

Perennial grain crop roots and nitrogen management shape soil food webs and soil carbon dynamics

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ABSTRACT

Perennial grain crops may confer greater ecosystem services relative to annual row crop systems due to their extensive roots systems and year-round ground cover. However, less is known about the extent to which perennial grain crops affect food web dynamics and soil carbon (C) cycling over time. Furthermore, many mechanistic questions remain regarding the influence of root quantity and quality on soil biological communities and C cycling function. In this study, we quantified root biomass and quality, bacterial and nematode community structure, and labile soil C pools of perennial intermediate wheatgrass [*Thinopyrum intermedium* (Host) Buckworth and Dewey] and annual winter wheat (*Triticum aestivum* L.) across three nitrogen (N) management systems (Organic, Low inorganic N, High inorganic N). After 4 years, the perennial grain crop had significantly greater root quantity and permanganate oxidizable carbon (POXC) relative to annual wheat. Mineralizable C was similar between the two plant systems but differed by N management. Perenniality more than management influenced nematode community structure and function; perennial grains increased the structure index (a measure of trophic complexity) by 55% compared to annual wheat. Both plant type and management influenced bacterial communities, but not until the final year of the experiment. Our findings demonstrate that established perennial grain crops increase labile soil C and support more highly structured and complex food webs relative to annual cropping systems.

1. Introduction

Distinct differences in plant physiology and ecosystem function between annual row crops and natural systems have provided a framework for the development of perennial grain crops as a way to transition to more sustainable agroecosystems (Crews et al., 2016; Glover et al., 2010). Differences in biomass allocation is one major distinction, whereby annuals allocate proportionally more biomass aboveground while perennial systems allocate more biomass towards extensive root systems (Dietzel et al., 2015; Sprunger et al., 2018a). Larger root systems in native grasslands are often associated with greater biological activity and enhanced soil physical structure (Culman et al., 2010; DuPont et al., 2014). The presence of larger roots stimulates greater root exudation and influences important nutrient cycling functions (Haichar et al., 2008; Culman et al., 2010). For example, roots

of perennial crops lead to significantly greater nitrogen (N) use efficiency relative to annual wheat (Sprunger et al., 2018a) and have contributed to reductions in nitrate leaching compared to annual row crops (Jungers et al., 2019; Syswerda et al., 2012; Culman et al., 2013a).

Because root production and decay are major regulators of the global C cycling, the introduction of perennial crops into working landscapes could significantly affect soil carbon (C) accumulation (Gill and Jackson, 2000). Furthermore, greater allocation toward root biomass likely contributes to enhanced C stabilization within perennial systems since root C persists longer in the soil than shoot C (Rasse et al., 2005). However, the extent to which perennial cropping systems contribute to soil C accumulation is mixed within the scientific literature. For example, early signs of soil C accumulation under perennial bioenergy crops relative to annual row crops has been shown in the upper

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Midwest (Tiemann and Grandy, 2015; Sprunger and Robertson, 2018). In contrast, differences in soil C have not been detected between annual wheat and perennial intermediate wheatgrass, despite twelve times the amount of root C within the perennial crop (Sprunger et al., 2018b). Increases in soil C are often associated with greater C quantity (Wang et al., 2017; Menichetti et al., 2015; Post and Kwon, 2000) and root chemical composition and quality effects on food web dynamics and soil C cycling are too often overlooked.

Understanding the relationship between C input quality and soil microbial communities has important implications for decomposition rates and subsequent soil C dynamics (Tiemann et al., 2015; Margenot and Hodson, 2016). For example, bacteria can decompose organic materials with low carbon to nitrogen (C:N) ratios more quickly than fungi (Mäkela et al., 2015). Nematode community structure can also play an important role in regulating soil organic matter dynamics and indicate whether decomposition pathways are primarily mediated by fungal or bacterial channels (Ferris et al., 2001; Ugarte et al., 2013). For example, through assessing the relationship between nematode indices and labile C fractions, Margenot and Hodson (2016) found that the ratio of fungal to bacterial-feeding nematodes (channel index) decreased with labile fractions but increased with aromatic C, indicating that fungal-feeders were associated with more processed C pools.

Belowground C inputs from plant roots also influence soil food web structure and function (Philippot et al., 2013). However, the relationship between belowground C inputs and microbial community dynamics across annual and perennial cropping systems remains understudied. Roots of perennial systems typically have a much higher C:N ratio compared to their annual counterparts (Dietzel et al., 2015; Sprunger et al., 2018a, 2018b). It is currently unclear how contrasting root C:N ratios in annual and perennial crops might influence food web structure and function in addition to short and long-term soil C dynamics.

Interactions between roots and the soil food web strongly influence soil C dynamics, and these relationships are further influenced by N fertilization. There is wide-spread evidence that inorganic N fertilization negatively influences microbial communities including arbuscular mycorrhizal fungi and nematode diversity in both annual and perennial cropping systems (Emery et al., 2017; Liu et al., 2016; Treseder, 2004). N fertilization in croplands also reduces nematode species richness and diversity (Liu et al., 2016). Such changes in nematode diversity can lead to shifts in microbial communities that reflect adaptation to stress and disturbance (Emery et al., 2017). In contrast, croplands receiving organic fertilizer additions such as manure or crop residues, typically experienced increased species richness, whereby organic C inputs have the ability to buffer stress and enhance soil food web structure and function (Liu et al., 2016). However, root biomass often increases with higher rates of inorganic sources of N, creating a greater source of C for microbial consumption (Liu et al., 2016). Total root biomass of an established perennial grain crop was greater in systems receiving inorganic N compared to poultry manure (Sprunger et al., 2018a). Thus, determining the trade-offs within food web dynamics and nutrient cycling that occur when growing perennials that receive inorganic fertilizer additions is important for evaluating net ecosystem benefits of perennial cropping systems. Addressing this uncertainty will become increasingly important with the advent of fertilized perennial grains and bioenergy cropping systems.

