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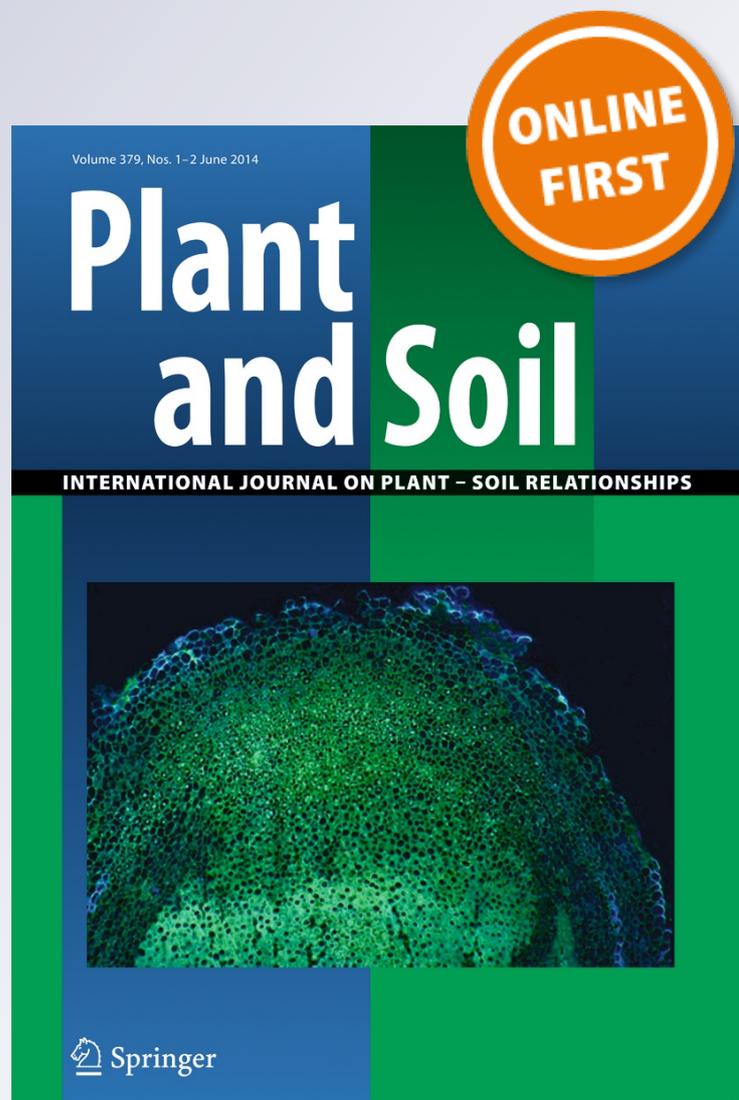
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Root traits and soil properties in harvested perennial grassland, annual wheat, and never-tilled annual wheat

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Abstract

Background and aims Root functional traits are determinants of soil carbon storage; plant productivity; and ecosystem properties. However, few studies look at both annual and perennial roots, soil properties, and productivity in the context of field scale agricultural systems.

Methods In Long Term and Conversion studies in North Central Kansas, USA; root biomass and length, soil carbon and nitrogen, microbial biomass, nematode and

micro-arthropod communities were measured to a depth of one meter in paired perennial grassland and cropland wheat sites as well as a grassland site that had been converted to cropland using no tillage five years prior.

Results In the Long Term Study root biomass was three to seven times greater (9.4 Mg ha^{-1} and 2.5 Mg ha^{-1} in May), and root length two times greater (52.5 km m^{-2} and 24.0 km m^{-2} in May) in perennial grassland than in cropland. Soil organic carbon and microbial biomass carbon were larger, numbers of Orbatid mites greater (2084 vs 730 mites m^{-2}), and nematode communities more structured (Structure Index 67 vs 59) in perennial grassland versus annual cropland. Improved soil physical and biological properties in perennial grasslands were significantly correlated with larger, deeper root systems. In the Conversion Study root length and biomass, microbial biomass carbon, mite abundance and nematode community structure differed at some but not all dates and depths. Isotope analysis showed that five years after no-till conversion old perennial roots remained in soils of annual wheat fields and that all soil fractions except coarse particulate organic matter were derived from C_4 plants.

Conclusions Significant correlation between larger, longer roots in grasslands compared to annual croplands and improved soil biological, physical and chemical properties suggest that perennial roots are an important factor allowing perennial grasslands to maintain productivity and soil quality with few inputs. Perennial roots may persist and continue to influence soil properties long after conversion to annual systems.

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S. T. DuPont (✉)
Penn State Extension, The Pennsylvania State University,
14 Gracedale Ave, Nazareth, PA 18064, USA
e-mail: tdupont@psu.edu

J. Beniston · R. Lal
Carbon Management and Sequestration Center,
The Ohio State University, Columbus, OH 43221, USA

J. D. Glover
Bureau for Food Security, U.S. Agency for International
Development, Washington DC 20523, USA

A. Hodson · S. W. Culman
Agricultural Sustainability Institute, University of California,
Davis, CA 95616, USA

H. Ferris
Department of Entomology and Nematology, University of
California, Davis, CA 95616, USA

Keywords Sustainable agriculture · Perennial grassland · Root biomass · Root length · Soil food web · Soil carbon · ^{13}C natural abundance

Abbreviations

MBC	Microbial Biomass
SOC	Soil Organic Carbon
POM	Particulate Organic Matter
BI	Basal Index
SI	Structure Index
EI	Enrichment Index
NMS	Non-metric Multidimensional Scaling

Introduction

Root functional traits (i.e. root biomass, length, surface area, ratio of fine to coarse roots, and placement of roots in the soil) are determinants of soil carbon storage; plant resource acquisition and productivity; and ecosystem properties. Yet few studies characterize root traits of perennial crops which have the potential to produce cereals, legumes, fibers and biofuels with few inputs and sustained ecosystem services (Boody et al. 2005; Fornara and Tilman 2008; Glover et al. 2007; Jordan et al. 2007; Tilman et al. 2006).

Root production and mortality are major regulators of soil C and nutrient fluxes (Gill and Jackson 2000; Nadelhoffer and Raich 1992; Silver and Miya 2001; Stewart and Frank 2008) and provide an opportunity to sequester the anthropogenic C that is threatening climate stability (Monti and Zatta 2009; Rasse et al. 2005; Tilman et al. 2006). Carbon from roots increases SOM and provides a flow of energy to the rhizosphere where it is a key controller of microbial community mass and structure (Farrar et al. 2003). Generally, larger root inputs of sugars, polysaccharides and amino acids result in increased microbial biomass in the rhizosphere (Marschner et al. 2001; O'Donnell et al. 2001b; Schweinsberg-Mickan et al. 2012). With long residence time in the soil due to lower tissue nitrogen, high tensile strength and placement deeper in the soil horizon (Craine et al. 2003), 1.5–2 times more soil organic carbon is root versus shoot derived (Balesdent and Balabane 1996; Molina et al. 2001; Rasse et al. 2005).

