Long-term impacts of high-input annual cropping and unfertilized perennial grass production on soil properties and belowground food webs in Kansas, USA

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ARTICLE INFO

Article history:
Received 10 May 2009
Received in revised form 2 November 2009
Accepted 5 November 2009
Available online xxx

Keywords:
Soil food webs
Perennial grasslands
Tallgrass prairie
Nematodes
T-RFLP
nifH

ABSTRACT

Soil ecosystem properties and processes which simultaneously maintain native fertility and sustain plant yields are of principal interest in sustainable agriculture. Native prairies in Kansas are relevant in this context, as some have been annually harvested for hay for over 75 years with no fertilization or detectable decline in yield or soil fertility. In contrast, annual crop production has resulted in significant reductions in soil fertility and now requires intensive inputs to maintain yields. Soil food webs were compared between hayed native grasslands and adjacent annual croplands in order to determine the long-term effects of these two production systems on soil ecosystem properties. Soil chemical and physical properties, bacterial and nematode community structure and abundance were measured across five paired sites at six depth intervals to 1 m. Soil organic carbon, total nitrogen, and water stable aggregates were all significantly greater in perennial grasslands than in annual croplands to a depth of 60 cm. Microbial biomass carbon was also greater in grasslands than in croplands, and shifts in δ13C indicated greater input of new carbon at lower depths in grasslands relative to annual croplands. Bacterial and nitrogen fixing communities in croplands and grasslands were significantly different in the surface 40 cm and nematode community differences persisted through 1 m. Nematode community indices suggested enhanced fungal decomposition pathways, fewer plant-feeding nematodes, and greater food web complexity and stability in grassland soils than in annual cropland soils. These data indicate that perennial grasslands in Kansas, even when annually harvested for decades, support higher levels of soil fertility and structure and more complex biological communities than annual cropping systems.

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1. Introduction

Nutrient cycling in natural ecosystems is characterized by tightly regulated processes which minimize losses due to leaching, runoff, denitrification, volatilization, and erosion. Plant nutrient acquisition in natural systems depends on (i) net inputs from atmospheric deposition, biological nitrogen fixation and mineral weathering, (ii) internal cycling of nutrients from decomposition of litter and soil organic matter, and (iii) microbial transformations of carbon (C), nitrogen (N), and phosphorus (P) (Grierson and Adams, 1999). In contrast, annual-based agricultural systems typically have much greater nutrient losses – partly due to the removal of agricultural products from the landscape, but also due to inefficiencies in internal nutrient cycling and poor synchronization of nutrient availability with plant demand (Crews, 2005). For example, annual crops commonly take up less than 50% of the N applied as fertilizer (Cassman and Dobermann, 2002). These losses require that substantial amounts of nutrients are continually applied to sustain agricultural productivity and, in turn, have created or exacerbated a suite of global environmental problems associated with intensive annual agriculture (Tilman et al., 2002).

Soil microbial communities form the foundation of soil food webs, with nearly all biogeochemical transformations directly resulting from microbial activity. Nematodes are not only active players in these food webs, but their community structure can...
serve as an indicator to other soil biota and the soil food web as a whole (Ferris et al., 2001). Collectively, soil food webs exert proximate controls on nutrient mineralization, regulating resources available for plant uptake. However, soil food webs are reciprocally shaped by the aboveground plant community, as they are largely reliant on the timing, location, quantity, and quality of carbon deposition by plant roots (Wardle et al., 2004). As a result, there are often large differences in soil food webs between annual croplands and native plant communities. The perenniality of plants appears to be an important factor in food web structure and function, as soil food webs in managed perennial production systems generally resemble those found in native plant communities more so than those in annual crop fields (Freckman and Ettema, 1993; Neher and Campbell, 1994; Neher, 1999; Ferris et al., 2001).

Herbaceous perennial crops only occupy 6% of harvested cropland worldwide (Montfreda et al., 2008), but have potential in coming decades to provide a range of agricultural products currently produced by annual crops. The development of new herbaceous perennial grasses, legumes, and forbs, and new uses for them, would facilitate their expanded implementation in production systems (Cox et al., 2006; Ragauskas et al., 2006; Tilman et al., 2006; Glover et al., 2007; Jordan et al., 2007; Nash, 2008; Schmer et al., 2008). For example, high-diversity, low-input (HDLI) perennial grasslands provided more usable biofuel energy, greater C sequestration and caused less pollution than corn grain ethanol or soy-biodiesel (Tilman et al., 2006), and net energy yields reported for high-input, low-diversity perennial systems were even greater than energy yields from the HDLI grasslands (Schmer et al., 2008). While questions persist as to the long-term ability of perennial systems to support high yields and high soil quality (Russelle et al., 2007), more than fifty years of annual harvesting of unfertilized perennial grasslands has not reduced soil organic carbon (SOC) or total soil N levels in the upper 2 m of soil as compared to non-harvested grasslands in the Russian Chernozem (Mikhailova et al., 2000; Mikhailova and Post, 2006). Other studies report that unfertilized grasslands at the Rothamsted Research Park Grass experiment have been hayed twice-annually for 150 years with no declines in yield (Jenkinson et al., 1994; Silvertown et al., 1994) or in total soil N for the past 120 years (Jenkinson et al., 2004).

Glover et al. (this issue) reporting on other results of this and related studies, concluded that unfertilized perennial grasslands provide comparable levels of harvested N in biomass as modern yields of adjacent high-input wheat fields provide in harvested grain. Over the approximately 75-year management history of the two systems, roughly 26% more N ha$^{-1}$ has been harvested from the unfertilized perennial grasslands than from the region’s annual crop fields. Despite the large annual rates of N removal, the unfertilized perennial fields maintained greater levels of soil C, reduced leaching losses of N, and had substantially lower energy requirements.