Here, we build on previous perennial grain crop research to identify how roots of annual and perennial cropping systems affect food web structure and function. This study provides insight into soil ecosystem processes, including short and long-term C dynamics in these systems. The perennial grain crop examined in this study is intermediate wheatgrass (IWG; *Thinopyrum intermedium* (Host) Barworkth and D.R. Dewey), a cool-season grass that has been intensively bred for grain production over the last several decades and will hereafter be referred to as perennial Kernza (DeHaan and Ismail, 2017). In order for perennial Kernza to reach optimal yields, sources of N will need to be

applied (Jungers et al., 2017). To this end, the objective of this paper is to disentangle the effects that perenniality and N management have on soil food web dynamics and soil C cycling. We hypothesize that perenniality will be a larger driver of soil food webs and soil C dynamics compared to N management due to changes in newly added root C. We also expect that perennial Kernza will accumulate more soil C relative to annual wheat in large part due to exhibiting greater root quantity and lower root quality.

2. Materials and methods

2.1. Site description, experimental design, and management

The study was conducted at the W.K. Kellogg Biological Station (KBS) in Hickory Corners, Michigan (42° 24' N, 85° 24' W, elevation 288 m). Mean annual precipitation is 890 mm and mean annual temperature is 9.7 °C. The soils at this site are Kalamazoo series (fine-loamy, mixed, mesic Typic Hapludalfs) with measured soil properties at 0–10 cm as follows: pH = 5.5, soil organic carbon = 9.5 g kg soil⁻¹, total soil nitrogen = 0.94 g kg soil⁻¹, sand = 558 g kg soil⁻¹, silt = 368 g kg soil⁻¹, clay = 74 g kg soil⁻¹. More detailed soil information can be found elsewhere (Syswerda et al., 2012) along with more general site information can be found at: http://lter.kbs.msu.edu/about/site_description/index.php. Previously, the field site was in a conventionally managed corn (*Zea mays L.*)–soybean (*Glycine max (L.) Merr.*)–wheat rotation.

In the fall of 2009, a split-plot design with four replicates was established with management as the main treatment and plant species as the nested plots. The management systems were: i) organically-managed treatment (Organic), ii) conventionally managed, recommended 90 kg N ha⁻¹ treatment (Low N), and iii) conventionally-managed 135 kg N ha⁻¹ treatment (High N). The primary differences between management scenarios were in the fertilizer forms and rates applied and in the weed management strategies (herbicides applied only in conventionally managed plots). Weed control was only a factor in 2010 during the establishment of perennial Kernza (Culman et al., 2013a,b). After establishment, Kernza filled any available space via tillers and rhizomes and weeds were no longer an issue. Annual wheat had negligible weed pressure throughout the study, due to its ability to suppress weeds. Given the negligible amount of weed pressure in these plots and no use of herbicides beyond the establishment year, it is unlikely that herbicides significantly impacted belowground dynamics. Organic and Low N received the same rate of N (90 kg N ha⁻¹), but in different forms (poultry manure vs. urea), while Low N and High N differed only by N application rate (90 kg N ha⁻¹ vs. 135 kg N ha⁻¹, respectively). The Low N treatment represents the recommended practice in Michigan (Vitousek et al., 1995), and the High N treatment received 50% more N than the Low N treatment. At planting, organic plots received 2240 kg ha⁻¹ of commercially available pelletized poultry manure (mix of layer poultry manure and sawdust; 4-3-2 N-P-K). This application rate supplied 90 kg ha⁻¹ total N equivalent. Low and High N treatments received urea as starter 34 kg N ha⁻¹ at planting, and then two top-dressing applications in the spring to reach the total N rates of 90 and 135 kg N ha⁻¹, respectively. In addition, both the Mid N and High N systems received a starter of 53.8 kg K ha⁻¹ as K₂O at planting. Within each main plot, two plant species were planted: annual wheat (*Caledonia*) and perennial intermediate wheatgrass (Kernza). The Kernza seed used was from a breeding population derived from one cycle of selection primarily for seed size and yield per spike (Cox et al., 2010). Parents used to create the population had previously been through one to two cycles of selection by the Rodale Institute and the USDA Big Flats Plant Materials Center. Plots were planted with a grain drill at a rate of 310 seeds m⁻² at 15 cm row spacing in the first year and the annual wheat seeding rate was increased to 432 seeds m⁻² each year thereafter. Plots measured 3.66 by 5.5 m.

2.2. Soil and root sampling and analyses

Soils were sampled in June 2011 (Year 2), June 2012 (Year 3) and June 2013 (Year 4). Soils were sampled 0–10 cm and 6-cm diameter cores were taken in three randomly selected locations in the plot. The samples were composited, sieved to 6 mm, and mixed until homogeneous. For each sample, a representative subsample was transferred to a sterile whirlpack bag and stored at -20°C until microbial analyses were conducted. A subsample of 400 g was also taken for root analysis. Coarse roots (> 6 mm, via dry sieving) and fine roots (1 mm–6 mm, via wet sieving) were recovered, dried, weighed and ground for C and N in a CHNS analyzer (Costech Analyzer ECS 4010, Costech Analytical Technologies, Valencia, CA). More in-depth details on root sieving and root washing are described in [Sprunger and Robertson, 2018](#).