Root architecture influences plant resource acquisition and productivity (Swemmer et al. 2006). Deep roots can significantly enhance access to deep soil water

supplying up to 20–60 % of transpired water during dry periods, and helping plants survive extreme low moisture conditions (Canadell et al. 1996; Gregory et al. 1978; Nepstad et al. 1994). Root systems with dense mats of thin roots and root hairs are better able to forage for less mobile phosphorus (Manske et al. 2000; Richardson et al. 2011) and can ensure tight nitrogen cycling (Dell and Rice 2005). Extensive roots systems taking up sufficient nutrients and water can sustain massive growth. For example, big bluestem (*Andropogon gerardii*) grows up to 2 cm per day in peak season forming 3–5 ton of biomass per acre per year (Weaver 1958b).

Roots affect soil physical structure, including aggregate stability (Czarnes et al. 2000; Haynes and Beare 1997; Jastrow et al. 1998) and pore size (Whalley et al. 2005). Soil structure in turn is important for water retention, drainage, root growth and erosion mitigation. In addition to improving soil quality, deep, abundant roots reduce agricultural impacts on water quality (Carter and Gregorich 2010; Craine et al. 2002; Glover et al. 2010; Randall and Mulla 2001; Tilman et al. 1996).

Seminal work by Weaver and colleagues provided a broad basis of information on grassland species root architecture, distribution, and longevity (Weaver and Zink 1946a; Weaver 1947; 1958a; b; Weaver and Zink 1946b). Notably, they documented how much root material was produced by grassland species, the distribution of roots throughout the soil column, and the temporal dynamics of root system establishment and decay. Weaver et al. found that perennial grass species roots alone yielded 1.6 to 5.5 Mg Ha⁻¹ with 70–80 % of biomass in the top 30 cm (Weaver 1958b). However root system standing biomass and architecture varied distinctly between species and ecosystems. For example big bluestem (*Andropogon gerardii*) reached an average of 30–60 cm deeper in moist lowland sites compared to the same species in upland sites, and had coarser roots (thinnest roots 1–3 mm) compared to more predominant upland grass little bluestem (*Andropogon scoparius*; thinnest roots 0.5–1.0 mm) (Weaver 1958b). Interestingly, turnover for some grassland roots was slow with no root death for big bluestem (*Andropogon gerardii*) in the first year of growth and an average of 20 % for three year old root systems (Weaver and Zink 1946a).

The next four decades of root studies has added key information to our understanding of root functional traits. However the majority of recent grassland root studies were conducted on shallow or poor soil

(Kitchen et al. 2009), with upland species (Gill et al. 1999; Singh and Coleman 1974), or in semi-arid sites (Busso et al. 2001; Coupland and Johnson 1965; Frank 2007). Most studies that relate root characteristics to soil properties are container studies (Swemmer et al. 2006) or immature monocultures (Craine et al. 2002, 2003; Fomara et al. 2009). Many studies which include fine vs coarse root comparisons consider forest (Majdi 2005; Nadelhoffer and Raich 1992) or tropical systems (Nepstad et al. 1994). Studies that compare perennial grass and annual crop species do so with global analysis comparing across ecosystems (Canadell et al. 1996; Jackson et al. 1996). Very few studies look at both annual and perennial roots, soil properties, and productivity in the context of field scale agricultural systems (Buyanovsky et al. 1987).

In this study we characterize root biomass, length, surface area, N and C content in hayed prairie, annual wheat, and never tilled annual crop fields and quantify relationships between root properties soil biological properties and soil organic matter fractions. We conducted these measurements in a unique comparison of long-term harvested perennial grassland and annual cropland. Glover et al. (2010) documented that these annually-harvested, unfertilized perennial grasslands sustain harvests comparable to those of conventional high-input wheat (*Triticum aestivum*). While producing similar yields, perennial grasslands required no fertilizer, and energy inputs were 11.75 times less than in annual croplands. This high yielding perennial system also maintained greater numbers and/or diversity of insect pollinators, herbivores, and detritivores, more soil carbon, more soil nitrogen and greater food web complexity than annual wheat fields (Glover et al. 2010; Culman et al. 2010). We hypothesize that root length and biomass will be greater in perennial grassland than annual cropland, that soil carbon pools will be greater with increasing root biomass and length, and that root properties can explain a large amount of the variation between annual and perennial cropping systems.

A unique aspect of this study is the inclusion of a “conversion study” where perennial grassland was converted directly to annual crops with no tillage. This study allows us to better isolate relationships between root parameters and soil properties without the complicating factor of tillage. We hypothesize that root biomass and length, SOC, MBC, nematodes and mites will be smaller in never-tilled no till annual fields than in perennial grassland and that root properties will explain

a large amount of the variation between annual and perennial cropping systems.

Methods

Site description

We used two studies in North Central Kansas to address our research objectives, a Long-term Study and a Conversion Study. Both studies examine historically harvested, never-tilled, native grasslands with paired annual croplands. The Long-term Study includes cropland sites that have been in annual production for 75 years or more, while the Conversion Study includes recently converted cropland. The Long-term Study design takes advantage of historical effects of annual cropping on soil properties and soil biota. Since management practices in croplands have improved dramatically since the sod-busting and dust bowl eras, the Conversion Study was designed to use best management practices from the start. It was implemented in 2003 by converting native perennial grasslands to annual croplands with herbicide and no-tillage practices, and thus represents a unique insight into the impacts of converting tall-grass prairie to annual cropland in the absence of tillage (DuPont et al. 2010).

Long-term study

The five field sites in the Long-term Study were located in five counties of North Central Kansas as described by Glover et al. (2010). Each field site consisted of a native prairie meadow (perennial grassland) and an adjacent agricultural crop field which had wheat in 2008. Perennial grassland sites have never been tilled or fertilized and have been harvested annually for hay production for 75 years or more. The perennial fields have typically been cut to a height of 8–10 cm once per year in mid-summer. They have not been re-seeded. Although all the sites are classified by the USDA's Natural Resources Conservation Service as Prime Farmland with few constraints for agricultural production, the sites have been maintained in perennial grass hay production because their irregular shape and/or relatively small size (2–20 ha) makes annual crop management impractical (the Five Creek, Buckeye and Niles sites) or because of long-standing landowner tradition (the New Cambria and Goessel sites). The New Cambria perennial field received infrequent application of herbicides in recent

years to control annual cool-season grasses although plant community composition there was consistent with the composition of other sites. None of the landowners/managers reported any fertilizer application to their perennial fields and only the New Cambria site was reported to have been grazed (sporadically in the late nineteenth century).

On average, 79 % of ground cover at the sites consisted of perennial grasses (69 %), legumes (7 %), and non-legume forbs (3 %) native to the tallgrass prairie region of North America. Non-native annual grasses accounted for an additional 4 % of ground cover.

Adjacent crop fields on similar landscape positions have been sown primarily to wheat for similar periods of time and have received fertilizer inputs for the past several decades or more. Agricultural practices in the cropland sites have varied over time but generally consist of continuous winter wheat or rotations of wheat with sorghum (*Sorghum bicolor*) and/or soybean (*Glycine max*). Some sites have had no-till management for varying periods. Annual wheat field management followed typical practices for the region (Service 1996, 1997). During the project period residues were tilled under at four sites but retained in all years at Niles where zero-tillage was used. Specific field site names and respective locations were: **Niles**, Ottawa Co. N' 38.58.145, W' 97.28.616; **Goessel**, McPherson Co. N' 38.15.333, W' 97.22.307; **New Cambria**, Saline Co. N' 38.53.54, W' 97.32.615; **Buckeye**, Dickinson Co. N' 39°2'344", W 97°7'798, **Five Creek**, Clay Co. N' 38.22.665, W' 97.18.788. For detailed site characteristics see Culman et al. (2010; Table 1). The Five Creek site did not have wheat growing in 2008 and as such root, nematode and mite characteristics were not measured for the site.