Here we build on the work of Glover et al. (this issue) by comparing key soil biological, chemical, and physical properties between unfertilized perennial grasslands and adjacent high-input annual cropping systems in Kansas. We hypothesize that soil food webs have played a major role in maintaining native fertility in the hayed grasslands and have directly enabled the sustained export of nutrients from these fields. This study represents the first step in testing this hypothesis by examining the long-term effects of the two annually harvested production systems on soil food webs and soil ecosystem properties through 1-m depth. We anticipated that this comparison would allow us to identify key characteristics of soil ecosystems that are associated with sustained, long-term nutrient removal.

2. Materials and methods

2.1. Site descriptions and soil sampling

The five field sites in this study were located in five counties of North Central Kansas as described by Glover et al. (this issue). Specific field site names and respective locations were: Buckeye, Dickinson Co. N 39.2344, W 97.7798; 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; Five Creek, Clay Co. N 38.22.665, W 97.18.788. Soil series are as follows: Ottawa Co., Geary silt loam; Dickenson Co., Hobbs fine-silty mesic; Goessel Co., Goessel silty-clay, Saline Co., Detroit silty clay-loam; and Clay Co., Muir silt-loam.

Each field site consisted of a native prairie meadow (perennial grassland) and an adjacent annually cropped field sown primarily or exclusively in wheat (Triticum aestivum), located on similar landscape positions and soil types. Prairie sites have never been tilled or fertilized and have been annually harvested for hay production for more than 75 years. Adjacent annually cropped fields have been in production for comparable periods of time and have received fertilizer inputs for the past several decades. In recent years at some sites, farm managers have used short rotations of wheat, sorghum (Sorghum bicolor), and/or soybeans (Glycine max) and/or have used no-tillage practices for varying periods. Field management followed typical practices for the region (KSUAE, 1996, 1997).

Soils were sampled three times: (i) June 18–22, 2006, (ii) October 5–9, 2006, and (iii) June 17–20, 2007. Four centimeter diameter cores were taken to a depth of 1 m along a 25 m transect across wheat and grassland sites. Five cores were taken from each field and separated into sections by depth: 0–10 cm, 10–20 cm, 20–40 cm, 40–60 cm, 60–80 cm, 80–100 cm. The five samples from each depth were bulked and mixed until homogeneous. Sub-samples of soils were air-dried for soil properties, refrigerated at 4 °C for microbial biomass and nematode analyses, or stored at –20 °C for molecular analyses.

2.2. Soil chemical and physical properties

Soil properties were analyzed at the June 2007 sampling at The Land Institute (TLI) and at the Soil Testing Laboratory at Kansas State University (STL-KSU). Analyses at TLI included: pH (Robertson et al., 1999), bulk density by weighing soils of known volume after drying at 105 °C to constant weight (Jareell et al., 1999), soil texture by hydrometer (Elliott et al., 1999), water stable aggregates (WSA) by wet-sieving (Seybold and Herrick, 2001), and readily oxidizable carbon (ROC) (Weil et al., 2003). Analyses at STL-KSU included soil organic matter (OM) by the Walkley-Black procedure, soil organic carbon (SOC) and total N by dry combustion on a LECO CN 2000 combustion analyzer, total P by a modified Kjeldahl digestion after an ammonium acetate extraction, and total K by flame atomic absorption. Further details on analyses performed at STL-KSU can be found at (Missouri Agricultural Experiment Station, 1998).

2.3. Root biomass

In June 2007, five 12.5-cm diameter soil cores were collected from the Niles perennial grass and annual crop fields to a depth of 2 m and separated into 0.2 m sections. Roots were dried and weighed to determine root mass per area. Because soil cores were collected from only one site, no statistical analyses were performed on the data. Arithmetic means are reported.
2.4. Microbial biomass

Microbial biomass carbon (MBC) was determined from June 2007 samples using the simultaneous chloroform fumigation extraction (scFME) method (Fierer et al., 2003). Briefly, 10 g of soil from each sample were weighed into two, 70 ml glass vials. Forty ml of 0.05 M K2SO4 were added to both vials, and 0.5 ml of EtOH-free CH3Cl were added to one of the vials. Soil-free blanks were also prepared. Vials were sealed and shaken at 150 rpm for 4 h. Exacts were centrifuged for 15 min at 1500 rpm and the supernatant was vacuum filtered through 0.45 μm Whatman filter paper. Microbial biomass extracts were bubbled for 30 min with air to remove any residual CH3Cl and stored at −20°C until analysis. Dissolved organic carbon (DOC) and the natural abundance 13C values of DOC were determined using an OI Analytical Model 1010 TOC Analyzer (OI Analytical, College Station, TX) interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the University of California Davis Stable Isotope Facility.

MBC was calculated as the difference between chloroform and non-chloroform (control) samples divided by a KEC-factor of 0.35 (Sparling et al., 1990). Analyzed blank samples contained extremely small amounts of C, so no correction was made for the non-chloroform N fraction. The 13C isotope composition was expressed in parts per thousand (‰) relative to the International PeeDee Belemnite (PDB), where δ13C = (Rsample/Rstandard − 1) × 1000 and R is the molar ratio of 13C/12C. The δ13C (‰) of MBC was calculated as follows: δ13C_MBC = [(13Csample + 13C control) − (13Ccontrol)]/(12Ccontrol), where 12C and 13C_sample is MBC (DOC kg ha−1) extracted from the chloroform and non-chloroform samples, and 12C and 13C_control is the 13C natural abundance of the chloroform and non-chloroform extracts (‰), respectively (Ryan and Aravena, 1994).

