Plant roots and GHG mitigation in native perennial bioenergy cropping systems

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Abstract

Native perennial bioenergy crops can mitigate greenhouse gases (GHG) by displacing fossil fuels with renewable energy and sequestering atmospheric carbon (C) in soil and roots. The relative contribution of root C to net GHG mitigation potential has not been compared in perennial bioenergy crops ranging in species diversity and N fertility. We measured root biomass, C, nitrogen (N), and soil organic carbon (SOC) in the upper 90 cm of soil for five native perennial bioenergy crops managed with and without N fertilizer. Bioenergy crops ranged in species composition and were annually harvested for 6 (one location) and 7 years (three locations) following the seeding year. Total root biomass was 84% greater in switchgrass (*Panicum virgatum* L.) and a four-species grass polyculture compared to high-diversity polycultures; the difference was driven by more biomass at shallow soil depth (0–30 cm). Total root C (0–90 cm) ranged from 3.7 Mg C ha^{-1} for a 12-species mixture to 7.6 Mg C ha^{-1} for switchgrass. On average, standing root C accounted for 41% of net GHG mitigation potential. After accounting for farm and ethanol production emissions, net GHG mitigation potential from fossil fuel offsets and root C was greatest for switchgrass (−8.4 Mg CO2e ha^{-1} yr^{-1}) and lowest for high-diversity mixtures (−4.5 Mg CO2e ha^{-1} yr^{-1}). Nitrogen fertilizer did not affect net GHG mitigation potential or the contribution of roots to GHG mitigation for any bioenergy crop. SOC did not change and therefore did not contribute to GHG mitigation potential. However, associations among SOC, root biomass, and root C : N ratio suggest greater long-term C storage in diverse polycultures vs. switchgrass. Carbon pools in roots have a greater effect on net GHG mitigation than SOC in the short-term, yet variation in root characteristics may alter patterns in long-term C storage among bioenergy crops.

Keywords: biofuel, carbon sequestration, diversity, greenhouse gases, prairie, species richness, switchgrass

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Introduction

The concentration of carbon dioxide (CO_2) and other greenhouse gases (GHG) in the atmosphere are increasing and altering global climate (IPCC, 2014). Two pathways to mitigate atmospheric GHG emissions include (1) reducing fossil fuel consumption and CO_2 related emissions and (2) increasing terrestrial carbon (C) sinks (Pacala & Socolow, 2004). Perennial grassland bioenergy crops can mitigate GHG emissions via both pathways by displacing fossil fuel use with renewable biofuels and sequestering C in roots and soils (Gelfand *et al.*, 2013; Tilman *et al.*, 2006). However, the relative contribution of fossil fuel displacement and belowground C sequestration to net GHG mitigation potential is not well known, especially in response to management options such as plant diversity and N fertilization.

Perennial grassland bioenergy systems – ranging from grass monocultures to diverse polycultures – can produce bioenergy with greater net energy yields compared to conventional biofuel crops such as corn (*Zea mays* L.; Tilman *et al.*, 2006; Schmer *et al.*, 2008). Switchgrass (*Panicum virgatum* L.) was identified as a widely adapted, high-yielding native perennial bioenergy crop during the late 20th century and is currently undergoing selection and breeding for greater biomass yields throughout the United States (Casler & Vogel, 2014). Mixing switchgrass with other grasses, legumes, and forbs in polycultures can increase diversity and other ecosystem services including soil C sequestration (Fornara & Tilman, 2008) compared with conventional biofuel systems (Tilman *et al.*, 2006). While ecological theory and experimentation also show that communities with more species should produce more biomass (Tilman *et al.*, 1996; Hector *et al.*, 1999; Loreau *et al.*, 2001), switchgrass monocultures often yield more biomass than polycultures in agricultural settings (Picasso *et al.*,

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2008; Zamora *et al.*, 2013; Jungers *et al.*, 2015b; Zilverberg *et al.*, 2014). The high performance of switchgrass monocultures is partly explained by the selection and breeding of germplasm along with advancements in agronomics (Casler, 2005). There is a persisting need to improve species selection and agronomic management of diversity and fertility of such polycultures to efficiently produce both bioenergy and ecosystem services including C sequestration.

Nitrogen is a limiting nutrient in most native grassland ecosystems, and thus, N fertilization has been frequently used to increase biomass yields in perennial grassland bioenergy systems (Lemus *et al.*, 2008; Kering *et al.*, 2011; Jarchow & Liebman, 2013; Jungers *et al.*, 2015a,b). Fertilizing grasslands also enhances net energy gain because greater biomass yields from fertilization provide more energy than is used in producing and applying N fertilizer (Schmer *et al.*, 2008). Nitrogen fertilization had a similar affect on the GHG balance of perennial grassland bioenergy systems; fertilized grasslands offset more C with increased biofuel yields despite additional emissions related to production and application of N fertilizer (Gelfand *et al.*, 2013). Negative environmental consequences related to N fertilization are less for perennial than annual bioenergy crops. Once established, perennial bioenergy crops leached ten times less nitrate (McIsaac *et al.*, 2010) and emitted significantly less nitrous oxide (Smith *et al.*, 2013) compared with a corn–soybean rotation in the Upper Midwest, USA. However, long-term N addition can reduce grassland diversity and have potential consequences for carbon storage (Fornara & Tilman, 2012) and productivity (Isbell *et al.*, 2013), which emphasizes the need to better understand these interactions.

Understanding the mechanisms and consequences of belowground C and nutrient dynamics in grassland bioenergy systems can improve agronomic management to enhance GHG mitigation. Soil organic carbon (SOC) has been identified as an important C sink for GHG mitigation (Lemus & Lal, 2005) and is dependent on the quantity and quality of root biomass inputs (Agostini *et al.*, 2015). During the early years of perennial bioenergy crop establishment, a significant amount of C can be transferred from the atmosphere to the relatively labile belowground pool of roots (Frank *et al.*, 2004). Root decomposition results in a complex set of feedbacks and interactions that can have both positive and negative consequences for GHG mitigation. Nutrient mineralization from root decomposition stimulates plant productivity (De Graaff *et al.*, 2010), which is especially important for sustaining yields in annually harvested, low-input systems (Garten & Wullschleger, 2000). However, GHGs are emitted in the form of CO_2 during decomposition, which can increase in the presence of mineralized nutrients and result in a net loss of ecosystem C (Mack *et al.*, 2004; Dijkstra *et al.*, 2013). During decomposition, some fraction of root C is also transferred to recalcitrant SOC for long-term storage (Horwath, 2007). Therefore, the relative importance of these processes in relation to net GHG mitigation partly depends on the timescale of assessment (Knops & Tilman, 2000). A range of SOC change values have been reported in both short-and long-term studies (Ma *et al.*, 2000; McLauchlan *et al.*, 2006; Adler *et al.*, 2009; Gelfand *et al.*, 2013) which emphasizes the importance of our need to understand the factors driving these processes (e.g., root C to N ratio (C : N), root and shoot C allocation, management activities) and how they interact through time.