2.3. Bacterial community structure

To characterize bacterial communities, we used an amplicon sequencing approach. We extracted soil DNA using the MO BIO PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA) from 0.25 g of homogenized soil. We amplified the V4–V5 region of the 16S ribosomal RNA gene using barcoded primers (bacterial/archaeal 515f/806r primer set; [Caporaso et al., 2012](#)). For each soil sample, PCR reactions were run in triplicate. Amplicons were cleaned using the AMPure XP purification kit, quantified using the QuantIt PicoGreen kit (Invitrogen), and libraries pooled at equal molar ratios. The pooled libraries were sequenced on the Illumina MiSeq platform using paired end reads (Illumina Reagent Kit v2, 500 reaction kit) at the Indiana University Center for Genomics and Bioinformatics Sequencing Facility. Raw sequences were processed using the *mothur* software package version 1.39.5. A standard *mothur* pipeline was used to process and analyze bacterial sequence data ([Kozich et al., 2013](#)). Contigs were assembled from the paired end reads, quality trimmed using a moving average quality score (minimum score 35), aligned sequences to the Silva Database (version 123), and removed chimeric sequences using the VSEARCH algorithm ([Rognes et al., 2016](#)). Operational taxonomic units (OTUs) were created by first splitting sequences based on taxonomic class and then binning into OTUs based on 97% sequence similarity.

2.4. Nematode community structure

Nematode communities were enumerated and identified in soil samples. Nematodes were extracted from 200 cc soil within 10 days from sample collection using a combination of decanting, sieving and Baermann funnel methods ([Barker, 1985](#)). Samples were sieved through a 250 μm sieve to remove organic fragments and larger particles and onto a 38 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 to genus or family by microscopy within one week of extraction.

Nematodes were assigned to trophic groups according to [Yeates et al. \(1993\)](#) and colonizer-persister 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. Soil food web indices were calculated after [Ferris et al. \(2001\)](#). The Structure Index (SI) is based on the relative abundance of nematodes in higher trophic groups and cp levels and indicates soil food web length and connectance. The Channel Index (CI) is calculated as the proportional abundance of fungal feeders to the abundance of enrichment opportunist bacterial feeders and reflects the primary decomposition channel in the soil, fungal mediated or bacterial facilitated. The Basal Index (BI) enumerates the predominance of nematode groups that are tolerant to disturbance. The Enrichment

Index (EI) measures the number of opportunistic bacterial and fungal feeders that respond quickly to the input of C and N sources. Dauer larvae were included in all soil food web indices because they reflect the potential for nematode communities to respond to enrichment and nutrient additions ([Bongers and Bongers, 1998](#)), which is a central objective of this study.

2.5. Mineralizable carbon

Mineralizable C reflects the pool of C that is most microbially available and is very sensitive to changes in management. One-day mineralizable C was determined on rewetted soils ([Franzleubbers et al., 2000](#); [Haney et al., 2001](#)). The amount of water needed to bring soils to 50% water-filled pore space (WFPS) was determined gravimetrically for each depth prior to the incubations. Ten grams of air-dried soil were weighed in duplicates into 100 mL beakers and placed inside a 237 mL canning jar. Deionized water was added to the soil, jars were capped tightly, and a zero-time CO_2 concentration was determined immediately by sampling 0.5 ml of air from the headspace and injecting into a LI-Cor LI-820 infrared gas analyzer (IRGA; Lincoln, Nebraska). The jars were incubated at 25°C for 24 h and a 1-day CO_2 concentration was determined. Carbon mineralization was determined as the difference between zero time and 1-day CO_2 concentration.

2.6. Permanganate oxidizable carbon

POXC is an indicator of the theoretical ‘active C pool’ but reflects a more processed pool compared to mineralizable C and serves as an early indication of soil C stabilization ([Culman et al., 2012](#); [Hurisso et al., 2016](#)). Permanganate Oxidizable Carbon (POXC) were based on [Weil et al. \(2003\)](#), and described fully here: <http://lter.kbs.msu.edu/protocols/133>. Briefly, 20 mL of 0.02 M KMnO_4 was added to a 50 mL centrifuge tube containing 2.5 g of air-dried soil. The tubes were shaken for exactly 2 min (240 rpm), allowed to settle for exactly 10 min, and 0.5 mL of the supernatant were transferred into a 2nd 50 mL centrifuge tube and mixed with 49.5 mL of deionized water. Sample absorbance was read with a spectrophotometer at 550 nm.

2.7. Statistical analyses

Analysis of Variance (ANOVA) was performed on soil, plant, nematode indices, and species richness data with the PROC MIXED procedure in SAS v.9 (Cary, NC). Sampling year, management, and plant species were treated as fixed effects and block as a random effect with significant differences determined at $\alpha = 0.05$. Means were compared with an adjusted Tukey's pairwise means comparison procedure in PROC MIXED in SAS. All graphing was performed with the package *ggplot2* ([Wickham, 2016](#)) in R (R Core Team, 2019).

To compare nematode and bacterial alpha diversity, we estimated Shannon Diversity indices and species richness. Bacterial OTUs were rarefied to 80,000 OTUs prior to estimating Shannon Diversity indices. Observed OTUs (at the 97% sequence cutoff) counts represented estimated bacterial richness values while observed genus counts represented nematode richness. To examine beta diversity, we visualized the community responses using principal coordinates analysis (PCoA) of bacterial and nematode community composition based on the Bray-Curtis dissimilarity. We used a permutational multivariate analysis of variance (perMANOVA) to examine among-treatment differences in bacterial and nematode communities. We performed perMANOVA using the *adonis* and *envfit* functions in the vegan package in R 2019 ([Dray et al., 2017](#); [Oksanen et al., 2007](#)). We examined correlations between the PCoA ordination of bacterial and nematode communities and soil variables. Finally, we used indicator species analysis to identify which bacterial OTUs and nematode taxa represented plant type. To identify specific community members indicative of each treatment, we performed a Dufrene-Legendre indicator species analysis using the

Table 1

Coarse and fine mean root biomass and C:N ratios (\pm standard error) of annual winter wheat and perennial Kernza across three N management regimes from the final year of the experiment (June 2013). Different lower-case letters denote statistical significance at $p < 0.05$.