Conversion study

The Long-term Study sites are limited by the fact that differences in soil properties observed may be artifacts of the region's early agricultural practices for which better alternatives have been more recently developed (e.g., no-tillage practices). In August 2003, researchers established three, 20 × 20 m research blocks in the perennial grass field at the Niles site, in order to conduct more detailed studies of soil and ecosystem properties following the conversion of perennial grass plots to annual cropping using no-tillage practices (hereafter referred to as the Conversion Study). A randomized

complete block design was imposed over the annually harvested grassland, with two treatments and three blocks. The two treatments included: (i) continuation of the annual harvest regime that had been in place for 75 years or more and (ii) conversion of the grassland into annual cropland using best management practices through herbicide application and without tillage. In converted plots the existing plant communities were sprayed multiple times with herbicide and were initially planted to soybeans (*Glycine max*) in Summer 2004. Soybeans were again planted in 2005 and followed by sorghum (*Sorghum bicolor*) in 2006, and wheat (*Triticum aestivum*) in 2006 and 2007. DuPont et al. (2010) provide specific seeding and application rates and harvested yields.

Root sampling and separation

Plant root biomass and length from annual cropland wheat and perennial grassland were quantified from May and June samplings at four sites (Niles, Goessel, New Cambria, Buckeye). Five soil cores of 6-cm diameter were collected on May 18 (boot stage, heading ½ complete, 10.3 Feeks scale) and June 23 (ripening: kernal hard, 11.3 Feeks scale) 2008 to a depth of 1 m in the following intervals: 0 – 0.1 m, 0.1 – 0.2 m, 0.2 – 0.4 m, 0.4 – 0.6 m, 0.6 – 0.8 m, 0.8 – 1.0 m. Each root core was bisected vertically into two sections which were used separately for root biomass and length measurements. Roots were separated from soil using a hydropneumatic root elutriator (Smucker et al. 1982). Soil and roots were deposited in a six manifold washer where gentle air and water bubbling removed soil, and roots were floated onto a submerged sieve (840 µm primary 420 µm secondary). Any surface residue that floated onto screens was removed manually with tweezers. Unlike studies of single crop species where roots have somewhat uniform appearance and bright white/yellow living roots are easily distinguished from dark dead roots, mixed species and perennial roots have varying color and texture. As such it was not possible to accurately distinguish between live and dead roots and they were not separated. Soil from root sample cores was discarded. Root biomass samples were oven-dried at 50 °C for 48 h and weighed to determine root mass per area. Small subsamples of dried root material were then ground for total C analysis. Root length samples were preserved in a fifty percent alcohol solution and stored at temperatures between 1 and 4 °C for no more

Table 1 Conversion Study. SOC, POM C fractions (g C kg⁻¹ soil) and Delta C¹³ (δ¹³C ‰) five years after no-tillage conversion from perennial grassland to annual cropland

depth	SOC (g C kg ⁻¹)			Coarse POM C >250 μm (g C kg ⁻¹)			SOC δ ¹³ C ‰			POM δ ¹³ C ‰		
	PR	NT	P value	PR	NT	P value	PR	NT	P value	PR	NT	P value
0 - 0.1 m	31.16	30.45	0.63	1.96	1.55	0.31	-15.9	-16.2	0.43	-18.6	-20.6	0.145
0.1-0.2 m	21.89	21.41	0.61	0.64	0.24	0.0006	-13.4	-13.3	0.87	-16.5	-20.5	0.003
0.2-0.4 m	15.74	15.98	0.87	0.25	0.14	0.087	-12.8	-12.8	0.63	-14.7	-18.3	0.01
0.4-0.6 m	10.01	10.8	0.55	0.20	0.08	0.11	-12.8	-12.9	0.42	-14.6	-18.2	0.007
0.6-0.8 m	7.00	7.33	0.61	0.11	0.05	0.057	-12.9	-13	0.92	-16.9	-17.9	0.29
0.8-1.0 m	8.04	6.68	0.36	0.38	0.15	0.017	-10.3	-11.5	0.66	-5.4	-11.2	0.41

PR, Perennial grassland; NT, Never-tilled annual cropland; SOC, Total soil organic carbon; POM, Particulate organic matter carbon. N=3

than 10 days prior to processing. Root length, surface area and volume were measured using Winrhizo digital scanning software (Whinrizo Pro 2003, Regent Instruments, Canada) after staining with Trypan blue. Natural abundance ¹³C signatures were measured for root biomass samples at the Stable Isotope Facility at the University of California at Davis. Due to a soil processing error Goessel root samples for May were lost and as such May Goessel data was not included in the analysis.

Soil sampling

Soils were also sampled on May 18 and June 23, 2008 with a geoprobe hydraulic sampler for determination of SOC, particulate organic matter (POM), MBC, and for assessment of nematode and micro-arthropod assemblages. Five cores (4 cm diameter, different cores for soil than for roots) were taken from each field and separated into the same depth intervals as the roots. Soil cores from each depth were bulked and mixed to obtain a homogenous composite sample. Subsamples were either air-dried for determination of soil properties, or refrigerated at 4 °C for MBC, micro-arthropod and nematode analyses. The mass and volume of cores was recorded for each depth for bulk density (ρ_b) measurements. Soil conditions, except at the high clay site (Goessel), were exceptionally good for taking accurate BD measurements. Moisture content was determined gravimetrically.

Soil organic carbon

At both sampling dates dried subsamples were ground and analyzed for total C and δ¹³ as described above.

Total SOC was calculated according to Lal et al. (1999) based on the depth interval and bulk density (ρ_b).

Particulate organic matter

Particulate Organic Matter (POM) was fractionated as outlined in (Sollins et al. 1999) and (Cambardella and Elliott 1992). Air-dried soil from the June sampling date was passed through a 2-mm sieve. A 20 g aliquot was placed in a 250 ml plastic bottle in 60 ml of 5 g L⁻¹ sodium hexametaphosphate and shaken overnight (16 h). The resulting solution was then passed through nested 250 μm and 53 μm sieves. Distilled water was used to wash the solution through the sieves and the material captured on the sieves was washed into pre-weighed glass beakers and oven dried (<45 °C) to constant weight. The resulting material was considered as the coarse POM (2 mm – 250 μm) and POM (250 – 53 μm) fractions. POM fractions were ground and analyzed for total C and δ¹³ as described above.

Microbial biomass C

The MBC was extracted by chloroform fumigation (Vance et al. 1987). Briefly, two 10 g samples of field moist soil, sieved through at 6.75 mm sieve, were taken from each sample. One sample was immediately placed in a 250 ml plastic bottle with 80 ml of 0.5 M potassium sulfate. The bottle was then shaken for 1 h and filtered through a 0.45 μm syringe filter. The second sample was fumigated in a vacuum desiccator with 50 ml of chloroform. The desiccator was evacuated until the chloroform boiled for 1 min, and then sealed for 24 h. Following fumigation, the sample was extracted the same way as the

non-fumigate sample. Control samples were extracted using the same process, but were not fumigated.