The relative effects of plant species composition and N fertilization – alone and in combination – on net GHG mitigation potential in bioenergy feedstock systems are not well known. Long-term research shows that diverse polycultures of native perennial grassland plant species accumulated more soil C than monocultures of the same species (Fornara & Tilman, 2008; Steinbeiss *et al.*, 2008; Cong *et al.*, 2014). Complementarity among species of varying functional traits can enhance SOC storage by increasing organic matter inputs from above- and belowground plant communities (Fornara & Tilman, 2008). Nitrogen fertilization increases aboveground, but not belowground, biomass in grassland bioenergy crops (Jarchow *et al.*, 2012), thus improving GHG mitigation by increasing fossil fuel offsets rather than belowground C storage. Besides altering biomass quantity, both species diversity and N fertilizer can alter SOC dynamics by influencing biomass quality. The C : N of biomass inputs like roots is important for determining decomposition rate and SOC storage and has been negatively correlated with species diversity and N fertilizer rates (Mueller *et al.*, 2013). As species composition and N fertilizer are important management factors in grassland bioenergy systems, it is essential to understand how they interact to influence above- and belowground productivity and the relative impact of these variables on net GHG mitigation.

Our objectives were to (1) determine the effects of plant species composition and N fertilization on root biomass and soil C; (2) determine the relative and absolute contribution of C stored in soil, belowground biomass, and fossil fuel offsets (i.e., aboveground biomass) to net GHG mitigation potential for all systems; and (3) compare the net GHG mitigation potential of five bioenergy cropping systems ranging in species diversity and managed with vs. without N fertilizer. Rather than comparing productivity and C dynamics from random assemblages of species across a diversity gradient like most dedicated diversity experiments, this study

presents findings from a dedicated bioenergy experiment that was designed and managed (no hand weeding) to resemble practical bioenergy cropping systems, replicated across a range of growing conditions, and span 7–8 years of production.

Methods

Study sites

This study was established in the spring of 2006 at Becker, Lamberton, and Waseca, Minnesota; and in 2007 at Crookston, Minnesota, USA. Data used for this study were collected from a subset of locations and treatments of a larger study (See Mangan *et al.*, 2011). We selected these locations because they represent a range of growing conditions including soil type, precipitation, and temperature gradients (Table 1). Data were analyzed for all available years at each location (2006–2013 or 2007–2013). Soils at Becker are sandy, mixed, frigid Entic Haploboroll, while soils at Crookston are coarse-silty over clayey, mixed over smectitic, superactive, frigid Aeric Calciaquolls. Soils at Lamberton and Waseca are Aauic Hapludolls and Typic Endoaquolls, respectively, and both described as fine-loamy, mixed, superactive, and mesic. The previous crop was corn (*Zea mays* L.) at Waseca and Lamberton, rye (*Secale cereale* L.) at Becker, and spring wheat (*Triticum aestivum* L.) at Crookston.

The experiment was a randomized complete block in a split-plot arrangement with three replications per location. Nitrogen fertilizer was applied to the main plots at either 0 or 67 kg N ha^{-1} as ammonium nitrate annually in spring from 2008 to 2013. Fertilization was delayed until 1 or 2 years after the seeding year to ensure establishment of sown species and prevent invasion of noxious weeds. Species mixture treatments were assigned to subplots (9 m^2; 3 m × 3 m) and included: switchgrass monoculture; four-species grass mix; eight species grass/legume mix; 12 species grass, forb, and legume mix; and high-diversity 24 species mix of species from all three functional groups (i.e., grasses, legumes and nonleguminous forbs; See Table S1 for complete list). All species represent unimproved ecotype seed collected in the upper Midwest. For information on establishment success and plant densities for each treatment and location, see Mangan *et al.* (2011). Annual

biomass yields and species composition are available in Jungers *et al.* (2015b).

The research sites were tilled in May prior to seeding. Sites at Becker, Lamberton, and Waseca received Vapam (Sodium N-methyldithiocarbamate) herbicide at a rate of 84 kg active ingredient ha^{-1} to suppress weeds prior to seeding. Plots were broadcast seeded at a rate of 12 g pure live seed m^{-2}. Weeds were manually removed in 2006 and 2007, controlled using a pre-emergent herbicide (acetochlor [2-Chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl) acetamide]) in 2008 and 2009, and then by hand weeding and infrequent spot-spraying of glyphosate [N-(phosphomonomethyl) glycine] to control sporadic outbreaks of Canada thistle (*Cirsium arvense* (L.) Scop.) for the remainder of the experiment.

Soil measurements

Prior to seeding (2006 or 2007), three replicates of ten soils cores (1.5 cm diameter) were collected to a depth of 60 cm at each site. Soil cores were divided into four 15-cm increments and combined by depth for each replicate. In the fall of 2013, three soil cores were collected per subplot and divided into the same depth increments. The three cores were combined by depth for each subplot. Samples were dried at 33 °C and passed through a 2-mm sieve. Carbonates were measured with an acid test consisting of 1 M HCL. Soil organic C and N were determined via dry combustion (Nelson & Sommers, 1996) using an Elementar Vario Max CN analyzer (Hanau, Germany).

Root measurement

In October 2013, three soil cores (4.1 cm diameter) were collected (equally spaced) along a 1-m transect in the middle of each subplot to a depth of 90 cm. Soil cores were divided into three 30-cm segments and frozen until processed for root mass determination. Plant biomass was separated from soil using a hydropneumatic elutriation system (Smucker *et al.*, 1982). This system uses pressurized air to force water through soil and a series of mesh screens to remove soil from biomass. Samples were further cleaned by hand sieving to remove sand and debris. Belowground biomass was dried at 60 ° until constant weight and then manually

Table 1 Soil and climate conditions at each experimental site in Minnesota, USA

| Location | Location | Precipitation (cm)* | | Temperature (°C)* | | Soil texture (%) | | | OM† | TOC‡ |
		May–July	August–October	May–July	August–October	> 2.0 mm	0.25–2.0 mm	< 0.25 mm	g kg^{-1}	
Becker	45.3870, −93.8818	30.0	25.3	18.6	14.8	12.1	80.7	7.2	14	9
Crookston	47.8103, −96.6145	23.0	17.8	17.6	13.5	NA	NA	NA	27	NA
Lamberton	44.2373, −95.3020	27.2	20.0	19.2	15.1	23.7	42.5	33.9	36	22
Waseca	44.0678, −93.5258	32.1	26.2	18.8	15.0	28.6	43.5	27.8	43	23

*30-year average (1971–2000).
†Organic matter.
‡Total organic carbon.

screened to remove all aboveground structures (i.e., leaves and stems). Some samples included crowns and rhizomes, which were included in the belowground biomass sample. Dry mass was recorded for all belowground biomass and hereafter referred to as roots. As we do not have repeated measures of root biomass across time, root biomass is presented as the net difference in root growth and root mortality after 7 (Crookston) or 8 (Becker, Lamberton, and Waseca) years since seeding.