Plant	Management	Coarse Roots		Fine Roots	
		Biomass (Mg ha ⁻¹)	C:N Ratio	Biomass (Mg ha ⁻¹)	C:N Ratio
Annual	High N	0.40 (0.13) ^c	21.6 (1.7) ^c	0.23 (0.13) ^c	23.5 (1.7) ^b
Annual	Low N	0.42 (0.12) ^c	21.0 (0.9) ^c	0.15 (0.03) ^c	22.1 (2.1) ^b
Annual	Organic	0.52 (0.12) ^c	25.3 (2.6) ^c	0.07 (0.01) ^c	23.4 (0.8) ^b
Perennial	High N	3.20 (0.32) ^a	35.7 (3.8) ^b	0.52 (0.11) ^a	21.4 (1.5) ^b
Perennial	Low N	1.97 (0.31) ^b	37.3 (3.6) ^{ab}	0.46 (0.05) ^b	25.1 (3.0) ^b
Perennial	Organic	1.31 (0.29) ^{bc}	54.1 (1.7) ^a	0.20 (0.05) ^c	33.5 (1.3) ^a
	Plant	80.7***	84.2***	11.3*	4.3
	Management	7.4**	11.2***	6.5*	6.8*
	Plant \times Management	9.5**	4.5*	1.0	6.4*

* Significance level: $P < 0.05$.

** Significance level: $P < 0.01$.

*** Significance level: $P < 0.001$.

indval function in the *labdsv* package in R (Roberts, 2016). For this analysis, we only included bacterial taxa with a relative abundance greater than 0.05 when summed across all plots.

3. Results

3.1. Root quantity and quality

Perennial Kernza had greater coarse and fine root biomass and greater coarse root C:N compared to annual wheat (Table 1). Kernza coarse and fine root biomass was 4.8 and 2.6 times greater than annual winter wheat when averaged across the three management systems. Kernza coarse root C:N was nearly twice that of annual wheat (42 vs. 23), when averaged across management. Nitrogen management also influenced perennial root biomass and C:N ratios but had no effect on annual wheat (Table 1). The High N treatment in Kernza had greater root biomass and lower C:N in both coarse and fine fractions relative to the Organic treatment with Low N being intermediate (Table 1).

3.2. Bacterial and nematode community composition

Nitrogen management influenced bacterial communities in 2012 and 2013, while plant species only influenced community structure in the 4th year (2013) (Table 2). In 2011, bacterial community composition was similar between perennial Kernza and annual winter wheat, and no differences were evident amongst the three managements. In 2012, there were distinct bacterial communities among management types as evidenced by separation of samples along the PCoA axis 1, (Table 2; Fig. 1). Then, during 2013, management and plant type both explained variation in bacterial community composition, where PCoA axis 1 represented plant type and PCoA axis 2 represented management along the high to low input treatment type (Table 2; Fig. 1). In addition, variation in bacterial composition was highest in the perennial Kernza in the High N treatment (Fig. 1). Indicator species analysis identified 12 bacterial taxa (OTUs) that were representative of plant type (Table S1). The indicator bacterial taxa associated with annual wheat were more taxonomically diverse and included representatives from the phyla *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, and *Firmicutes*, while indicator taxa associated with Kernza included representatives of *Actinobacteria* (orders Frankiales and Micrococcales) and *Proteobacteria* (order Burkholderiales) (Table S1).

Nematode community structure was minimally influenced by plant species and N management until 2013, when both had a significant influence (Table 2). In 2013, both plant and management explained variation in nematode community composition (Fig. 2). The indicator

nematode taxa associated with annual wheat included *Aphelenchus*, *Paratylenchus*, and *Pratylenchus*, while nematode indicator taxa associated with Kernza included *Monhysteridae* and *Plecticus* (Table S2). *Dauerlarvae* was the only nematode indicator taxa associated with a type of N management and was associated with the Organic system (Table S2).

3.3. Bacterial and nematode diversity and richness

Plant type had no effect on bacterial diversity and richness in 2011 and 2012 (Table S3), but perennial Kernza was associated with higher bacterial diversity compared to the annual wheat in 2013 (Table 3). Bacterial richness and diversity were similar across management type over the course of three years (Table S3 and Table 3). There was no difference in nematode diversity between perennial Kernza and annual winter wheat in 2011 and 2012 (Table S3), but a significant plant effect, where perennial Kernza had greater diversity compared to annual wheat in the Low N and High N treatments (Table 3). Nematode diversity was similar across all three managements in 2011, 2012, and 2013 (Table S3 and Table 3). However, in 2012 and 2013, there were significant plant by management interactions as the Organic system had greater diversity compared to the Low N and High N treatments for annual wheat. Nematode species richness was similar across plant and management in 2011, but in 2012 Organic annual wheat had greater species richness than most treatments (Table S3). In 2013, perennial Kernza had greater species richness than did annual wheat (Table 3).

3.4. Nematode indices

The structure, channel, and basal nematode indices were significantly influenced by both plant and management (Table 4), while the Enrichment Index was only influenced by management in 2012 and 2013. The Enrichment Index (EI, higher values reflecting greater nutrient cycling) was similar between the two plants, except for 2013, within the Organic treatment where perennial Kernza had significantly greater values than annual wheat (Table 4). When averaged across year, EI values were substantially higher in the Organic treatment compared to the conventional systems for both plants. Perennial Kernza within the High N treatment had significantly greater Structure Index (SI, higher values reflecting greater trophic complexity) values compared to annual wheat in 2012 and 2013 and had greater SI values within the Low N treatment in 2013 (Table 4). The two plants had similar SI values within the Organic treatment across the three years. On average, SI values were highest in the Organic treatment compared to the two conventional treatments. Channel Index values (CI, higher values reflecting

Table 2
Permutational multivariate analysis of variance (perMANOVA) of bacterial and nematode communities.