Filtered samples were frozen in 50 ml centrifuge tubes analyzed for total C and $\delta^{13}\text{C}$ as described above. The MBC was calculated according to Voroney et al. (1993). For the MBC samples, $\delta^{13}\text{C}$ signatures were calculated according to an equation from Ryan and Aravena (1994; Eq. 1).

$$\delta^{13}\text{C}_{\text{MBC}} = (\delta^{13}\text{C}_{\text{FUM}} \times C_{\text{FUM}} - \delta^{13}\text{C}_{\text{CONT}} \times C_{\text{CONT}}) / (C_{\text{FUM}} - C_{\text{CONT}}) \quad (1)$$

where C_{FUM} and C_{CONT} are the mass of C in the fumigated and control samples, and $\delta^{13}\text{C}_{\text{FUM}}$ and $\delta^{13}\text{C}_{\text{CONT}}$ are the $\delta^{13}\text{C}$ values of the fumigated and control samples, respectively.

The fraction of MBC derived from wheat or C_3 C was calculated according to the following equation (modified from Ryan and Aravena 1994, Eq. 2):

$$x = (\delta_{\text{S}} - \delta_{\text{C4}}) / (\delta_{\text{C3}} - \delta_{\text{C4}}) \quad (2)$$

where, δ_{S} is the $\delta^{13}\text{C}$ value of the sample, and δ_{C3} and δ_{C4} reflect the $\delta^{13}\text{C}$ values of the MBC in soils under only C_3 and C_4 vegetation, respectively. These references values were obtained from a study focused on the long-term sites (Beniston et al. 2014).

Micro-arthropods

Soil micro-arthropods were extracted in three samples of 80 ml for each time-point using modified Berlese-Tullgren funnels at 10 °C. The apparatus consisted of 240 ml plastic funnels (10 cm diameter) supported in 950 ml glass jars under 20 watt bulbs with heat reflectors. Each sample was placed in a plastic sleeve and set on a screen (6.35 mm) inside each funnel. Below each funnel, arthropods were collected in 20 ml glass vials containing 70 % ethanol. After 3 days, when the soil was completely dry, vials were removed. Recovered arthropods were preserved in 70 % ethanol and identified to family or genus.

Nematodes

Nematodes were extracted from 200 to 300 g soil within 10 days of sample collection using a combination of decanting, sieving and Baermann funnel methods (Barker 1985). Samples were sieved through a 0.246 mm sieve to remove larger particles and onto a 36 μm sieve to

separate nematodes from excess water. Samples were washed into beakers and placed on Baermann funnels for 48 h. Nematodes were counted using a dissecting microscope and the first 200 nematodes encountered in the sample identified at 200 \times to 400 \times to genus or family within one week of extraction.

Nematodes were assigned to trophic groups according to Yeates et al. (1993) and colonizer-persister (cp) groups based on Bongers (1990), and (Bongers and Bongers 1998). The cp scale classifies nematodes into five groups from microbial feeders with short life cycles and high fecundity (cp 1 and 2) to omnivores and predators with long life cycles and greater sensitivity to perturbation (cp 4 and 5). Standardized indices of food web structure and function based on characteristics of nematode assemblages allow the effects of environmental stress, dominant decomposition channels, and soil suppressiveness to plant parasites and pathogens to be determined (Bongers 1990; Ferris et al. 2001; Ferris and Matute 2003; Lenz and Eisenbeis 2000; Wardle et al. 1995). Soil food web indices were calculated after Ferris et al. (2001). The Structure Index is based on the relative abundance of nematodes in higher trophic groups and cp levels and indicates soil food web length and connectance. The Basal Index enumerates the predominance of nematode groups that are tolerant to disturbance. The Enrichment Index is based on the relative abundance of opportunistic bacterivore and fungivore nematodes and is considered an indicator of nitrogen cycling and availability.

Statistical analysis

Analysis of Variance (ANOVA) was performed on root and biological data using the PROC MIXED procedure in SAS v.9 (Cary, NC). Depth and management history were treated as fixed effects and block as a random affect. For root biomass and length, repeated measures were used for depth but dates were analyzed separately due to interactions between date and treatment. Mite data, available only for the top 0.10 m was analyzed using a repeated measure for date. Nematode indices and MBC, which had no interaction with depth, were analyzed using repeated measures for date. SOC, POMC, and $\delta^{13}\text{C}$ data, available only for the June sampling date, were analyzed as one way ANOVA comparing the effect of treatment (land use) by each sampling depth using JMP v. 9 software. Least squared means are reported for soil physical, chemical,

biological and root data. Significant differences were determined at $P=0.05$ or 0.10 .

The influence of root properties on treatment differences was measured using a series of mixed model analysis of covariance (ANCOVAs) procedures in SAS v.9 (Cary, NC). ANCOVAs were performed for the June sampling date only due to incomplete data for the May date.

Nematode communities were analyzed with non-metric multidimensional scaling (NMS) ordination. NMS is an ordination technique that summarizes complex multidimensional data in a low dimensional space. NMS uses the rank information in a distance matrix to calculate scores for a specified number of axes, and does not assume linear relationships among variables (McCune and Grace 2002). NMS analyses with the Bray-Curtis distance measure were performed on presence/absence for 56 nematode taxa by using PCORD via the *NMS function* with default parameters (50 runs with real data) except for the following: user defined start 58, 2D analysis. The final stress value was 11.7. The final instability was less than the 0.00001 stability criterion. Correlations between the NMS ordination of nematode communities and soil variables were tested using Pearson and Kendall correlations. Significance was determined using Multi-Response Permutation Procedures (MRPP). Final multivariate analysis was performed on three sites excluding "Goessel" which was an outlier in initial analysis, likely due to extremely clay based soil and loss of one replication during sampling.

Results

Root biomass

In the Long-term Study, average root biomass was 3.7-6 times greater in perennial grassland than in annual wheat fields in the first meter of soil (9.4 Mg ha^{-1} and 2.5 Mg ha^{-1} in May; 10.5 Mg ha^{-1} and 1.8 Mg ha^{-1} in June respectively; $P=0.004$; Fig. 1, Supplementary Table 1). The surface 0.4 m of soil contained 68 % and 74 % of the total root biomass in the first meter of soil for the grassland and wheat sites, respectively in May and June. Deeper depths of grassland sites (0.4-1.0 m) contained significant root biomass with 2.6 Mg ha^{-1} comparable to the entire first meter of soil in annual wheat. Annual wheat contained 0.6 Mg ha^{-1} in deeper soil horizons (0.4-1.0 m).

In the Conversion Study where perennial grassland had been killed and annual crops planted with no tillage five years prior in 2003, root biomass differed between annual wheat and perennial grassland at some but not all depths and dates. In May average biomass in the first meter was 2.3 times greater ($P=0.04$; Fig. 2; Supplementary Table 2) in grassland than never-tilled wheat. However, in the surface 0.2 m differences were not significant. In June differences were not consistent at deeper depths (Fig. 2). The presence of taproots resembling those of compass plant (*Silphium laciniatum*) at deeper depths suggests that even five years after no-tillage conversion to annual crops, perennial roots were still present in the annual system and potentially providing a belowground energy source (Fig. 2 June, depth 0.6 m).