After dried root samples were weighed separately, the three replicates were combined by depth for each subplot and ground to pass through a 1-mm screen. The samples were then analyzed for C and N using a combustion analyzer.

GHG mitigation potential by roots was calculated as the product of root biomass, root C content, and the CO_2 e constant, and then annualized by dividing the value by the number of years since seeding (7 years for Crookston and 8 years for Becker, Lamberton, and Waseca; Eqn S1). The GHG mitigation potential from root biomass was annualized so that it can be compared to other components of the net GHG mitigation potential equation (Eqn S7).

Fossil fuel offsets

Biomass yield was measured annually after the seeding year (from 2007 or 2008 to 2013) in all plots from each location following a killing frost as described by Jungers *et al.* (2015yb). In 2008, all dried biomass samples were ground and scanned using near infrared spectroscopy (NIRS) to estimate the concentration of cell wall carbohydrates using calibrated equations based on wet chemistry results. A subset of biomass samples was processed using wet chemistry to validate NIRS results. A detailed explanation of this method is described by Vogel *et al.* (2010). Cell wall carbohydrate concentrations were used to predict theoretical ethanol yield (Eqn S2) and adjusted to assume 90% conversion efficiency. GHG mitigation from fossil fuel offsets of ethanol production was determined using published estimates from the GREET life cycle analysis model (Gelfand *et al.*, 2013; Eqn S3).

GHG emissions from bioenergy crop management

GHG emissions from managing bioenergy crops come from farm inputs (N fertilizer production and application), biomass handling (transporting and processing biomass for cellulosic energy production), and N_2O emissions from farmland. For GHG emission estimates related to N fertilizer production and application, biomass harvest and handling, and cellulosic ethanol conversion, we used GREET model estimates published in Gelfand *et al.* (2013; Eqn S4). GHG emissions from N_2O emissions were estimated using the empirical model MGRASS, which is based on N fertilizer rate and the mean temperature (°C) in January, February, and March (Roelandt *et al.*, 2005; Eqn S5). We estimated N_2O emissions using daily temperature readings at each location for each year. Therefore, estimates are reported by location and do not include subplot level variation from species mixture treatments.

Net GHG mitigation

Net GHG mitigation (Eqn S7) is the average annual net total of the GHG sinks minus GHG emissions measured as Mg CO_2e ha^{-1} yr^{-1}. GHG sinks include (1) root C: the annualized difference in root growth and root mortality, and (2) fossil fuel offsets: average annual GHG mitigation potential from offsetting fossil fuels. Greenhouse gas sources include average annual GHG emissions related to N fertilizer production and application, biomass harvest and handling, cellulosic ethanol conversion, and N_2O emissions from management for each bioenergy crop/N fertilizer treatment combination. As not all of these GHG components were measured at the same spatial scale, we chose not to conduct statistical tests on the sum of these components (i.e., net GHG mitigation), but rather statistically test only the variables measured for each experimental unit (subplot).

Statistics

Data were analyzed using the open-source statistical software R (version 3.1.3; R Development Core Team, 2014). We used mixed-effects analysis of variance models to explain variation in root biomass, C, N, and C : N ratio by fitting the fixed effects of species mixture and N fertilizer treatments as categorical variables, and depth as a continuous variable, along with all possible interactions. We used this same fixed effects structure to explain variation in final SOC, and changes in SOC from the first year to 2013. When there were interactions between depth and either of the other main effects (species mixture or N fertilizer treatment), we fit separate models with depth as a categorical variable to evaluate interactions.

For all response variables, we fit a nested random effects structure that included species mixture subplots nested within fertilizer treatment main plots, within block, within site to account for spatial autocorrelation and pseudo-replication. Mixed-effects models were conducted using the R package LME4 (Bates *et al.*, 2014). We fit this model 9999 times to bootstrapped datasets for each response variable. Each bootstrapped dataset included *n* values that were randomly sampled with replacement from the original dataset of size *n*. From the 9999 models, we used the distribution of the coefficients to estimate the mean effect size and 95% confidence intervals (CIs) for all main effect treatment levels for each response variable. *Post hoc* comparisons of treatment level means were made by comparing overlap of CIs across treatment combinations of interest and verified with Tukey adjusted *P* values ($\alpha = 0.05$) to control the experimentwise type 1 error rate (Ruxton & Beauchamp, 2008). Mean comparisons by CI overlap and Tukey were mostly consistent, and when they differed, we reference the Tukey *P* value in the text. Pairwise comparisons were made with tools in the R package multcomp (Torsten *et al.*, 2008).

We tested for relationships among response variables to further explore observed patterns in the data. We used mixed-effects linear regression models to test for associations among root biomass, root C : N, and changes in SOC. The same random effects structure was used as in the ANOVA models (i.e., subplot within plot within block within site).

Table 2 Analysis of variance table for root biomass (Mg ha^{-1}), root carbon (C) content (g kg^{-1}), root nitrogen (N) content (g kg^{-1}), and root carbon to nitrogen ratio (C : N)

Source	Root biomass			Root C			Root N		Root C : N	
	DF*	*F* ratio	*P* value	DF	*F* ratio	*P* value	*F* ratio	*P* value	*F* ratio	*P* value
Trt	4, 88	22.52	**<0.001**	4, 88	6.17	**<0.001**	34.45	**<0.001**	39.72	**<0.001**
Nfert	1, 11	0.77	0.4	1, 11	1.12	0.313	10.65	**0.008**	19.36	**0.001**
Depth	2, 709	1301.45	**<0.001**	2, 229	117.13	**<0.001**	250.28	**<0.001**	335.20	**<0.001**
TrtXNfert	4, 88	0.83	0.507	4, 88	0.71	0.586	0.44	0.777	2.62	0.058
TrtXDepth	4, 709	21.06	**<0.001**	4, 229	2.15	0.076	1.03	0.394	7.99	**<0.001**
NfertXDepth	1, 709	4.47	**0.035**	1, 29	0.04	0.848	4.92	0.168	0.68	0.411
TrtXNfertXDepth	4, 709	2.84	**0.024**	4, 229	0.74	0.569	2.82	**0.026**	0.36	0.837

*Number of degrees of freedom in the numerator and denominator of *F* test.
Bold values indicate significance at *P* = 0.05.