Factor	Bacteria			Nematode		
	2011	2012	2013	2011	2012	2013
Block	0.040	0.033	0.057	0.099	0.086*	0.065*
Plant	0.041	0.058	0.081**	0.109	0.069	0.143***
Management	0.065	0.141*	0.127**	0.114	0.070	0.148*
Plant*Management	0.081	0.074	0.073	0.071	0.074	0.101

R² values represent the relative proportion of variation each factor contributes to the total variation in the dataset.

* Significance level: $P < 0.05$.

** Significance level: $P < 0.01$.

*** Significance level: $P < 0.001$.

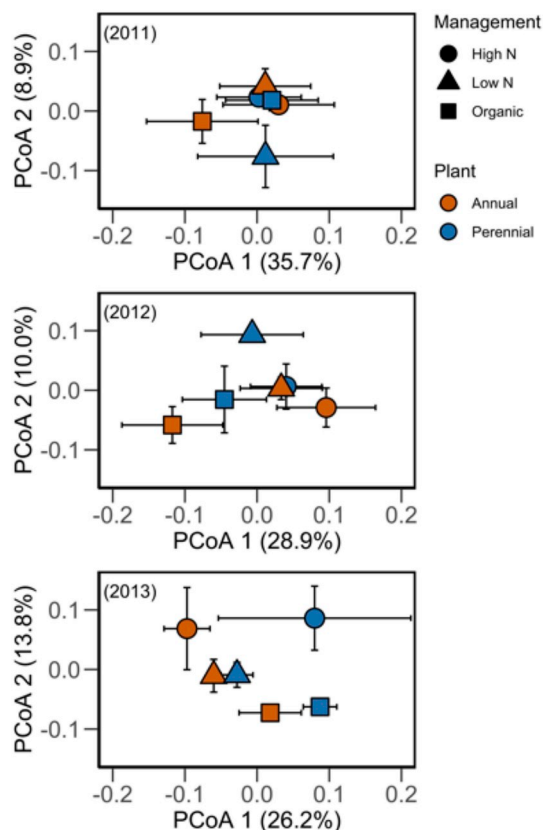


Fig. 1. Principal coordinate analysis (PCoA) ordinations for bacterial community composition in annual wheat and perennial Kernza across three N management systems (High N, Low N, and Organic) in 2011, 2012, and 2013. Error bars represent the standard error from the mean.

greater fungal decomposition) were significantly greater in the annual wheat system for both High N and Low N treatments in 2012 and 2013 (Table 4) compared to perennial Kernza. In contrast, CI values between perennial Kernza and annual wheat were similar in the Organic treatment across all three years (Table 4). Annual wheat Basal Index (BI, higher values reflecting greater food web disturbance) values were significantly greater than perennial Kernza BI values in 2012 and 2013 within the High N treatment and in 2013 for the Low N treatment (Table 4). Noteworthy differences were also visible across management. For instance, in 2013, the Organic annual wheat system had values that were substantially lower than that of the annual Low N and High N treatments (Table 4). The plant effect F statistic was larger than that of the management F statistic for all indices, except the enrichment index (data not shown).

3.5. Labile soil carbon

Labile soil C measurements responded differently to plant and management effects. Over the course of three years, POXC was greater in Kernza compared to annual wheat in the High and Low N treatments, but no different in the Organic treatment (Fig. 3). For instance, when averaged by year, perennial Kernza had 18% and 15% greater POXC compared to annual wheat for High N and Low N, respectively. In addition, there was no overall management effect on POXC, as values were statistically similar across the three treatments (Fig. 3). POXC values differed by year (Fig. 3) and generally increased over time in both plant systems. Mineralizable C was significantly influenced by management (Fig. 4) but not by plant or year (Fig. 4). Mineralizable C rates were greatest in the Organic treatment for both plants (Fig. 4).

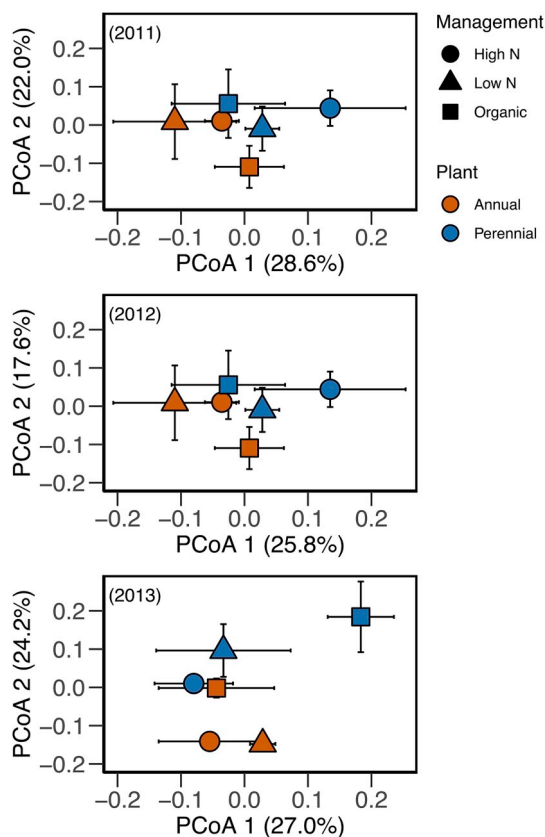


Fig. 2. Principal coordinate analysis (PCoA) ordinations for nematode community composition in annual wheat and perennial Kernza across three N management systems (High N, Low N, and Organic) in 2011, 2012, and 2013. Error bars represent the standard error from the mean.

