.36)
Marsden	S	47.34	(3.54)
VICMS	L	96.63	(9.78)
VICMS	S	64.18	(4.52)
WICST	L	141.37	(7.16)
WICST	S	112.21	(10.33)

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