Results

Belowground biomass production

The effect of species mixtures and N fertilization on root biomass varied by depth as indicated by the two-way and three-way interactions among these variables (Table 2). Root biomass was greater in the switchgrass, grass mix, and grass/legume mix compared to the 12-species and high-diversity mixtures in the 0–30 cm soil profile, but root biomass was similar for switchgrass and the mixtures in the 30–60 and 60–90 cm profiles (Fig. 1). Switchgrass root biomass from 0 to 30 cm was greater in unfertilized compared with fertilized plots (t_{11} = 4.51; *P* = 0.019), but root biomass for the other species mixtures were similar across fertilizer treatments and depths.

Root C and N concentration

There was a significant effect of species mixture treatments and depth on root C concentration, but root C was not affected by N fertilizer. There were no two-way or three-way interactions among variables (Table 2). Root C concentration increased with depth for all species mixture treatments (Fig. S1). At the shallow depth (0–30) where roots were most abundant, mean root C concentration was largest in switchgrass (mean = 40.5%; SE = 0.6) compared with the other species mixture treatments (mean = 37.8%; SE = 0.3).

Species mixture, N fertilizer, and depth all affected root N concentration alone and in combination (Table 2). Root N concentration was greater in grass/legume, 12-species, and high-diversity mixture treatments compared to the grass mix and switchgrass
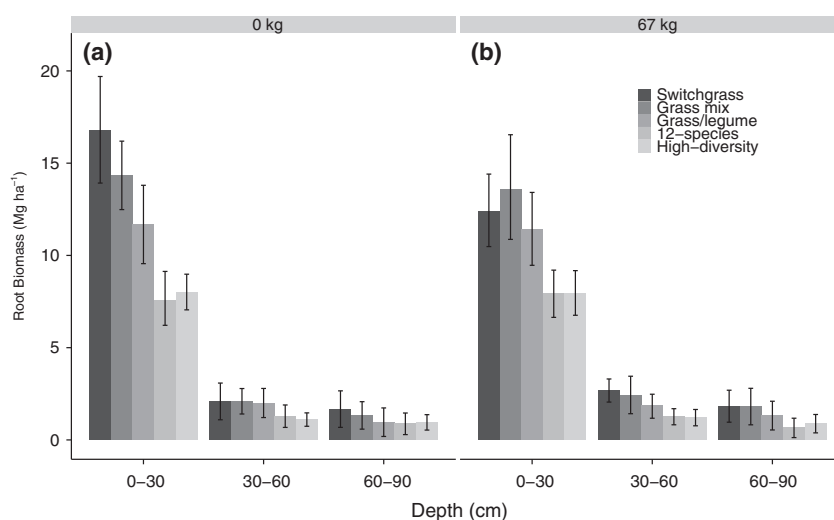

Fig. 1 Root biomass (95% CIs) at three depth intervals for five native perennial bioenergy crops managed with 0 (a) and 67 (b) kg N ha^{-1}. Measurements reflect seven (one site) or eight (three sites) years of root growth after seeding.
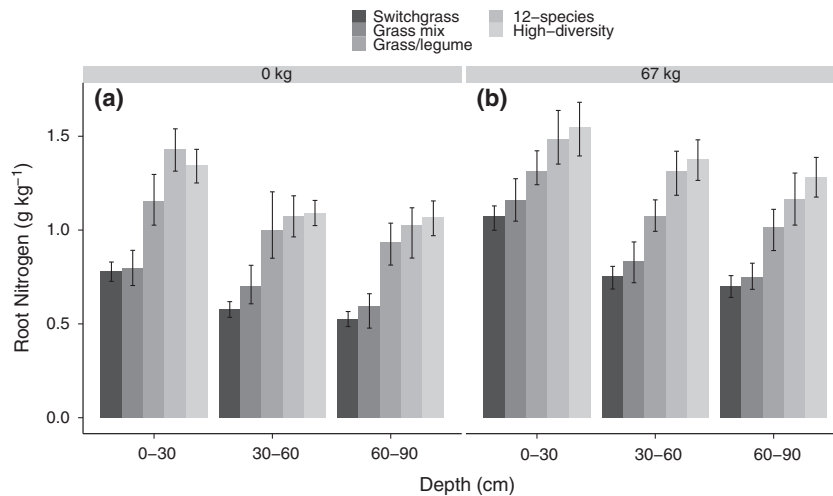
Fig. 2 Root nitrogen content (95% CIs) at three depth intervals for five native perennial bioenergy crops managed with 0 (a) and 67 (b) kg N ha^{-1}.

treatments at most depth/N fertilizer rate combinations (Fig. 2). Root N concentration increased with N fertilizer and decreased with depth. Root N varied more within and across treatments than root C and therefore contributed more to variation in the root C : N ratio (Fig. 3).

Species mixture and N fertilization affected root C : N, both alone and interactively; species mixture effects also varied by depth (Table 2). Root C : N was greatest in the switchgrass and grass mix and lowest in the 12-species and high-diversity mixtures at most depths. Root C : N was lower in the fertilized vs. unfertilized switchgrass treatment at all depths, and lower in the grass mix at 0–30 and 60–90 cm depths. Root C : N

was similar across fertilizer treatments for the grass/legume, 12-species, and high-diversity mixtures. Root C : N increased with depth, but more so for switchgrass and the grass mix (Fig. 3). Root C : N was positively associated with root biomass at the 0–30 ($P < 0.001$; Fig. 4a), 30–60 ($P < 0.001$), and the 60–90 ($P < 0.001$) cm depth increments.

Soil organic carbon

Final SOC was similar across all treatments but decreased with depth ($F_{1,347} = 597$; $P < 0.001$). Averaged across treatments final SOC was 22.5, 17.7, 14.5, and 11.3 g kg^{-1} at the 0–15, 15–30, 30–45, and 45–60 cm
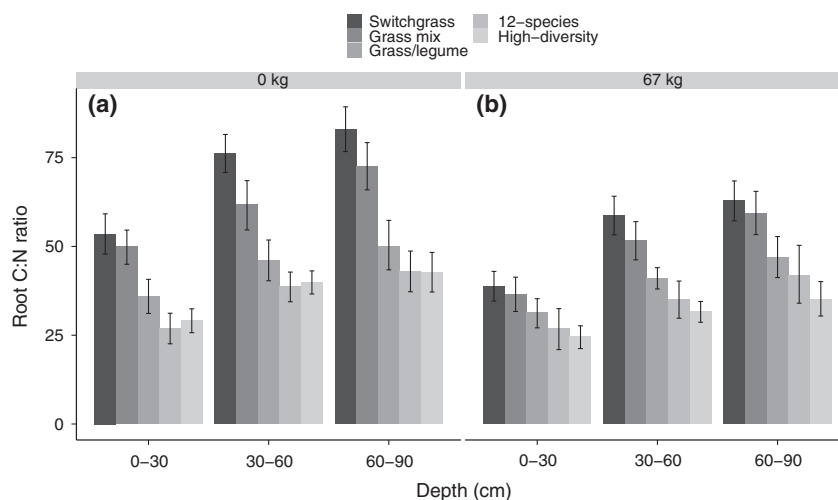