4. Discussion

The objective of this study was to determine how perenniality and management impacted root quantity and quality, food web structure and function, and labile C pools. Perenniality influenced roots, whereby perennial Kernza had greater root biomass relative to annual wheat, while management only affected root growth in the fourth year when root biomass was greatest under the High N system. Nematode communities followed a similar trend, where perenniality explained changes in nematode structure and function early in the experiment, while N management only impacted nematode community composition in the final year. Although there were distinct bacterial communities amongst N management, it was not until 2013, that both management and perenniality significantly accounted for variation in bacterial composition. Finally, perenniality influenced POXC, while management

influenced mineralizable C. This study is one of the first that works to untangle the different impacts that perenniality and management have on root dynamics, food web structure, and labile soil C pools.

4.1. Perenniality and management effects on surface root quantity and quality

Four years post establishment, perennial Kernza had 4.8 times the amount of coarse root biomass and 2.6 times the amount of fine roots relative to annual wheat. These margins are similar to those found in years two and three of this experiment (Sprunger et al., 2018a) and are consistent with other annual and perennial plant comparisons (Dietzel et al., 2015; DuPont et al., 2014; Jarchow and Liebman, 2012). Most noteworthy, was the greater perennial Kernza coarse and fine root biomass under the high N treatment. Root biomass often increases with enhanced inorganic N due to greater nutrient uptake in nutrient rich environments (Ryser and Lambers, 1995; Heggenstaller et al., 2009).

While it has been well established that perenniality leads to increases in root quantity (Sprunger et al., 2018a; Dietzel et al., 2015; DuPont et al., 2014), much less is known regarding how different management practices effect root quality. In our study, both perenniality and N management influenced coarse root C:N, while fine root C:N was only driven by management. Perennial Kernza consistently had greater coarse root C:N ratios relative to annual wheat. This demonstrates that perennials have a greater capacity to store carbon in their roots relative to annual systems (Dietzel et al., 2015; Sprunger and Robertson, 2018). Most noteworthy, is the significant difference in perennial Kernza coarse root C:N across N managements. Perennial Kernza under organic management had significantly greater coarse and fine root C:N ratios compared to perennial Kernza under the high N treatment. This trend is consistent with previous studies, showing greater root C in organic relative to conventionally-managed systems (Chirinda et al., 2012). Greater C:N ratios are often associated with longer mean residence times and could have important implications for long-term soil C storage (Ryan et al., 2018; Jungers et al., 2017).

4.2. Bacterial and nematode communities respond differently to perenniality and N management

Our hypothesis stating that perenniality would be a larger driver of soil food webs compared to N management could only be partially supported because the bacterial and nematode responses were different and more nuanced. Bacterial and nematode communities responded differently to perenniality and N management. Perenniality significantly explained variation in nematode community structure after one year of establishment, while N management explained bacterial community composition more than perenniality. In this study, the nutrient limitation of these soils was a common stressor across the site. Therefore, N additions were a stronger environmental filter on bacterial communities than plant type. This response to nutrient enrichment is

Table 3

Mean diversity (Shannon Diversity Index) and species richness of bacterial (OTU counts) and nematode communities (genus counts) (± standard error) from annual winter wheat and perennial Kernza systems across three N management regimes in the final year of the experiment (June 2013). Different lower case letters denote statistical significance at p < 0.05.

Plant	Management	Bacteria		Nematode ^a	
		Diversity	Richness	Diversity	Richness
Annual	High N	7.28 (0.08) ^b	6884 (617)	1.70 (0.1) ^b	13.0 (1.7) ^b
Annual	Low N	7.35 (0.05) ^{ab}	7445 (332)	1.69 (0.07) ^b	14.8 (0.8) ^{ab}
Annual	Organic	7.38 (0.02) ^{ab}	7364 (349)	2.15 (0.05) ^a	15.3 (0.8) ^{ab}
Perennial	High N	7.32 (0.07) ^{ab}	6793 (256)	2.10 (0.1) ^a	17.5 (1.3) ^a
Perennial	Low N	7.41 (0.03) ^a	7413 (126)	2.01 (0.1) ^a	15.3 (0.5) ^{ab}
Perennial	Organic	7.43 (0.05) ^a	7358 (398)	2.00 (0.06) ^a	17.3 (1.3) ^a

^a Nematodes were assessed at the genus level.

Table 4

Nematode indices (\pm standard error) from annual wheat and perennial Kernza across three N managements over the course of three years. Different lower case letters denote statistical significance at $p < 0.05$.

	Structure Index		Channel Index		Basal Index		Enrichment Index	
	Annual	Perennial	Annual	Perennial	Annual	Perennial	Annual	Perennial
2011								
Organic	61.6 (2.5)	68.9 (7.8)	44.3 (25.1)	36.0 (21.5)	21.7 (2.7)	21.5 (7.2)	66.1 (5.6)	60.2 (13.5)
Low N	62.4 (11.1)	65.9 (9.6)	50.1 (25.4)	34.3 (9.6)	23.3 (5.6)	22.3 (5.5)	55.4 (14.0)	57.2 (5.7)
High N	62.2 (2.5)	48.1(14.8)	28.3 (8.2)	29.1 (6.3)	25.1 (3.1)	36.3 (11.0)	56.8 (0.7)	48.1 (7.7)
2012								
Organic	69.8 (5.1) ^{ab}	63.3 (5.1) ^{ab}	28.1 (9.1) ^b	48.4 (7.9) ^b	20.9 (3.9) ^{ab}	27.0 (9.5) ^{ab}	58.7 (7.9) ^a	46.2 (3.8) ^{ab}
Low N	66.5 (9.3) ^{ab}	72.0 (5.2) ^{ab}	77.7 (6.5) ^a	49.6(10.2) ^b	27.9 (7.4) ^{ab}	21.7 (3.2) ^{ab}	34.5(5.4) ^b	47.1 (2.7) ^{ab}
High N	55.9 (5.3) ^b	78.6 (15.3) ^a	64.0 (12.9) ^a	48.4 (7.9) ^b	32.8 (3.4) ^a	17.3 (4.1) ^b	40.9 (5.9) ^b	53.4 (6.5) ^{ab}
2013								
Organic	55.5 (5.1) ^a	60.3 (9.3) ^a	31.7 (4.6) ^b	18.7 (7.0) ^b	27.2 (1.3) ^b	19.4 (4.4) ^b	57.1 (2.6) ^{ab}	72.2 (4.6) ^a
Low N	31.1 (5.4) ^b	55.8 (10.0) ^a	74.3 (6.1) ^a	38.5 (7.4) ^b	46.6 (3.6) ^a	30.9 (6.2) ^b	39.4 (2.7) ^c	46.2 (5.8) ^c
High N	21.4 (5.4) ^b	51.5 (2.3) ^a	77.1 (13.3) ^a	32.7 (6.4) ^b	53.3 (3.4) ^a	33.5 (3.3) ^b	37.6 (2.1) ^c	47.7 (6.2) ^{bc}