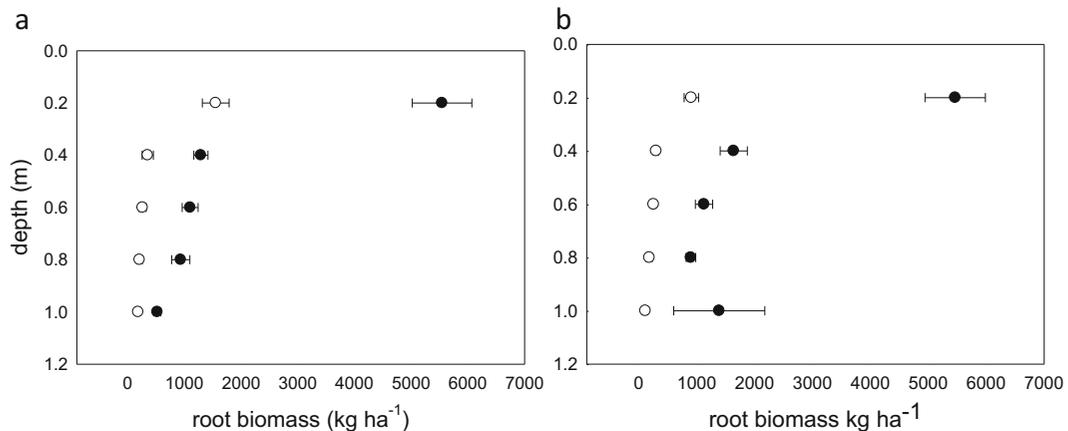


Fig. 1 Long Term Study - Root biomass (a) May 2008 and (b) June 2008 in grassland (closed circles) and cropland wheat (open circles) sites at 0 – 0.2 m, 0.2 – 0.4 m, 0.4 – 0.6 m, 0.6 – 0.8 m, and

0.8 – 1.0 m. $p=0.0003$; 0.00037 . Error bars=standard error of the mean

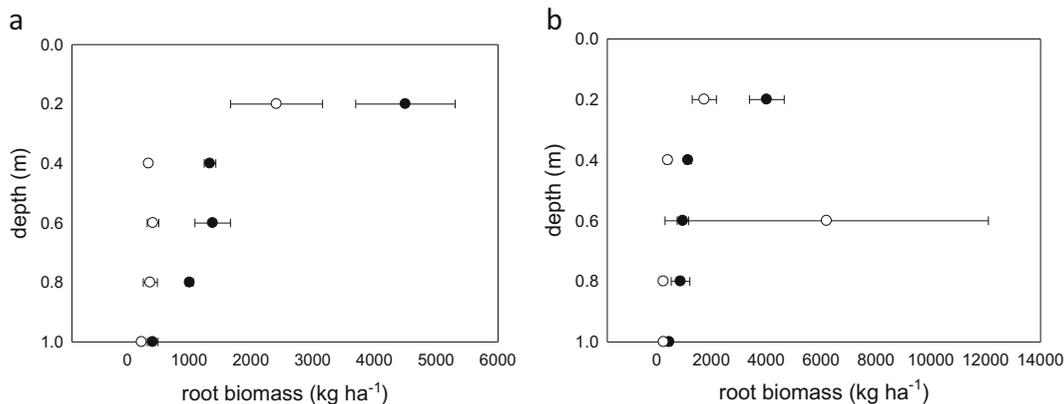


Fig. 2 Conversion Study - Root biomass (a) May 2008 and (b) June 2008 in grassland (closed circles) and never tilled cropland wheat (open circles) sites at 0–0.2 m, 0.2–0.4 m, 0.4–0.6 m, 0.6–0.8 m, and 0.8–1.0 m. $p=0.039$; 0.9364 . Error bars=standard error of the mean

Root length

In the Long-term Study total root length was 2.2–2.3 times greater in perennial grassland than annual wheat fields in the first meter of soil (52.5 km m⁻¹ and 24.0 km m⁻¹ in May; 44.8 km m⁻¹ and 19.2 km m⁻¹ in June respectively; $P=0.083$ and $P=0.017$; Fig. 3, Supplementary Table 3). In May perennial grassland had 33 km m⁻¹ of roots in the top 0.4 m compared to 14.7 in annual wheat, and 19.4 km m⁻¹ compared to 9.2 km m⁻¹ from 0.4 to 1.0 m. In June grasslands had 27.7 km m⁻¹ and cropland 11.2 km m⁻¹ (0–0.4 m) and 17.14 km m⁻¹ versus 8.05 km m⁻¹ (0.4–1.0 m).

Fine and coarse roots are often examined separately in order to better interpret roles of the root system in plant function. We analyzed root length and surface area in eight size classes. Root length in perennial grasslands

was significantly greater in all size classes than in cropland, with the exception of the smallest classes 0.00–0.10 and 0.10–0.20 mm diameter in May (Supplementary Table 3). Although differences were not significant for 0.10–0.20 mm, the trend was for longer length in grasslands. Interestingly in May the trend was towards greater length in wheat for the thinnest (0.00–0.10 diam) roots.

In the Conversion Study, total root length was not significantly different between perennial grassland and never-tilled wheat in May, except between the 0.4–0.6 m and 0.6–0.8 m depths (Fig. 4). When analyzed by size fractions the smallest fraction 0.00–0.10 was not significantly different for most depths (Supplementary Table 3). Additionally, differences were not significant in the surface 0.20 m of soil for any size fraction. In June total length was not significantly different. When

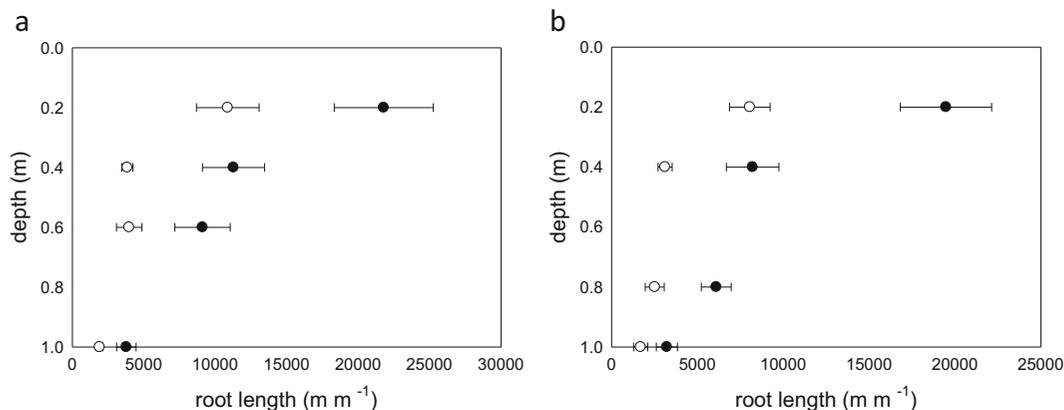


Fig. 3 Long-term Study - Root length (a) May 2008 and (b) June 2008 in grassland (closed circles) and cropland wheat (open circles) sites at 0–0.2 m, 0.2–0.4 m, 0.4–0.6 m, 0.6–0.8 m, and 0.8–1.0 m. $p=0.083$; 0.017 . Error bars=standard error of the mean