Fig. 3 Root carbon to nitrogen ratio (95% CIs) at three depth intervals for five native perennial bioenergy crops managed with 0 (a) and 67 (b) kg N ha^{-1}.
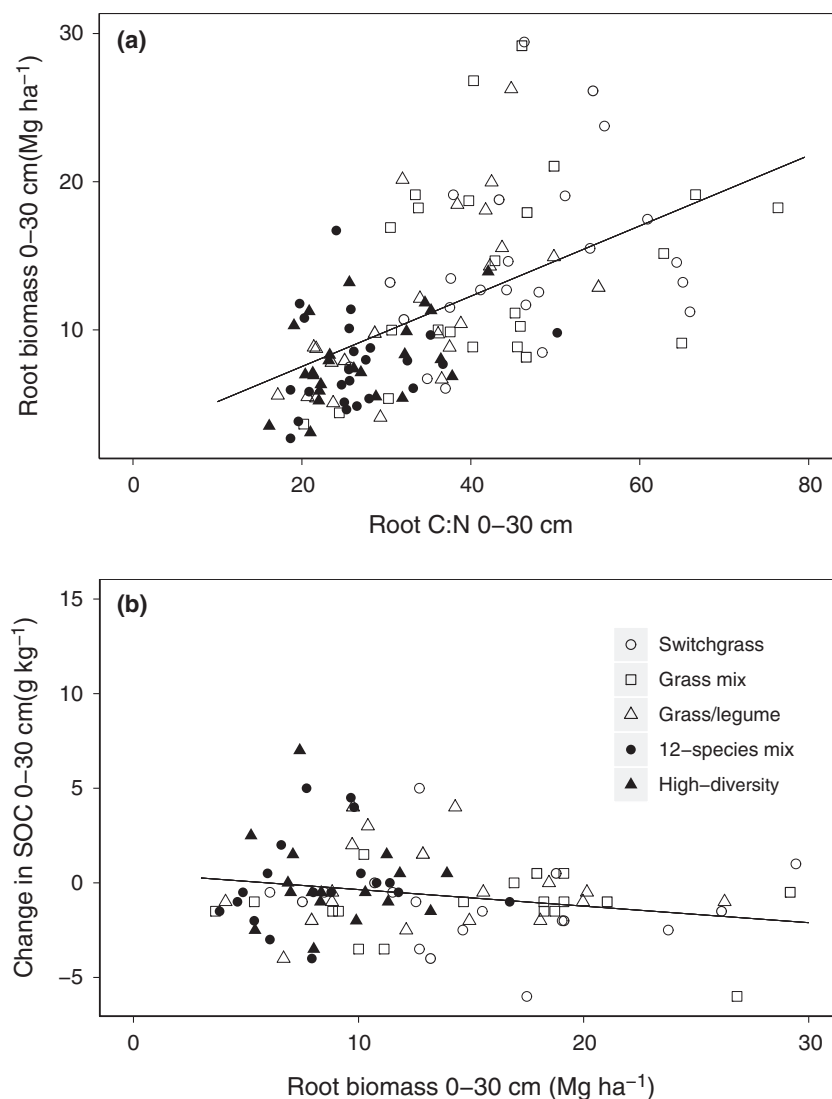
Fig. 4 Relationship between root biomass and C : N from 0 to 30 cm (a) and relationship between the change in shallow soil organic carbon (SOC; average of 0–15 and 15–30 cm intervals for each subplot) and total root biomass from 0 to 30 cm (b) in five native perennial bioenergy crops.

depth increments, respectively. Comparing final to initial SOC values showed no change across treatments (Fig. S3). When analyzing the data by discrete depth increments, SOC decreased in the 15–30 cm interval for all treatments in the unfertilized plots, and for all treatments except the high-diversity in the fertilized plots (mean = 2.66 g kg^{-1}; SE = 0.22). Change in SOC averaged across 0–15 and 15–30 cm depth intervals was negatively associated with root biomass from 0 to 30 cm (Fig. 4b; $P = 0.026$; Y-intercept = 2.781; $\beta = 0.237$).

Greenhouse gas mitigation potential: root biomass

The GHG mitigation potential by roots varied by species mixture treatment ($F = 27.824$, $P < 0.001$, Fig. 5) but

not N fertilizer ($F = 0.452$, $P = 0.549$). Averaged across fertilizer treatments, the GHG mitigation potential of carbon storage in root biomass was largest for switchgrass (mean = −3.6 Mg CO_2 e ha^{-1} yr^{-1}, SE = 0.2) and the grass mix (mean = −3.2 Mg CO_2 e ha^{-1} yr^{-1}, SE = 0.3). The grass mix was similar to the grass/legume mix (mean = −2.6 Mg CO_2 e ha^{-1} yr^{-1}, SE = 0.2), but different than the 12-species (mean = −1.8 Mg CO_2 e ha^{-1} yr^{-1}, SE = 0.1) and high-diversity (mean = −1.8 Mg CO_2 e ha^{-1} yr^{-1}, SE = 0.1) mixtures. Variation in root biomass with soil depth (Fig. 1) contributed significantly to GHG mitigation potential with roots from shallow soil depth (0–30 cm) contributing considerably more to GHG mitigation than other soil depths.
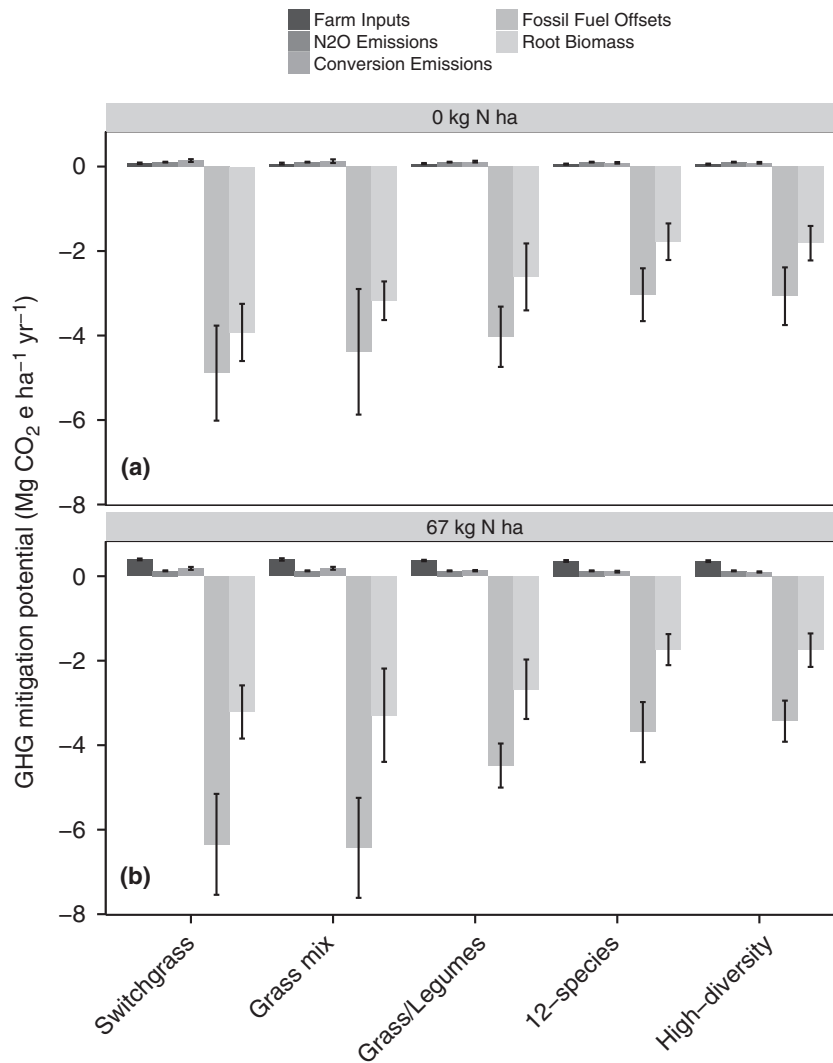
Fig. 5 Average annual greenhouse gas (GHG) mitigation potential of five native perennial bioenergy crop mixtures managed with 0 (a) and 67 (b) kg N ha^{-1}. Farm inputs include fossil fuel requirements for fertilizer production and application and biomass harvest. N$_2$O emissions are modeled using winter temperatures and N inputs. Conversion emissions include GHGs related to converting biomass to cellulosic ethanol. Fossil fuel offsets are based on harvested biomass yield and fermentable carbohydrate content. Root biomass includes root carbon from 0 to 90 cm depth.