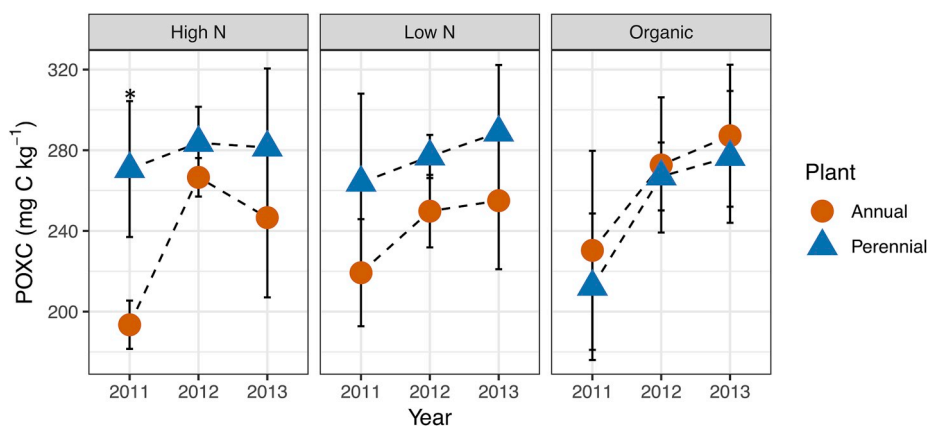


Fig. 3. Permanganate oxidizable carbon (POXC) for annual wheat (circles) and perennial Kernza (triangle) across three N management systems (High N, Low N, and Organic) from 2011, 2012, and 2013. Error bars represent the standard error from the mean and asterisks denote statistical significance at $p < 0.05$.

commonly observed in natural environments that are subject to atmospheric deposition of nutrients (Ramirez et al., 2012; Leff et al., 2015; Wang et al., 2018). Furthermore, treatment differences were only detected in 2012 and 2013 when perennial Kernza was in its third and fourth year. The more established perennials offer more substantial C resources which is also a strong determinant of changes to bacterial communities. This highlights that perennials often need to be fully established (i.e., > 3 years) before changes in microbial community

structure are detectable (Hargreaves and Hofmockel, 2014; Checinska Sielaff et al., 2018). The SI index was substantially greater under perennial Kernza relative to annual wheat. Furthermore, predatory and omnivorous nematodes in this guild are typically intolerant to stress and more abundant in systems with greater resource availability (Ferris et al., 2001; Margenot and Hodson, 2016). Thus, characteristics like those found in perennials, including greater root biomass and no-till likely determine in part these greater SI values.

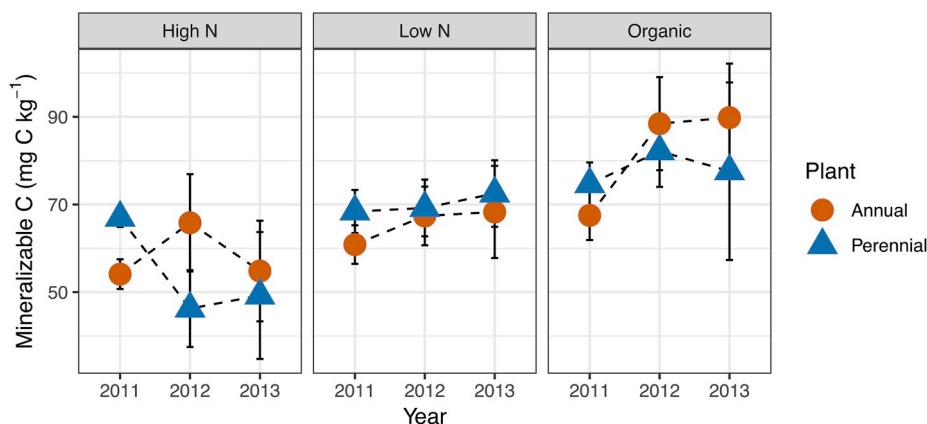


Fig. 4. Mineralizable carbon for annual wheat (circles) and perennial Kernza (triangle) across three N management systems (High N, Low N, and Organic) from 2011, 2012, and 2013. Error bars represent standard errors from the mean.

In contrast, a basal food web system is one that has experienced stress and disturbance, including limited resources and adverse environmental conditions (Ferris et al., 2001). The annual wheat system is considered a more stressful environment, given increased disturbance due to tillage and reduced root inputs. It is likely that the surviving organisms in this guild have adapted to a stressed environment. Thus, it is not surprising that BI values were significantly greater within the annual wheat system compared to perennial Kernza. Furthermore, plant parasitic nematodes (*Paratylenchus* and *Pratylenchus*) were found to be key indicator taxa of the annual system which can be emblematic of a disturbed system (Table S2). Others have reported similar patterns, whereby native perennial grasslands have substantially lower basal indices relative to annual cropland (Culman et al., 2010; DuPont et al., 2010). Reduced C inputs and exposure to fallow periods could also explain the greater channel index (CI) values within the annual wheat (Culman et al., 2010).