2.5. T-RFLP analyses

Bacterial community composition was assessed by terminal restriction fragment length polymorphism (T-RFLP) (Liu et al., 1997) on soil samples collected June 2006, October 2006, and June 2007. At each sampling, DNA was extracted from 0.25 g soil with the MoBio PowerSoil™ DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA). Using previously described methods (Culman et al., 2006), 16S rRNA genes from bacteria were amplified by polymerase chain reaction (PCR) using 27F and 1492R primers. Three 50 μl reactions of each sample were pooled and amplified DNA was subject to separate restriction enzyme digestes with both Hhal and Sau96 I restriction enzymes. Digested DNA was purified and submitted for terminal fragment-size analysis to Cornell University’s Biotechnology Resource Center, Ithaca, NY. Multiple enzymes confirmed trends found in bacterial data and so only data generated from Hhal are reported.

T-RFLP analyses were also performed to characterize free-living diazotrophic populations in soils on June 2007 samples. Soil DNA extracts were amplified by PCR using the forward primer nifH-b1-112F (Burgmann et al., 2004) and the reverse primer CDHP Nif723R (Steward et al., 2003). These primers target nifH, the structural gene for nitrogenase reductase, in the extracted soil DNA and the amplification products in products of approximately 600 bp. Three 50 μl reactions of each sample were amplified as follows: initial denaturation at 95°C for 10 min; 35 cycles of denaturation at 95°C for 30s, annealing at 61°C for 30s, and extension at 72°C for 45s; and a final extension step at 72°C for 10 min. Reaction concentrations were: 0.05 U μl−1 AmpliFerg Gold™ DNA polymerase, 1× PCR buffer, and 2.5 mM MgCl2, 0.8 mM dNTPs, 0.5 μg μl−1 BSA, both primers at 0.25 μM, nuclelease-free water and 50 ng DNA template reaction−1. Amplified DNA was digested with the restriction enzymeMspI (New England Biolabs) in the following reaction concentrations: 5 U enzyme, 1× of supplied buffer, 0.1 μg μl−1 BSA, 450 ng DNA, and nuclease-free water.

2.6. Nematode analyses

Nematode communities were enumerated and identified in soil samples collected June 2006, October 2006, and June 2007. Nematodes were extracted from 200 to 300 g 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 beaters 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 1 week of extraction or fixed in 4% formalin until identification.

Nematodes were also extracted and identified from the duff layer—the surface detrital layer consisting of mostly dead plant litter. Duff was collected at the June 2007 sampling date by placing five 15 cm diameter rings in each plot and removing the residue and partially decomposed plant material within each ring. Subsamples were mixed and nematodes were extracted from 30 to 80 g of duff using the same methods as described above for soil. Duff layers were sampled because they provide the organic source for nutrient cycling at the soil surface.

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 3–5). Herbivores were divided into two groups based on functional traits: plant associates (PA), composed of the family Tylenchidae that purportedly graze on plant root hairs, algae and fungi (Bongers and Bongers, 1998), and plant feeders (PF) composed of ecto-and endoparasites. Standardized indices of food web structure and function based on characteristics of nematode assemblages yield insight into ecosystem processes, such as the effects of environmental stress, dominant decomposition channels, and levels of organic enrichment (Bongers, 1990; Lenz and Eisenbeis, 2000; Ferris et al., 2001; Ferris and Matute, 2003; Ferris and Bongers, 2006). Soil food web indices were calculated after Ferris et al. (2001).

N mineralization rates were estimated using the soil food web community model developed by Hunt et al. (1987) and adapted by De Ruiter et al. (1993). The model derives C and N mineralization rates from biomass measurements of individual genera by portioning consumption into the rate at which feces or prey remains are added to detritus, the rate at which elements are incorporated into their biomass, and the rate at which minerals are excreted as waste. Calculations for total N mineralized are from bacterivores, fungivores, omnivores and predators. Herbivores are not included in the model due to their possible negative impacts on plant productivity.

2.7. Statistical analyses

Analysis of Variance (ANOVA) was performed on soil chemical and physical data, MBC data, and nematode data using the PROC MIXED procedure in SAS v.9 (Cary, NC). Depth and management history were treated as fixed effects and site a random effect, with significant differences determined at α = 0.05. Nematode trophic group and index data were analyzed over all dates, with depths treated as repeated measures. All soil data were converted into mass per hectare, in order to account for differences in soil volume...
and bulk density. Least squared means are reported for all univariate data.

Bacterial and nematode communities were analyzed with nonmetric 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. NMS is amendable to many types of ecological data (McCune and Grace, 2002), including T-RFLP data (Culman et al., 2008). NMS analyses with the Bray-Curtis distance measure were performed on presence/absence data for 298 terminal restriction fragments for 16S rRNA genes, 124 fragments for nifH, and abundance data for 76 nematode taxa in R (2008) via the metaMDS function in the vegan package with default parameters except for the following: autoptransorm = false, 100 runs, pc = false. The respective final stress values were 18.0, 24.7, and 19.3 for 16S T-RFLP, nifH T-RFLP, and nematode taxa, respectively. Correlations between the NMS stress values were 18.0, 24.7, and 19.3 for 16S T-RFLP, nifH T-RFLP, and nematode taxa, respectively. Correlations between the NMS ordination of bacterial and nematode communities and soil variables were tested with 1000 permutations in the envfit function in the vegan package in R (2008).