Greenhouse gas mitigation potential: fossil fuel offsets

The GHG mitigation potential by fossil fuel offsets varied by species mixture treatment ($F = 29.204$; $P < 0.001$) and N fertilizer ($F = 11.453$; $P = 0.043$; Fig. 5). There was a significant interaction between species mixture and N fertilizer ($F = 3.141$; $P = 0.019$). In unfertilized plots, GHG mitigation by fossil fuel offsets was similar for switchgrass, grass mix, and the grass/legume mixture (Fig. 5). In fertilized plots, GHG mitigation by fossil fuel offsets was greatest in switchgrass and the grass mix compared to grass/legumes, 12-species, and high-diversity mixtures. This is a result of lower biomass yields and ethanol potential of the 12-species and high-diversity mixtures compared to switchgrass (Table S2 and S3). Within 12-species and high-diversity mixtures, fossil fuel offsets were similar for fertilized and unfertilized plots.

GHG sources and net mitigation

On average, GHG emissions estimated at the site level or from literature sources were 7% of the GHG mitigated from fossil fuel offsets and root biomass. In fertilized plots, farm inputs (which include N fertilizer production and application) were 570% greater compared with unfertilized plots. Nitrous oxide emissions were estimated at the site level annually
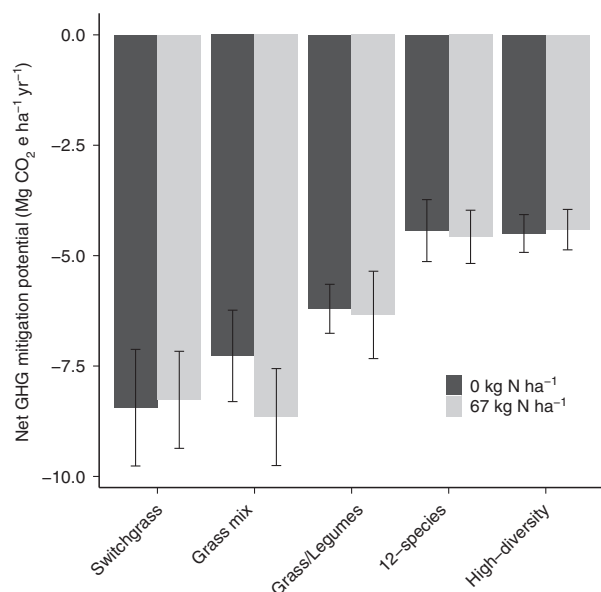
Fig. 6 Average annual net GHG mitigation potential based on the sum of all components (Fig. 5) for five native perennial bioenergy crops managed with 0 and 67 kg N ha^{-1}. Error bars are 95% CI of the mean.

based on N fertilizer rate and late winter temperatures, and averages ranged from 81 to 139 kg CO$_2$e ha^{-1} yr^{-1}.

Net GHG mitigation was largely driven by fossil fuel offsets and total root C. Net GHG mitigation potential was similar for switchgrass, grass, and grass/legume mixtures, and greater than the 12-species and high-diversity mixtures (Fig. 6). Within species mixture treatments, GHG mitigation potential did not vary by N fertilizer rate.

Discussion

This study is among the first to show the relative contribution of standing root biomass to GHG mitigation in perennial grassland bioenergy systems. Our results show that after seven or eight years of production, root carbon in unfertilized grassland monocultures and mixtures had nearly an equal effect on GHG mitigation as fossil fuel offsets by converting aboveground biomass to cellulosic ethanol (Fig. 5). Accounting for GHG emissions and fossil fuel offsets, root C contributions to net GHG mitigation ranged from 13 to 69%. On average, there were 13.7 and 22.5 Mg of CO$_2$ e ha^{-1} in the form of root C after seven or eight years of perennial bioenergy crop production.

Our estimates of root biomass are larger than estimates from other studies on native perennial bioenergy crops, which may be related to the longer timespan of our study. For example, our estimates of switchgrass root biomass were three times larger than estimates by Jung & Lal (2011) for the 0–30 cm layer, a study that sampled roots 4 years after seeding. Similarly, our estimates for grass mixture root biomass in the 0–100 cm layer were two times greater than those by Jarchow & Liebman (2012) who sampled 2 years after seeding. Root biomass and C storage in roots increased linearly through time for up to 12 years after restoring formally cultivated fields to native perennial grasses (Baer *et al.*, 2002); therefore, it is likely that the extended time period of our study contributed to the relatively high root biomass yields compared with more abbreviated studies.

An alternative explanation for why we observed more root biomass compared with other studies may be related to soil texture. Although we focus on the average effect of species mixture and N fertilizer treatments on root biomass across locations, there were site-level differences in root biomass responses to treatments (Fig. S2). The sites with the coarser soils – Becker and Crookston – tended to have more root biomass than the Lamberton and Waseca sites with finer soils, but this also depended on species mixture treatments. Root biomass values in the fine-loamy soils at Lamberton and Waseca were closer to those measured by Jarchow & Liebman (2012), who also conducted their study on fine-loamy soils. Root density tends to increase in coarse- compared to fine-textured soils because water-holding capacity is usually less in coarse-textured soil (Schenk & Jackson, 2002).