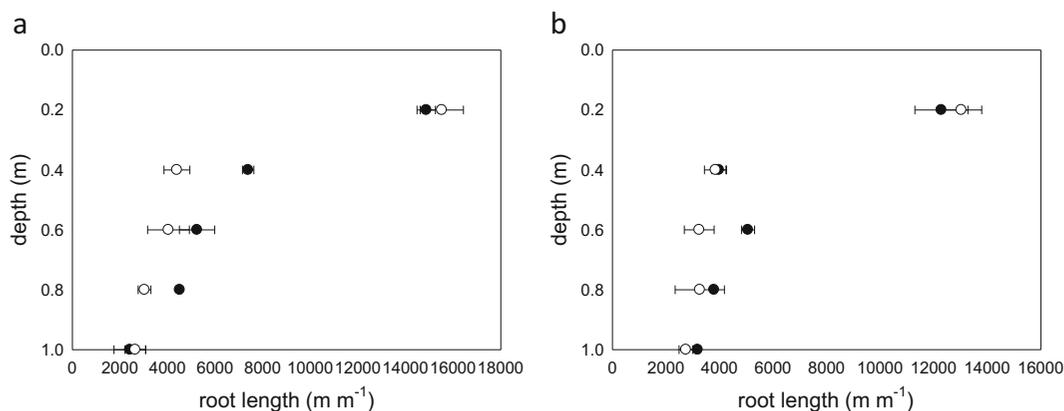


Fig. 4 Conversion Study - Root length (a) May 2008 and (b) June 2008 in grassland (closed circles) and never tilled cropland wheat (open circles) sites at 0–0.2 m, 0.2–0.4 m, 0.4–0.6 m, 0.6–0.8 m, and 0.8–1.0 m. $p=N.S.$ Error bars=standard error of the mean

analyzed by size fractions, only medium fractions 0.35–2.0 mm were significantly different for depth intervals from 0.4 to 1.0 m. Similar values in surface depths in annual never-tilled wheat and perennial grassland mirrors the trend seen in root biomass and in the Long-term Study suggesting a concentration of roots in surface depths in annual systems. However, due to the presence of identifiable perennial plant roots in deeper depths of annual wheat fields, wheat root lengths in the Conversion Study may be confounded by remnant grassland roots.

Soil biological, chemical and physical properties

Soil organic carbon

SOC pools were significantly degraded in long-term annual cropland compared to perennial grasslands to a depth of 0.60 m (78 Mg C ha⁻¹ vs 109 Mg C ha⁻¹; Beniston et al. 2014). Over the 1.0 m profile grasslands

contained 153 Mg C ha⁻¹ and croplands contained 115 Mg C ha⁻¹. In the Conversion Study five years after no-tillage establishment of annual crops, total SOC was not significantly different between never-tilled cropland and perennial grassland (Table 1). However, coarse POM (>250 μm) was significantly fewer in never-tilled cropland than perennial grassland between 0.1 m to 0.6 m (Table 1).

Microbial biomass

In the Long-term Study MBC pools were greater in perennial grassland soils to a depth of 0.80 m (3.5 Mg ha⁻¹ vs 1.4 Mg C ha⁻¹; Beniston et al. 2014). Perennial grassland soils had approximately three times as much MBC as cropland soils in all sample depths between 0 and 0.40 m and twice as much MBC at 0.60 and 0.80 m. In the Conversion Study, MBC was significantly greater in grassland than never-tilled annual cropland only in the surface 0.4 m in May (Table 2).

Table 2 Microbial biomass C (kg ha⁻¹) from mid May and late June in the Conversion Study

Depth	May MBC (kg ha ⁻¹)		P value	June MBC (kg ha ⁻¹)		P value
	PR	NT		PR	NT	
0 - 0.1 m	619.6	420.8	0.029	824.8	710.1	0.102
0.1-0.2 m	557.1	384.7	0.028	768.9	660.1	0.071
0.2-0.4 m	432.2	312.7	0.042	657.1	560.1	0.351
0.4-0.6 m	307.3	240.6	0.234	545.2	460.1	0.552
0.6-0.8 m	182.4	168.6	0.847	433.4	360.1	0.195
0.8-1.0 m	57.5	96.5	0.689	321.5	260.1	0.420

PR, Perennial grassland; NT, Never-tilled annual cropland. $N=3$

At June sampling dates MBC was not significantly different at any depth.

Soil micro-arthropods

In the Long-term Study Oribatid mites were significantly greater in the perennial grasslands (Grassland=2084 mites m⁻², Cropland=730 mites m⁻²; *p*=0.0047; Table 3). Numbers of collembolan were not significantly different. In the conversion study, Oribatid mites trended towards larger numbers in never-tilled cropland compared to perennial grassland in the surface 0.1 m (Never-tilled Cropland=5044 mites m⁻²; Grassland=2001 mites m⁻², *p*>0.05). Prostigmatid mites were extremely rare, present in only 10 % of samples. Predatory Mesostigmatid mites were also not significantly different but the trend was for larger populations of these indicators of more complex soil systems in grassland (Grassland=1126 mites m⁻², Never-tilled Cropland=625 mites m⁻²; *p*>0.05). Numbers of collembolans were not significantly different between treatments.

Soil nematodes

In the Long-term Study, nematode communities in perennial fields trended toward higher Structure and lower Basal Index ratings than annual fields in the surface 0.40 m (Structure Index: Grassland=67 vs Cropland=59; Basal Index: Grassland=23 vs Cropland=32; Table 3). At the Conversion Site, never-tilled annual wheat plots had a higher Basal Index and lower Structure Index than perennial grassland to 1.0 m (Basal Index: Never-tilled Cropland=37.6; Grassland=33.06; Structure Index: Never-tilled Cropland=49.6; Grassland=54.4; *p*<0.1). The Enrichment Index was not

significantly different between sites (Enrichment Index: Grassland=42.3; Never-tilled Cropland=37.6).

Relationships between nematode communities, roots, soil properties and soil micro-arthropods in long-term perennial grassland and annual cropland

We analyzed relationships between management practices, soil properties, nematode and soil micro-arthropod communities, and roots amongst data collected from paired harvested perennial grassland and annual wheat sites at three depths (0–0.10 m, 0.10–0.20 m and 0.20–0.40 m) (Fig. 5). The data points represent nematode communities from individual samples made up of 56 genera analyzed with NMS. Vectors represent correlations of soil properties, roots, mite trophic groups and nematode indices with nematode communities. The length and angle of each vector represent the strength and direction of the relationship to the nematode community. Nematode communities were significantly different between perennial and annual systems (Fig. 6; *p*<0.0001). Treatment differences are represented in Axis 1 (61 % of the variation), while differences in depth are represented in Axis 2 (22 % of the variation).