Permutational multivariate analysis of variance (perMANOVA) was also employed to test significance of the experimental factors (depth, management, site) for bacterial and nematode community datasets and to assess the relative proportion of variation that each factor contributed (Anderson, 2001). PerMANOVA analyses were performed in R (2008) with the adonis function in the vegan package with the default parameters (Bray-Curtis distance measure, 100 permutations).

### 3. Results and discussion

#### 3.1. Soil chemical and physical properties

Significant degradation of soil chemical and physical properties occurred in annual crop fields, relative to the unfertilized and annually harvested perennial grasslands. Soils under annual crops contained significantly lower amounts of SOM, SOC, readily oxidizable carbon (ROC), and total soil N than the perennial grasslands at each depth to 60 cm of soil (Table 1). These results are consistent with other studies that show degradation of soil characteristics in cropland following conversion from perennial grassland (Lamb et al., 1985; Ajwa et al., 1998; Elliott et al., 1994; David et al., 2009). Despite the fact that annual crop fields have received approximately 70 kg N ha$^{-1}$ year$^{-1}$ for the past several decades, relative to harvested perennial fields, levels of SOC have been reduced by 28% and total N by 27% in the upper 60 cm of soil. Soil physical properties were also influenced by management history. Bulk density and water stable aggregates (WSA), important physical indicators of soil function and plant root activity (Elliott et al., 1999), were significantly reduced in annual croplands compared to perennial grasslands (Table 1).

In contrast, the annual haying of prairie sites did not result in the same rates of degradation. Because no control soil exists, it is unclear whether the decades of annual removal of harvested biomass has degraded soil conditions in the grasslands relative to their pre-harvest conditions. However, other studies of unfertilized, harvested perennial grasslands indicate long-term annual harvests may not result in measurable depletion of SOM, SOC or total P pools (Shortridge, 1973; Jenkinson et al., 1994, 2004; Silvertown et al., 1994; Mikhailova et al., 2000; Mikhailova and Post, 2006). If the annual harvesting of the grasslands resulted in significant degradation in soil fertility, yields would be expected to decline over time. The greater soil quality measured in the perennial fields, despite high levels of nutrient removal in the absence of fertilizer, suggests fundamental differences in plant community functioning, nutrient cycling and associated soil biology between these two systems.

#### 3.2. Root biomass

Average root biomass was 6.7 times greater in the perennial grassland than in the annual wheat field in the first meter of soil at the Niles site (19.26 Mg ha$^{-1}$ and 2.87 Mg ha$^{-1}$, respectively; Fig. 1). The top 0.4 m of soil contained 65.1% and 77.3% of the total root biomass in the first meter of soil for the grassland and wheat site, respectively. Although root biomass was only measured at one site, the findings are typical with other grassland and cropland

### Table 1

Soil properties of field sites in this study$^a$.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Management history</th>
<th>pH</th>
<th>Clay (%)</th>
<th>SOM (Mg ha$^{-1}$)</th>
<th>SOC (Mg ha$^{-1}$)</th>
<th>ROC (kg ha$^{-1}$)</th>
<th>Total N (Mg ha$^{-1}$)</th>
<th>Total P (kg ha$^{-1}$)</th>
<th>Total K (kg ha$^{-1}$)</th>
<th>WSA (Mg m$^{-3}$)</th>
<th>BD (kg m$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–10</td>
<td>Grassland</td>
<td>5.9</td>
<td>21</td>
<td>62.0</td>
<td>36.8</td>
<td>1040</td>
<td>3.1</td>
<td>427.6</td>
<td>431.4</td>
<td>0.93</td>
<td>1.22</td>
</tr>
<tr>
<td></td>
<td>Cropland</td>
<td>5.4</td>
<td>24</td>
<td>43.6</td>
<td>25.3</td>
<td>766</td>
<td>2.2</td>
<td>545.8</td>
<td>451.2</td>
<td>0.67</td>
<td>1.48</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>0.005</td>
<td>0.004</td>
<td>0.008</td>
<td>0.005</td>
<td>0.019</td>
<td>N.S.</td>
<td>&lt;0.001</td>
<td>0.026</td>
<td></td>
</tr>
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<td>10–20</td>
<td>Grassland</td>
<td>5.6</td>
<td>25</td>
<td>59.8</td>
<td>35.3</td>
<td>964</td>
<td>3.0</td>
<td>457.0</td>
<td>388.2</td>
<td>0.90</td>
<td>1.44</td>
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<td></td>
<td>Cropland</td>
<td>5.5</td>
<td>27</td>
<td>42.5</td>
<td>24.8</td>
<td>716</td>
<td>2.1</td>
<td>505.1</td>
<td>442.7</td>
<td>0.67</td>
<td>1.66</td>
</tr>
<tr>
<td></td>
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<td>0.002</td>
<td>0.006</td>
<td>0.003</td>
<td>N.S.</td>
<td>N.S.</td>
<td>&lt;0.001</td>
<td>0.030</td>
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<tr>
<td>20–40</td>
<td>Grassland</td>
<td>5.7</td>
<td>33</td>
<td>55.5</td>
<td>32.2</td>
<td>812</td>
<td>2.7</td>
<td>920.3</td>
<td>843.3</td>
<td>0.85</td>
<td>1.56</td>
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<td></td>
<td>Cropland</td>
<td>6.0</td>
<td>32</td>
<td>40.4</td>
<td>23.7</td>
<td>615</td>
<td>2.0</td>
<td>850.6</td>
<td>883.2</td>
<td>0.68</td>
<td>1.64</td>
</tr>
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<td>32</td>
<td>51.1</td>
<td>29.1</td>
<td>660</td>
<td>2.5</td>
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<td>867.3</td>
<td>0.80</td>
<td>1.64</td>
</tr>
<tr>
<td></td>
<td>Cropland</td>
<td>6.4</td>
<td>30</td>
<td>38.3</td>
<td>22.7</td>
<td>515</td>
<td>1.9</td>
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<td>830.6</td>
<td>0.70</td>
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<td>46.8</td>
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<td>508</td>
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<td>884.2</td>
<td>0.75</td>
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<td>6.6</td>
<td>32</td>
<td>36.1</td>
<td>21.7</td>
<td>414</td>
<td>1.8</td>
<td>827.2</td>
<td>911.0</td>
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<td>42.4</td>
<td>22.9</td>
<td>357</td>
<td>1.9</td>
<td>739.3</td>
<td>850.3</td>
<td>0.69</td>
<td>1.66</td>
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<tr>
<td></td>
<td>Cropland</td>
<td>6.8</td>
<td>33</td>
<td>34.0</td>
<td>20.6</td>
<td>314</td>
<td>1.7</td>
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<td>944.6</td>
<td>0.72</td>
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<td>N.S.</td>
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</tbody>
</table>