Variation in root biomass across studies can also be related to planting configuration and root sampling. Seeds were broadcast across plots in this study, while many studies measuring switchgrass biomass planted seeds in rows and sampled for roots between rows where crowns and rhizomes are minimal (Jung & Lal, 2011; Garten *et al.*, 2011). As crowns and rhizomes can account for 10–20% of switchgrass total plant biomass, comparing root biomass samples with and without these organs could result in very different results (Garten *et al.*, 2011).

Variation in root biomass across species mixture treatments may have been related to differences in root morphology among species of various functional groups. Root biomass samples included fine, coarse, live and dead roots, and rhizomes. Rhizomes were infrequently observed, but may have contributed to the greater root biomass values in switchgrass and grass mixtures in the shallow depth compared to the 12-species and high-diversity treatments because the forbs in the polycultures did not form rhizomes. In 2013 when the roots were sampled, 49% of the 12-species and 57% of the high-diversity mixtures were composed of forbs. Forbs in our mixtures included Maximilian sunflower

(*Helianthus maximiliani* Shrad.), yellow coneflower (*Ratibida pinnata* (Vent.) Barnhart), and stiff goldenrod (*Solidago rigida* L.), which produce long taproots instead of shallow fibrous systems that are known to colonize soils well beyond 1 m deep (Weaver, 1958). Our study was consistent with others in that it restricted sampling to a 90 cm depth, but this may not have captured total root biomass beneath treatments with deep-rooting forbs (Garten *et al.*, 2011).

Net GHG mitigation represented the total annual contribution from all observed and literature-based sources of GHG emissions and sinks in native bioenergy cropping systems. The GHG sinks were fossil fuel offsets from converting annual aboveground biomass to cellulosic ethanol and root C accumulation. Our measure of GHG mitigation potential of fossil fuel offsets was similar to those reported by Gelfand *et al.* (2013) for fertilized (6.2 Mg of CO_2 e ha^{-1} yr^{-1}) and unfertilized prairie (4.6 Mg of CO_2 e ha^{-1} yr^{-1}), but more than those measured by Adler *et al.* (2009) for switchgrass (about 1.5 Mg of CO_2 e ha^{-1} yr^{-1}). In our study and those by Gelfand *et al.* (2013) and Adler *et al.* (2009), fossil fuel offset values were the largest contributors to net GHG mitigation. However, our results differ from those of Gelfand *et al.* (2013) and Adler *et al.* (2009) in that we did not observe changes in SOC, which they report as a substantial sink for GHG mitigation. Instead, we found that C in root biomass was the next most significant GHG mitigation pathway, which was nearly similar in its effect as SOC in the Gelfand *et al.* (2013) study. Potential reasons for this are discussed below.

Nitrogen fertilization did not affect net GHG mitigation potential for any of the bioenergy cropping systems (Fig. 6). Although N fertilization significantly increased aboveground biomass yields (Jungers *et al.*, 2015b), the associated GHG benefit was somewhat offset as a result of increased fossil fuel emissions related to producing and applying N fertilizer, as well as decreases in belowground biomass in some species composition treatment × depth combinations. Even though N fertilization did not have a positive or negative effect on net GHG mitigation, a shift in biomass from belowground to aboveground components from N fertilization should be economically beneficial for producers as aboveground biomass has marketable value (Mooney *et al.*, 2009). However, bioenergy crops with less root biomass may be more susceptible to drought stress, which also has economic implications if aboveground biomass is reduced (Mann *et al.*, 2013).

Net GHG mitigation potential varied by species mixture treatments, which was mostly due to treatment variations in above- and belowground biomass. Farm inputs, N_2O, and conversion emissions did not vary across species mixtures. Without fertilizer, there was little variation in fossil fuel offsets among mixture treatments despite a difference in biomass yield (Table S2). For example, average biomass yields were greater in switchgrass and the grass/legume mixture compared to other treatments without fertilizer (Jungers *et al.*, 2015b) yet these treatments did not have greater fossil fuel offsets. Differences in the concentration of fermentable sugars across locations and species mixture treatments may have introduced variability to the fossil fuel offset potential (Table S3). Although short-term studies have shown that annual fossil fuel offsets are mostly dependent on biomass yield and not theoretical ethanol yield (Jungers *et al.*, 2013; Jarchow & Liebman, 2012), the cumulative effect of relatively small alterations in theoretical ethanol yield compared with biomass yield over 7 years of production suggests that theoretical ethanol yield is important when considering long-term bioenergy production.

Although the potential for perennial bioenergy crop roots to offset GHGs is similar to converting aboveground biomass to bioenergy in the absence of fertilizer, the actual impact of these GHG mitigation pathways is uncertain. First, it is not known if an equal volume of fossil fuels will actually be displaced with the production of cellulosic ethanol. The availability of cellulosic ethanol could increase overall energy use, which could limit fossil fuel displacement and subsequent GHG mitigation. Second, the C sink of roots is highly susceptible to decomposition in response to land conversion, thus the actual GHG mitigation potential of this sink is dependent on long-term land use decisions and management practices. Tillage to replace the perennial bioenergy crop with an annual crop could emit a large volume of GHGs (Gelfand *et al.*, 2011), and it is not clear how long perennial bioenergy crops will remain in a field before being converted. Nonetheless, results from this study could inform future policies designed to maximize GHG mitigation in bioenergy cropping systems.

Soil organic C accumulation is regarded as a significant contributor to GHG mitigation (Pacala & Socolow, 2004). Similar studies of perennial bioenergy crops show that SOC can mitigate from 4.0 to 7.3 Mg CO_2 e ha^{-1} yr^{-1} for 13-yr-old prairie and 9-yr-old switchgrass stands, respectively (Gelfand *et al.*, 2013; Follett *et al.*, 2012). However, SOC did not change much in this study, especially across treatments, during 7 or 8 growing seasons (Fig. 4b; Fig. S3). Previous studies agree that changes in SOC as a result of converting row crop fields to perennial cover are highly variable and dependent on, among other variables, initial soil conditions (Bransby *et al.*, 1998; Zan *et al.*, 2001; Sartori *et al.*, 2006; Adler *et al.*, 2009; Schmer *et al.*, 2011; Bandaru *et al.*, 2013; Agostini *et al.*, 2015). Although all our research

sites were tilled following row crop production prior to establishment of native perennials, initial soil C levels were relatively high at most sites (Table 1). High initial C levels may have limited C accumulation by perennial crops (Tiemann & Grandy, 2015). In other studies where initial SOC levels were low and aboveground biomass was not removed, SOC increases occurred as early as 5 years after converting land from row crops to native perennial cover (Reeder *et al.*, 1998; Chimento *et al.*, 2014). In even more studies from other environments, researchers reported no change in SOC beneath switch-grass during 5 years of growth (Garten & Wullschleger, 2000; Ma *et al.*, 2000), and only after 10 years of switch-grass production did Follett *et al.* (2012) observe an increase in SOC on marginal land. Even in soils with low initial SOC values, short-term changes (<5 yr) in SOC are unlikely to be observed as the mean residence time can range from 1.2 to 2.4 years for roots (Agostini *et al.*, 2015).