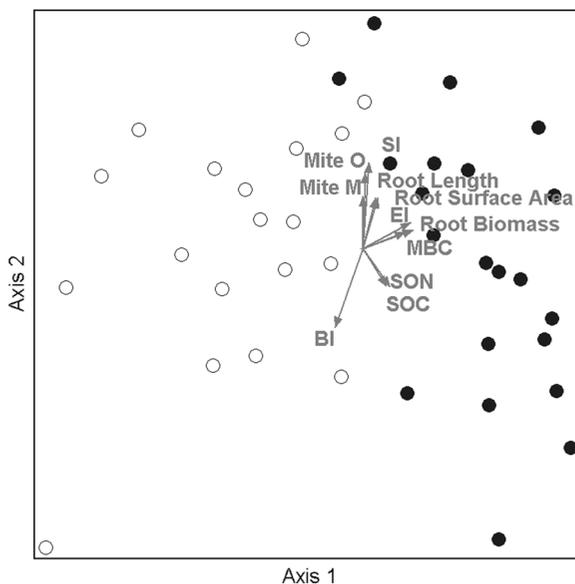


Fig. 5 NMS ordination of nematode communities in grassland (closed circles) and cropland sites (open circles) 0–0.4 m depth. Vectors represent the relationship of soil, root and nematode factors to nematode communities; basal index (BI), soil nitrogen (SON), soil organic carbon (SOC), microbial biomass (MBC), root biomass, nematode enrichment index (EI), nematode structure index (SI), root length, root surface area, predator mites oribatid (Mit O), fungal feeding mites (Mite M) for May and Jun 2008

Table 3 Soil biological properties in the Long-term Study

	Grassland	Annual Cropland
Structure Index	67.3±4.9	59.3±4.7
Basal Index	23±2.9	31.7±2.8
Oribatid mites m ⁻²	2,084±188	730±188
Mesostigmatid mites m ⁻²	875±434	138±434
Collembola m ⁻²	1,209±584	709±584

Nematode structure and basal indices at depths 0–0.4 m (mean: no-interaction with depth). Mites and collembola measured to 0.1 m. Means plus or minus the standard error. *N*=4

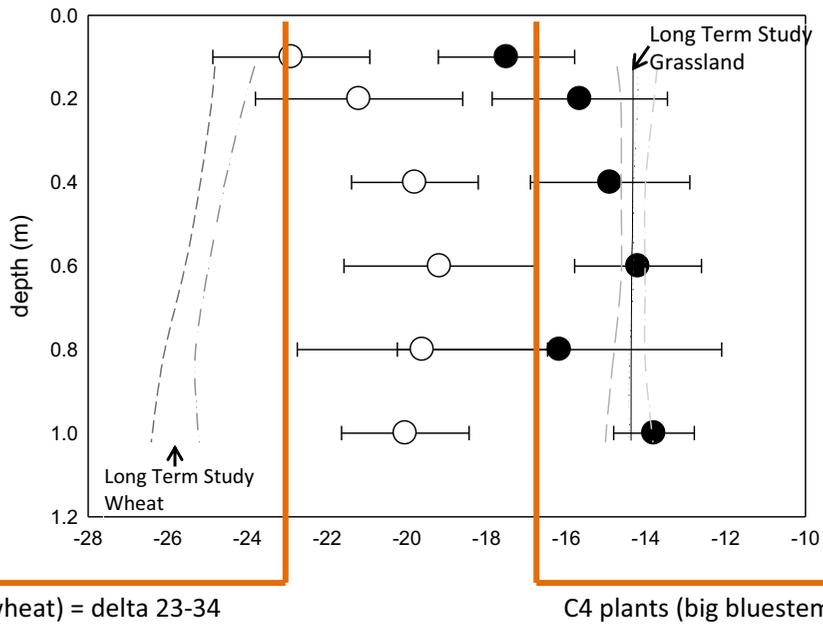


Fig. 6 Carbon isotope analysis of roots (Delta PDB) in Conversion Study grassland (closed circles) and never-tilled cropland wheat (open circles) sites. C3 plants such as wheat have a delta PDB between 23 and 34. C4 plants such as the most predominant

grass in grassland sites, big bluestem, have a delta PDB between 9 and 17. Average delta PDB in Long Term Study grassland sites -14.7 and in wheat was -25.7 (lines=average, dashed lines=standard error)

Vectors represent a strong positive relationship between perennial grassland nematode communities and high nematode Structure Index; root length, root surface area, root biomass, soil C and N. The nematode Basal Index has a strong positive relationship with annual wheat nematode communities. Total number of Orbatid (predator) and Mesostigmatid (decomposer) mites were related to upper depths because they were only sampled in the surface 0.1 m.

Relationships between soil physical, biological and root properties were further explored with Pearson's correlations. Root length and biomass were positively correlated with MBC, SOC, POM C, and nematode Enrichment Indices (Table 4; $p < 0.01$). Root length

was correlated with MBC (0.75), SOC (0.56), POM C (0.65), nematode enrichment index (0.61), and total nematodes (0.38). Root biomass was correlated with MBC (0.56), SOC (0.42), and POM C (0.74).

The influence of root properties on treatment differences was measured using a series of mixed model analysis of covariance (ANCOVAs). The ANCOVA for perennial grassland versus annual cropland controlling for root biomass was found to have no significant main effects for soil properties SOC, POMC, MBC in June ($p > 0.05$). The ANCOVA for perennial grassland versus annual cropland controlling for root length was found to have no significant effects for SOC and MBC ($p > 0.05$) and significant differences for

Table 4 Pearson's correlation coefficients for the June date from the Long-term Study

	MBC (kg ha ⁻¹)	MBC/SOC Ratio	Total Nematodes (1,000 nem/m ²)	Nematode EI	SOC Pool (Mg ha ⁻¹)	POM C (g C kg ⁻¹ soil)
Root Length (total length, Km m ⁻²)	0.75	0.71	0.38	0.61	0.56	0.65
Root Biomass (kg ha ⁻¹)	0.56	0.52	0.29	0.51	0.42	0.74
SOC Pool (Mg ha ⁻¹)	0.76	0.4	0.03	0.32	*	0.26

Values in bold are significant at $p < 0.01$. $N=4$

MBC, Microbial biomass carbon; SOC, Soil organic carbon; EI, Enrichment index; POM C, Particulate organic matter carbon

POMC ($P=0.46$). In contrast when root biomass or length covariables were not included in the analysis soil properties SOC, MBC, and POMC were significantly different in June ($p<0.05$).

Discussion

Long-term grassland and annual cropland

A better understanding of ecosystems that are able to maintain both productivity and soil quality is necessary to design future agro-ecological systems. Here we report root properties of a grassland ecosystem that has been harvested annually for seventy five years or more, exporting similar amounts of nutrients as high yielding annual systems, and still maintained high soil quality compared to agricultural cropland.

Root biomass and length were significantly greater in long term perennial grassland than annual cropland. Biomass values of 9.4 Mg ha^{-1} vs 2.5 Mg ha^{-1} in May and 10.5 Mg ha^{-1} vs 1.8 Mg ha^{-1} in June in grassland compared to wheat are typical for studies in the region. Tallgrass prairie root biomass values typically range from 7 to 21 Mg ha^{-1} (Rice et al. 1998). Grassland root biomass was 9.5 and 2.7 times greater than wheat root biomass in the surface 0.3 m (Frank et al. 2006) and the surface 0.5 m (Buyanovsky et al. 1987), respectively, demonstrating the large allocation of belowground resources in these perennial grasslands, relative to their annual counterparts. Globally, temperate grasslands average more than nine times greater root biomass than croplands (Jackson et al. 1996).

Root lengths in this study were longer than those found for grasslands in the nearby Konza prairie (Manhattan Kansas, US) where Nippert et al. (2012) measured $16\text{--}28 \text{ km m}^{-2}$ in the first meter of soil.