$^a$ The first two rows at each depth display the least squares means of the fives sites; the third row contains the p-value of those means. N.S. = not statistically significant (p ≥ 0.10); SOM = soil organic matter; SOC = soil organic carbon; ROC = readily oxidizable carbon; Total P = total phosphorus; Total K = total potassium; WSA = water stable aggregates; BD = bulk density.

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comparisons in the region. For example, grassland root biomass was 9.5 and 2.7 times greater than wheat root biomass in the surface 30 cm (Frank et al., 2006) and the surface 50 cm (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).

3.3. Microbial biomass

Perennial grasslands maintained significantly greater amounts of microbial biomass carbon (MBC) than annual croplands at all depths, except 80–100 cm (Fig. 2a). MBC in the surface soils of grasslands are typically at least twice as great as in the surface of cultivated fields (Jenkinson and Powlson, 1976; Lynch and Panting, 1980; Schimel et al., 1985; Acosta-Martinez et al., 2007), but few comparative studies have reported MBC through multiple depths (Allison et al., 2007a). Microbial biomass is a chief component of the active SOM pool (Smith and Paul, 1990) regulating nutrient and energy cycling, and ultimately plant and ecosystem productivity (Wardle, 1998). It has been used as a measure of belowground resource availability and an indirect measurement of belowground inputs from plants (Wardle, 1992; Paterson, 2003; Waldrop et al., 2006). The greater MBC in the grassland sites coincides with the greater root biomass at the Niles site (Fig. 1) and the findings of similar ecosystem comparisons (Buyanovsky et al., 1987; Jackson et al., 1996), suggesting greater availability of resources due to root activity (exudation and rhizodeposition) and increased diversity of plant substrate input (Wardle, 1992; Zak et al., 2003) in grassland soils. Microbial biomass plays an important role in N retention in tallgrass prairies (Blair et al., 1998), as this source has been shown to vary temporally, acting as both a source and a sink of N for plants in these systems (Garcia and Rice, 1994).

Isotope ratio mass spectrometry was used to determine $\delta^{13}C$ values of the microbial biomass. This approach takes advantage of the difference in photosynthetic pathways (and resulting $\delta^{13}C$ values) between C4-dominated prairies and C3 annual wheat (Ehleringer and Rundel, 1989). Since prairies have dominated this region for the last 5000 years or more (Axelrod, 1985), SOC should be primarily derived from C4 photosynthesis. However, since the conversion of prairies into agricultural fields, C3 annual wheat has historically been the primary crop grown in this region. Hence, new additions of C in annual fields should have C3 $\delta^{13}C$ signatures, relative to the background of C4 $\delta^{13}C$ values.

In the surface depths, clear differences in $\delta^{13}C$ values exist between grasslands and croplands (Fig. 2b), indicating the MBC was derived from the different photosynthetic pathways in these two systems. Soils from the C4-dominated grasslands yielded $\delta^{13}C$ values much higher than soils collected from the C3-dominated croplands. However, $\delta^{13}C$ values from deeper profiles in the annual crop fields increasingly become enriched in $\delta^{13}C$, indicating the MBC at these depths was derived increasingly more from C4 photosynthesis. This trend suggests two phenomena. First, very little C3-derived C from annual croplands is reaching these lower soil profiles. Wheat grown in this region has been shown to lack rooting activity at these lower depths (Fig. 1; Buyanovsky et al., 1987). Since isotopic signatures of microbial biomass have been shown to shift within weeks after the incorporation of litter with a different signature (Gregorich et al., 2000; Potthoff et al., 2003; John et al., 2004), $\delta^{13}C$ of microbial biomass is generally viewed to reflect the signature of the most recent input (Dijkstra et al., 2006).

Second, the more enriched $\delta^{13}C$ values of microbial biomass in annual croplands at these lower depths suggests this C source was derived from SOC sequestered by C4 prairie plants prior to agricultural conversion. This phenomenon would help explain the lower SOC values measured in cropland relative to grassland sites—after 75 years, our data suggest that heterotrophic bacteria are mineralizing remnant SOC that is not being replaced through plant deposition, resulting in a net SOC loss in the annual crop fields.