Because we did not find differences in SOC change across treatments, we omitted this component from the GHG mitigation potential equation. Even if we had included SOC change to the GHG mitigation equation, the best-case N fertilizer/species mixture treatment response would have resulted in −0.14 kg CO2e ha^{-1} of storage, which is <0.5% of the net GHG mitigation potential for any treatment combination. Summing SOC changes across soil depths for each treatment combination usually resulted in net SOC loss, which in the worst-case scenario would have been equal to 15% of net GHG mitigation potential. Steinbeiss *et al.* (2008) found that converting agricultural fields to perennial grasslands resulted in a net C loss 2 years after conversion, but C stocks recovered and exceeded initial values by year 4. Spring tillage and decomposition of the previous years' crop residue combined with low biomass production during the seeding year of perennial bioenergy crops may have led to SOC losses (Zhang *et al.*, 2010). As C inputs from aboveground biomass are less in an annually harvested bioenergy system, SOC recovery may take longer compared with an unharvested system (like those studied in Steinbeiss *et al.*, 2008).

Mechanisms responsible for the negative or neutral SOC change beneath the perennial bioenergy cropping systems could be related to the complex interactions between plant litter inputs and SOM decomposition known as rhizosphere priming effects (Dijkstra *et al.*, 2013). Positive priming effects are when SOM decomposition is accelerated by the addition of plant organic matter, such as roots (Kuzyakov, 2002). Perennial bioenergy cropping systems with more root biomass exude more organic substances that fuel microbial growth, which then begin to consume old SOM. This could explain the negative association in SOC change and root biomass we observed (Fig. 4b). However, net SOC losses by root-mediated SOM decomposition is often short term, and sequestration of root-derived C to humi-fied SOM can offset those losses within one growing season (Kuzyakov, 2002). Research quantifying respiration and SOM turnover through time in long-term experiments is needed to understand these complex plant–soil–microbe interactions and their consequences for GHG mitigation in bioenergy systems.

Soil organic C could be increasing in the grass/legume, 12-species, and high-diversity treatments more than the switchgrass and grass mix in the 0–15 cm layer (Fig. 4), but large variation in measurements led to sta-tistically insignificant results when analyzing treatments as factored variables with $\alpha = 0.05$. Initial SOC measure-ments occurred at the site level, not subplot, which may have reduced our ability to detect changes in SOC among species mixtures. We did, however, find statisti-cally significant relationships between root quality and SOC change that support this trend. Individual subplots with SOC increases in the top 30 cm of soil typically had fewer total roots (Fig. 4b), and the roots that were in the soil had a lower C : N ratio (Fig. 4a). The 12-spe-cies and high-diversity polycultures had less root bio-mass and lower C : N ratios than the other treatments (Table 2, Fig. 3 and 4). If SOC increases are mainly dri-ven by root C inputs (because aboveground litter inputs are less in these annually harvested systems; Rasse *et al.*, 2005), it is expected that roots with a lower C : N ratio would be more susceptible to decomposition com-pared with high C : N roots (Johnson *et al.*, 2007). In turn, plots with lower C : N root tissue (e.g., 12-species and high-diversity mixtures) should have less root bio-mass and potentially more SOC buildup if there is also greater decomposition.

Our data suggest that the low C : N ratio roots in diverse mixture bioenergy grasslands may be decom-posing faster than high C : N ratio roots of grass-domi-nated systems. This could reduce the short-term C stock in standing root biomass under diverse polyculture sys-tems, but may lead to greater SOC in the long-term compared with grass-dominated systems (Johnson *et al.*, 2007). Nutrient removal during annual biomass harvest is substantial (Jungers *et al.*, 2015a; Gillitzer *et al.*, 2012) and could affect soil nutrient stocks and subsequent productivity (Schmer *et al.*, 2011). Low-input systems with lower root mean retention times may have greater mineralization rates that help maintain yields longer than those with more recalcitrant roots in the absence of fertilizers. Therefore, the benefit of species diversity for SOC accumulation and yield may not be realized until a decade or more after establishment.

This study is one of the first to compare above- and belowground factors contributing to GHG mitigation by

grasslands of varying diversity levels managed for bioenergy. Without N fertilizer, root C contributed as much GHG mitigation as fossil fuel offsets by aboveground biomass converted to cellulosic ethanol for some species mixtures. N fertilizer increased aboveground biomass and fossil fuel offsets, but generally had no effect on root biomass except for switchgrass, and the overall effect of N fertilizer on net GHG mitigation was small. Grass-dominated plantings (e.g., switchgrass monocultures, grass mixtures, and grass/legume mixtures) showed greater net GHG mitigation potential than more diverse mixtures. Changes in SOC did not vary across species mixture or N fertilizer treatments and did not influence net GHG mitigation. However, the 12-species and high-diversity mixtures had lower root C : N ratios, which were associated with less root biomass and increases in SOC. This study underscores the importance of short-term (roots) and long-term (SOC) belowground C pools in relation to fossil fuel offsets in native perennial bioenergy systems.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Plant species sown into species mixture treatments.

Table S2. Average annual biomass yield (g m^{-2}; ±SE) of five perennial grassland bioenergy crops with and without 67 kg N ha^{-1} at four locations in Minnesota from 2007 (Becker, Lamberton, and Waseca) or 2008 (Crookston) to 2013.

Table S3. Average theoretical ethanol potential (l Mg^{-1}; ±SE) from biomass of five perennial grassland bioenergy crops with and without 67 kg N ha^{-1} at four locations in Minnesota. Value represent 90% carbohydrate conversion efficiency.

Fig. S1. Root carbon content (± 95% CIs) at three depth intervals for five native perennial bioenergy crop mixtures managed with and without 67 kg N ha^{-1}.

Fig. S2. Variation in root biomass (mean ± SE) within treatments and across locations in depth intervals from 0 to 30 cm (a), 30–60 cm (b), and 60–90 cm (c). The scale of the y-axes were adjusted for each depth interval to better highlight site-level variation within each species mixture treatment.

Fig. S3. Change in soil organic carbon (± 95% CIs) at three depth intervals for five native perennial bioenergy crop mixtures managed with and without 67 kg N ha^{-1}.