However, Konza soils are shallow and rocky, unsuitable for agriculture, which likely restricts roots to upper depths. Additionally, Nippert et al. washed roots with sieves which tends to destroy more small lateral roots, nodules and other fragile roots structures compared to the hydropneumatic root elutriator used in this study (Smucker et al. 1982). The values reported here are also greater than those cited in a review by Gregory (2006). Gregory and colleagues found average root lengths of 20 m m^{-2} in grasses and $5\text{--}10 \text{ m m}^{-2}$ in cereals in the surface 0.1 m of soil. This is considerably less than the 7.3 and 5.2 km m^{-2} in the surface 0.1 m of wheat fields

and 13.2 and 11.9 km m^{-2} in the surface 0.1 m of perennial grasslands in this study (May and June respectively). This discrepancy may be due to large variability across seasons, years and biomes (Gill and Jackson 2000; Hayes and Seastedt 1987). The larger, longer roots systems found in perennial grassland compared to annual cropland in this study are not surprising given regional and global data. However, they provide a rare example where both annual and perennial systems are positioned on deep, prime lowland soils typical of one of the most productive agricultural regions in the world.

Our study supports the hypothesis that root length, and biomass are positively related to soil carbon and biological factors. Root length, surface area and biomass were positively correlated to nematode communities, SOC, POMC, MBC, SON and nematode indicators of nutrient cycling (EI). Roots provide large belowground C sources known to affect MBC (Paterson 2003; Waldrop et al. 2006; Wardle 1992), microbial community structure (O'Donnell et al. 2001a) nematode communities (Ilieva-Makulec et al. 2006; Wardle et al. 2005) and SOC (Puget and Drinkwater 2001; Rasse et al. 2005). For example, Rasse et al. (2005) in a review of 19 studies concluded that much of soil C is root C since mean residence time of root C is 2.4 times greater than shoot C due to recalcitrance, physical protection in deep layers, protection in aggregates by mycorrhizae and root hairs, and chemical interactions. Roots affect belowground dynamics through exudates, sloughing and root death. While estimates of root turnover are highly variable (e.g., ranging from 0.20 to 2.5 years^{-1}) (Gill and Jackson 2000; Partel and Wilson 2002), using a conservative estimate of 20 %, $2.0 \text{ Mg ha}^{-1} \text{ year}^{-1}$ of root biomass would be supplied as a belowground resource in perennial grassland plots. Our analysis shows that differences in soil carbon, MBC, POMC between management practices are only significant when variation from root biomass or length is not accounted for. This supports the hypothesis that root properties can explain a large amount of the variation between annual and perennial cropping systems.

Root and soil properties five years after no-tillage conversion to annual cropland

The Long-term Study sites are limited by the fact that differences in soil properties observed may be artifacts of the region's early agricultural practices for which better alternatives have been more recently developed

(e.g., no-tillage practices). In order to look more closely at the effects of annual and perennial crops on soil properties with fewer interactions from management, researchers established plots which converted grassland to annual crops using best management practices through herbicide application and without tillage. Prior research showed that readily oxidizable carbon and microbial biomass were significantly less and nematode communities significantly different in never-tilled annual cropland versus perennial grassland in the surface 40 cm (DuPont et al. 2010). However, in this study differences between SOC and bacterial communities were not significant. Trends were similar with significant reductions in more dynamic soil properties including POMC, and MBC as well as nematode indicators of soil food web structure (SI) after no-till conversion to annual crops. But the magnitude of differences were not as disparate as in long term systems. Contrary to our hypothesis root length was not significantly different at most depths and root biomass was greater in grassland but not in the top 0.2 m.

Due to identifiable tap roots in deeper soil fractions we suspected annual cropland sites may have remnant perennial grassland roots contributing to belowground dynamics. In order to identify the source of belowground carbon; roots, MBC and SOC fractions were analyzed according to their stable carbon isotope ratios. Natural abundance $\delta^{13}\text{C}$ signatures provide a means for determining the relative carbon contribution from either C_3 or C_4 photosynthesis. Values of C_3 plants range between -23 and -34 ‰ whereas C_4 plants range from -9 to -17 ‰ (Eleki et al. 2005). Wheat is a cool season C_3 grass and thus never-tilled cropland root isotope values should have ranged from -23 to -34 ‰. In related sites which have had annual crop rotations for more than 75 years the average root value was -25.7 ‰. Perennial grasslands had an average value of -14.7 ‰. However, the majority of never-tilled annual wheat root samples had intermediate isotope signatures (-19.2 to -23 ‰; Fig. 6). This suggests that roots found in never-tilled cropland sites contain a mixture of wheat roots and old perennial grass roots.

Five years after the no-till conversion from harvested perennial grassland to no-till wheat, isotope analysis of soil carbon showed that all fractions except the most labile fraction, coarse POM, were derived from C_4 plants. As such, the majority of soil carbon in the never-tilled annual cropland sites was derived from the prior perennial grassland community.

Significant changes in ^{13}C signatures were observed in the coarse (0.25–2 mm) POM fraction which is considered to be the most labile size fraction, composed of recent plant inputs. Natural abundance ^{13}C values were distinct from the 0.1–0.2 m (Table 1; $p=0.003$) through the 0.4–0.6 m depths (Table 1; $p=0.007$). This suggests a significant input of wheat (C_3) biomass, in these layers. As no tillage had been performed to incorporate above ground residue, the source was likely roots. Coarse POM ^{13}C signatures did not differ in the soil surface (0–0.1 m) layer which may indicate that a significant quantity of prairie derived biomass persisted in the soil surface.

In June, below 0.1 m, natural abundance ^{13}C values for MBC did not differ between the two treatments (Grassland= -13.1 ‰, Never-tilled Cropland= -13.3 ‰; $p=0.26$). This trend suggests that the microbes extracted in the MBC from these lower depths in the never-tilled cropland plots were gaining C primarily through breaking down root material from the perennial grasslands that previously covered the plots.

Statistically distinct values were observed in the MBC $\delta^{13}\text{C}$ values for the 0–0.1 m depth of the conversion plots (Grassland= -14.8 ‰, Never-tilled Cropland= -16.8 ‰; $p=0.025$). This indicates that microbes in the never-tilled cropland plots are using some C_3 , or wheat-derived C, at this depth. Interestingly, estimation of the C source in the MBC at the 0–0.1 m depth in the never-tilled cropland plots suggests that only 43 % of the C in those samples is C_3 or wheat C. Thus the $\delta^{13}\text{C}$ signatures imply that even in the soil surface (0–0.1 m) the majority of C being utilized by the microbial biomass is C_4 from perennial grassland.

Conclusions

Root biomass and length were greater in perennial grasslands compared to annual wheat. Significant correlation between larger, longer roots in grasslands compared to annual croplands and improved soil biological properties and organic matter fractions suggest that perennial roots are an important factor allowing perennial grasslands to maintain productivity and soil quality with few inputs.

Fields recently converted to annual crops from perennial grassland showed some small reductions in soil quality indicators in annual cropland compared to perennial grasslands, even without tillage. The magnitude

of these differences is likely not as great (compared to long-term grass and cropland) because old perennial roots are still present in never-tilled annual plots and much of soil C is still C₄ derived from perennials plant communities that used to occupy these fields. Perennial roots may persist and continue to influence soil properties long after conversion to annual systems.

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