3.4. T-RFLP analyses

Analyses of bacterial communities via perMANOVA demonstrated all experimental factors (sampling time, depth, manage-
ment history and site) to be significant drivers of community structure (all $p < 0.01$, Table 2). Despite the large amount of variation and heterogeneity observed in these communities, NMS analyses reveal management history as a major driver of structure in the surface depths (0–40 cm). Ordination along the first and second axes (Fig. 3a) separated samples by depth and management history at the June 2007 sampling. These same patterns were observed in both June 2006 and October 2007 samplings (data not shown). Depth, management history and site accounted for 30%, 13%, and 16% of the total variation in the surface depths of the June 2007 bacterial dataset, respectively (Table 2). There were no differences between management history in the lower three depths (40–100 cm), as structure was primarily driven by site and depth (data not shown).

Nitrogen fixing bacterial communities were also significantly affected by depth, management history and site (all $p < 0.01$, Table 2). Similar to bacterial communities, N fixers were influenced significantly by management history in the soil surface (Fig. 3b; Table 2). However, depth had less influence on the community structure of N-fixing bacteria (Fig. 3b; 10% of the total variation) than on general bacterial communities. Differences in management history and depth were not observed below 40 cm depth in these N-fixing communities (data not shown).

Links between microbial communities, nutrient cycling, plant communities and ecosystem processes are never unidirectional and are rarely explained with just a few factors (Hooper et al., 2000; Naeem et al., 2000; Carney and Matson, 2005). However, studies have shown that shifts in microbial communities are often related to changes in ecosystem processes such as decomposition, nitrification, denitrification, nitrogen fixation, and specific enzyme activity (Cavigelli and Robertson, 2000; Waldrop et al., 2000; Carney et al., 2004; Carney and Matson, 2005; Kramer et al., 2006; Hsu and Buckley, 2009). For example, Patra et al. (2005) showed that community composition changes in free-living N fixers, nitrifiers, and denitrifiers corresponded to changes in the activities of these communities in unfertilized grasslands. Although we did not directly measure nitrogen fixation here, future work is needed to determine the effects of these altered communities on rates of N fixation and N cycling.

3.5. Nematode analyses

There were 76 nematode genera identified in this study (Appendix 1). As with bacterial communities, all experimental factors were significant drivers of nematode community structure (all, $p < 0.01$, Table 2). Despite the large amount of variance, there were 76 nematode genera identified in this study (Appendix 1). As with bacterial communities, all experimental factors were significant drivers of nematode community structure (all, $p < 0.01$, Table 2). Despite the large amount of variance, all factors measured were significant at $\alpha = 0.01$.

![Fig. 3. Nonmetric multidimensional scaling ordinations of June 2007 bacterial community structure (a) and nitrogen fixing community structure (b) in the surface 40 cm of soil. Closed symbols represent grassland sites and open symbols represent cropland sites; circles = 0–10 cm, squares = 10–20 cm, triangles = 20–40 cm.](image)

![Fig. 4. Nonmetric multidimensional scaling ordinations of the nematode communities at all depths averaged over all sites and sampling times. Closed circles represent grassland sites; open circles represent cropland sites; 10 cm = 0–10 cm, 20 cm = 10–20 cm, 40 cm = 20–40 cm, 60 cm = 40–60 cm, 80 cm = 60–80 cm, 100 cm = 80–100 cm. Error bars represent standard errors of the means.](image)

| Table 2 | R² values from perMANOVA of bacterial, nitrogen fixing and nematode communities.
<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td>All dates, 0–1 m</td>
<td>June 2007, 0–40 cm</td>
<td></td>
<td></td>
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<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Bacteria</td>
<td>0.15</td>
<td>0.05</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
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<td>0.12</td>
<td>0.30</td>
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<tr>
<td>Management</td>
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<td>0.13</td>
<td>0.13</td>
<td>0.11</td>
</tr>
<tr>
<td>Site</td>
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<td>0.12</td>
<td>0.16</td>
<td>0.25</td>
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<tr>
<td>Depth x management</td>
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<td>0.06</td>
<td>0.06</td>
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<tr>
<td>Depth x site</td>
<td>0.16</td>
<td>0.11</td>
<td>0.08</td>
<td>0.22</td>
</tr>
</tbody>
</table>

a $R^2$ values represent the relative proportion of variation each factor contributes to the total variation in the dataset. All factors measured were significant at $\alpha = 0.01$. Please cite this article in press as: Culman, S.W., et al., Long-term impacts of high-input annual cropping and unfertilized perennial grass production on soil properties and belowground food webs in Kansas, USA. Agric. Ecosyst. Environ. (2009), doi:10.1016/j.agee.2009.11.008
management history was the largest driver of nematode communities, accounting for 13% of the total variation (Table 2; Fig. 4). When nematode genera were classified into trophic groups, a number of differences between management histories were observed that persisted through depth (Fig. 5). The relative abundance of bacterial-feeding nematodes and the ratios of bacterial to fungal-feeding nematodes were larger in cropland than in grassland sites \((p = 0.002, 0.009, \text{respectively, over all depths; Fig. 5})\). These findings are consistent with a number of studies (Hendrix et al., 1986; Lenz and Eisenbeis, 2000; Yeates, 2003) and reflect lower bacteria: fungi ratios typically found in the low-input grassland sites (Yeates et al., 1997; Allison et al., 2007b). Increased fungal biomass in grasslands is likely a result of higher C:N ratios of plant inputs (e.g., rhizodeposition, root turnover) in grasslands which favor fungal populations, having lower N requirements than bacteria (Griffiths, 1994; Paterson, 2003). Nematode community structure and abundance in the duff layers were markedly different than those in soil, with duff layers containing up to 2.4 million nematodes m\(^{-2}\). In general, duff layers in the grasslands were dominated by fungal-feeding nematodes, while duff layers in the croplands were dominated by bacterial-feeding nematodes (data not shown).