Soil nematode indices can also be used to measure rapid responses to nutrient and resource additions. The EI values reflect how quickly bacteria respond to organic inputs and has been shown to be a good indicator of soil productivity and nutrient cycling. In the present study, perennality had no impact on EI values, which suggests that annual wheat and perennial Kernza have similar rates of nutrient cycling (Culman et al., 2010). However, two of the perennial indicator taxa were bacterial feeders (*Plectus* and *Monyhsteridae*), which could be an indication of strong nutrient cycling in the perennial Kernza system (Table S2). Also noteworthy is that out of all the indices, EI was the only index to respond to changes in N management. In the third and fourth year, EI values were higher within the organic system compared to the conventional systems in both cropping systems. In line with this finding, is our PCoA analysis on bacterial communities in 2012 and 2013, where bacteria are distinctly grouped by management. This suggests that in this study, bacteria seem to respond more quickly to changes in N management since perennality did not strongly explain variation in bacterial composition. The observed changes due to management demonstrate that bacteria are responding to the additional organic C inputs from the poultry manure and roots.

4.3. Perennality and N management have altering effects on soil carbon pools

Labile C pools responded differently to perennality and N management, which has important implications for short and long-term C cycling. Perennial Kernza had significantly greater POXC values relative to annual wheat within the conventionally managed systems. In contrast, N management was a larger driver of mineralizable C rates. Organic N respired more CO₂ than conventionally managed systems, especially in 2012 and 2013, which is consistent with studies that demonstrate suppression of mineralizable C rates with inorganic N fertilization (Jia et al., 2010; Grandy et al., 2013; Ward et al., 2017). Though POXC and mineralizable C reflect the labile C pool, recent work has demonstrated that these measures are functionally different (Culman et al., 2012; Hurisso et al., 2016). For instance, POXC reflects a more processed pool of C that is highly correlated with soil organic C and soil C stabilization (Hurisso et al., 2016). In contrast, mineralizable C reflects mineralization processes that are highly sensitive to N management (Culman et al., 2013b; Hurisso et al., 2016).

Individual soil C pools are dynamic in nature and thus respond differently to various agricultural management practices (Cates and Ruark, 2017; Hurisso et al., 2016). For instance, conservation management practices such as perennial ley system (alfalfa, red clover, timothy, orchardgrass and alsike clover) with minimal tillage influences POXC more so than mineralizable C (Hurisso et al., 2016). In contrast, Hurisso et al. (2016) found that manure additions had a greater influence on mineralizable C. Our findings corroborate this trend, whereby perennality (no-till by nature) had a greater influence on POXC, while the addition of poultry manure substantially influenced mineralizable

C. This consistent trend highlights the beneficial effects of deep root systems and reduced disturbance for accumulating soil C in more protected or stabilized pools of C. In contrast, manure additions tended to have a greater influence on short-term C cycling and nutrient availability.

4.4. Linking root dynamics to microbial communities and soil C cycling

Plant roots have been shown to stimulate microbial activity and decomposition processes, however, the interactive effects between plant roots, soil biota, and soil C remain understudied, especially across different cropping systems receiving different sources and rates of N. In our study, we found that despite substantially larger root systems under perennial Kernza early on in the study (Sprunger et al., 2018a), notable changes in soil biota only occurred in the last two years. Nematode communities appear to be grouped more by plant species, with the exception of the Organic annual system, which appeared to be more closely associated with the perennial systems compared to the conventional (Low N and High N) annual systems. Bacteria appeared to be explained by both management and perennality but this trend was only evident in the final year. The lag in microbial response to perennality and management could be explained by the process of root production and decay, whereby decomposers are interacting with roots of varying quality, creating a more complex chemical environment, which has profound impacts on microbial composition through time (Wickings et al., 2012).

Soil and root properties further explain patterns in bacterial and nematode communities (Figs. S1 and S2). During the 2013 season, root quantity was significantly correlated with bacterial communities within the High N perennial system (Fig. S1), while coarse and fine root C:N (root quality) significantly correlated with nematode communities within the Organic perennial system (Fig. S2). Surface roots of Organic perennial Kernza consistently had greater C:N ratios compared to conventionally managed perennial Kernza and annual wheat. This indicates that nematode communities are strongly associated with root quality and possibly N limited resources (Song et al., 2016). Further, the Organic annuals and perennials grouped together in this study, which suggests that greater sources of plant-derived C drives nematode community structure and function as demonstrated in previous studies (Bonkowski et al., 2009; Pollierer et al., 2007). In contrast, bacterial communities responded more rapidly to N management (2012, 2013), compared to perennality (2013). This observation could be due to bacteria response to soil nutrient pools (Ferris et al., 2001). Nutrient pools in our study clearly changed with management, as mineralizable C increased under organic poultry additions relative to the conventional systems. That root biomass, mineralizable C, and POXC were all significantly correlated with bacterial communities, provides evidence that labile C pools are important controls on bacterial communities.

5. Conclusions

This study examined the effect that perennality and N management have on soil food web dynamics and belowground cycling. Overall, perennial Kernza supported more highly structured food webs compared to annual wheat, while also appearing to store greater amounts of C in roots and stabilized C pools. A noteworthy finding from this study appears to be that perennality and management influence labile C pools initially and after perennial Kernza matures, food web structure and function begin to shift. This delayed response to perennality and management in microbial composition could be explained in large part to chemical complexities in the root system, which has important implications for short and long-term C cycling in perennial crops.

Data accessibility statement

All data analyzed in this study and code for sequence processing and

statistical analyses are publicly available at https://github.com/PeraltaLab/PWES_SoilBio. Raw sequence files are available on the NCBI SRA (BioProject PRJNA548308).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2019.107573>.

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