The relative abundance of nematode plant associates (non-parasitic root hair, algal and fungal feeders) was significantly higher in perennial grasslands than in croplands \((p < 0.001\) over all depths; Fig. 5), with significant differences detected at each depth \((p < 0.05)\). Commonly dominant in grassland systems (Todd, 1996; Todd et al., 1999), the PA family Tylenchidae averaged 35% of total abundance in this study. Plant associate nematodes, such as the Tylenchidae which do not cause measurable plant damage, have been reported to increase C and N mineralization, microbial activity and plant growth (Bardgett et al., 1999). Omnivorous and predatory nematodes were also greater in the grassland sites than in croplands over all depths examined \((p = 0.075; \text{Fig. 5})\). In contrast, relatively more plant-feeding nematodes were found in croplands than grasslands over all depths \((p < 0.05; \text{Fig. 5})\). Since larger numbers of plant feeders have been shown to reduce plant primary productivity (Stanton et al., 1981; Ingham and Detling, 1986, 1990) regulation of these nematodes is an important agronomic concern. The lower numbers of plant feeders in grasslands may result from the regulation and predation of nematode pests by omnivorous and predatory nematodes (Khan and Kim, 2005; Sánchez-Moreno and Ferris, 2007).

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Nematode indices are standardized ratings based on groupings of nematode taxa according to our best understanding of their function and trophic group; they provide insight into soil food web structure and ecosystem services (Ferris et al., 2001). Predatory and omnivorous nematodes, with long lifecycles, large body size, and low fecundity, are intolerant of stress and disturbance. The Structure Index (SI) quantifies proportional abundance of these functional guilds. Nematode communities in grasslands had significantly greater SI values for all depths, indicating that perennial grasslands supported more highly structured soil food webs than those supported in croplands \((p < 0.001; \text{Fig. 6a})\). The Basal Index (BI) indicates the predominance of nematode groups that are tolerant to disturbance. Nematode communities in annual croplands had higher BI values over all depths \((p < 0.001; \text{Fig. 6b})\), reflecting the greater disturbance in the annually cropped fields. The average differences in the SI and BI between grassland and cropland sites were smallest in the two surface depths \((0–20 \text{ cm})\) and increased in the lower depths.

The Channel Index (CI) measures the weighted abundance of fungal-feeding to bacterial-feeding nematodes. CI values in the duff layer and at 10–20 cm depth were higher in grasslands than in crop lands \((p = 0.004 \text{ and } p < 0.001, \text{respectively; Fig. 6c})\). These differences complement the trophic group data (Fig. 4), indicating...
that fungi play a greater role in mediating decomposition in the perennial grasslands than they do in croplands.

Bacteria respond quickly to organic inputs and bacterial-feeding nematodes can rapidly increase in response (Ferris et al., 2001). The Enrichment Index (EI) quantifies this increase and serves as an indicator of soil productivity. In general agricultural soil food webs are characterized by higher EI values relative to unmanaged natural areas (Ferris et al., 2001; Ferris and Bongers, 2006; Leroy et al., 2009). However, we measured no differences in the EI values between grassland and cropland soils (Fig. 6d), suggesting that the two systems support comparable nutrient cycling rates.

![Fig. 6. Nematode indices through depth: (a) Structure Index, (b) Basal Index, (c) Channel Index, and (d) Enrichment Index. Closed circles represent grassland sites; open circles represent cropland sites. Error bars represent standard errors of the means. * Indicates differences between management histories for each depth.](image)

![Fig. 7. Nonmetric multidimensional scaling analysis of bacterial community structure (a) and nematode community structure (b) in grassland soils (closed circles) and cropland soils (open circles). Bacterial community data points represent the average of five samples from June 2007; nematode community data points represent the average of 15 samples taken from June 2006, Oct 2006 and June 2007. Error bars represent standard errors of the means. 10 cm = 0–10 cm, 20 cm = 10–20 cm, 40 cm = 20–40 cm. Correlations of key soil properties with bacterial and nematode community structure are depicted by the vectors. The length and angle of the vector represent the strength and direction of the relationship to the bacterial or nematode community. All vectors depict statistically significant correlations (p < 0.001). SOC = soil organic carbon; ROC = readily oxidizable carbon; N = total soil nitrogen; WSA = water stable aggregates.](image)

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The relationship between EI and SI can serve as a conceptual tool to characterize the functioning of agronomic soil food webs (Ferris et al., 2001). A model agricultural system would simultaneously exhibit high EI and SI values, coupling high nutrient cycling and ecosystem productivity with highly structured food webs which regulate opportunistic species. We initially hypothesized that annually harvested perennial grasslands would have soil food webs that exhibited high SI values, but low EI values typical of natural grasslands (Ferris et al., 2001), while annually cropped sites would have relatively lower SI and higher EI values. Food web SI values in grassland sites were in fact, high at all depths, however, EI values were not significantly different between the two systems. SI values in annually cropped sites were high in the top 20 cm, but sharply declined at lower depths (Fig. 6a). This reduction in SI values in the annual cropland sites coincides with reductions in root biomass at the Niles site (Fig. 1) and those reported elsewhere (Buyanovsky et al., 1987; Jackson et al., 1996), reflecting the potential role that plant roots may play in maintaining structured soil food webs.

3.6. Linking bacterial and nematode communities with soil properties

Relationships between soil nutrient pools, bacterial communities and nematode communities are intricately linked through complex energy flows and trophic interactions. Correlations between the NMS ordinations of bacterial and nematode communities and soil properties were tested in order to examine patterns between these pivotal components of the soil ecosystem (Fig. 7). The length and angle of each vector represents the strength and direction of the relationship of soil properties to the bacterial and nematode communities. All vectors depict statistically significant correlations ($p < 0.001$). Differences in bacterial and nematode community structure due to depth and management history are readily apparent in the NMS ordinations along the first and second axes, respectively. Vectors of total N, SOC, ROC indicate that these soil properties are correlated both with surface depths and grassland sites. WSA appear to be strongly correlated with the measured communities of grassland sites, but not with depth. Collectively, these data illustrate the relationships between and the differences in food web structure and soil properties between the cropland and grassland sites.

Our data suggest that while the response of bacterial and nematode communities in the sites we have examined are related, some distinct differences occur between these communities, especially with respect to depth. For example, MBC (Fig. 2) and bacterial community structure (Fig. 3) closely track the soil chemical and physical properties described in Table 1. Differences in most of these key properties (e.g., SOC, ROC, total N, WSA) between grassland and cropland sites were found in the upper 60 cm of soil. Likewise, differences in microbial biomass and bacterial community structure between these systems generally diminished with soil depth. In contrast, differences in nematode community structure did not diminish with depth (Figs. 4–6), indicating they are perhaps more strongly affected by the rooting structure of the plant communities than by chemical and physical soil properties.

3.7. Putative evidence for increased synchrony of soil nutrients and plant demand in grasslands?

Our data point toward several potential mechanisms that could increase the synchrony of plant nutrient demand and soil nutrient release, possibly contributing to the sustained export of nutrients in the harvested grasslands. Although further work will be required to confirm and elucidate these mechanisms, we present a number of possibilities.

The greater SI values in grassland sites demonstrate soil food webs which are more complex and contain more top predator and omnivore groups than those found in cropland sites. These apex nematode groups are capable of mineralizing substantial amounts of N, accounting for as much as 8.5% of total N mineralized (Sbrot et al., 2003). From our data, the calculated N mineralization rate due to nematode communities was estimated at 28 kg N ha year$^{-1}$ in soils of grasslands, a significantly greater amount ($p = 0.007$) than the estimated 16.6 kg N ha year$^{-1}$ mineralized in soils of the annual croplands (Appendix 2). As nematodes typically mineralize only a small fraction of the total N mineralized by the entire soil food web, soil biota may play a pivotal role in synchronizing rates of N availability to grassland primary producers.

The higher CI values and proportions of fungal-feeding nematodes in grasslands relative to croplands suggest a greater contribution of fungal decomposition pathways. Buyanovsky et al. (1987) noted lower decomposition potential in tallgrass prairie sites, reporting CO$_2$ losses from litter decay in prairie sites to be half those found in wheat fields. A decreased rate of decomposition would suggest slower release of nutrients and energy which might be more effectively immobilized and retained in the prairie soil ecosystem, leading to enhanced potential for synchrony and coupling between plant and microbial activity in the field. Buyanovsky et al. (1987) suggested this synchrony resulted from increased competition for resources in the summer, particularly for N and water. Seasonal water use data measured from our study sites confirms greater water use in the 40–80 cm depths in grassland sites (see Fig. 6 in Glover et al. (this issue)). Lower soil water contents likely decreases decomposition rates at these depths in the perennial grasslands.

Other possible mechanisms of nutrient conservation include relatively lower N losses through leaching and denitrification. For example, Glover et al. (this issue) reported 24% less NO$_3$-N leached below rooting depths in perennial grass fields relative to losses below annual crop fields. These potential mechanisms of conservation have large scale implications (e.g., lower greenhouse gas emissions and conservation of groundwater quality) beyond field-level soil health.

4. Conclusions

This study compared belowground soil food webs in order to assess the long-term effects of annual cropping on landscapes once dominated by perennial grasslands. Despite receiving no fertilization, these grasslands have remained remarkably productive, sustaining annual harvests of biomass for nearly a century or more. Perennial grasslands supported significantly greater levels of key soil properties (SOC, total N, ROC, and WSA), greater levels of microbial biomass, more highly structured soil food webs and significantly different communities of soil bacteria and nematodes than annual croplains. Collectively, these findings suggest fundamental differences in belowground processes between these two systems.

Appendix 1

See Table A1.
Table A1
Mean nematode counts per m² soil for croplands and grasslands at each depth averaged across all dates.

<table>
<thead>
<tr>
<th>Nematode genus</th>
<th>CP group</th>
<th>0–10 cm</th>
<th>10–20 cm</th>
<th>20–40 cm</th>
<th>40–60 cm</th>
<th>60–80 cm</th>
<th>80–100 cm</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Cropland</td>
<td>Grassland</td>
<td>Cropland</td>
<td>Grassland</td>
<td>Cropland</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bunonema</td>
<td>b1</td>
<td>0.13</td>
<td>0.00</td>
<td>0.00</td>
<td>0.06</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Dauerlavae</td>
<td>b1</td>
<td>15.57</td>
<td>0.16</td>
<td>3.41</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Diplagasteridae</td>
<td>b1</td>
<td>0.13</td>
<td>0.07</td>
<td>0.00</td>
<td>0.07</td>
<td>0.13</td>
<td>0.10</td>
</tr>
<tr>
<td>Diplagasteriana</td>
<td>b1</td>
<td>0.00</td>
<td>0.14</td>
<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
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<td>b1</td>
<td>0.27</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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<td>0.00</td>
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<tr>
<td>Diplocladius</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
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<td>39.17</td>
<td>16.75</td>
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<td>b2</td>
<td>72.33</td>
<td>22.95</td>
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<td>b2</td>
<td>72.33</td>
<td>22.95</td>
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<td>14.92</td>
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