Coupled biochar amendment and limited irrigation strategies do not affect a degraded soil food web in a maize agroecosystem, compared to the native grassland

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Abstract

Climate change is predicted to increase climate variability and frequency of extreme events such as drought, straining water resources in agricultural systems. Thus, limited irrigation strategies and soil amendments are being explored to conserve water in crop production. Biochar is the recalcitrant, carbon-based coproduct of biomass pyrolysis during bioenergy production. When used as a soil amendment, biochar can increase soil water retention while enhancing soil properties and stimulating food webs. We investigated the effects of coupled biochar amendment and limited irrigation on belowground food web structure and function in an irrigated maize agroecosystem. We hypothesized that soil biota biomass and activity would decrease with limited irrigation and increase with biochar amendment and that biochar amendment would mitigate the impact of limited irrigation on the soil food web. One year after biochar addition, we extracted, identified, and estimated the biomass of taxonomic groups of soil biota (e.g., bacteria, fungi, protozoa, nematodes, and arthropods) from wood-derived biochar-amended (30 Mg ha⁻¹) and nonamended soils under maize with limited (two-thirds of full) and full irrigation. We modeled structural and functional properties of the soil food web. Neither biochar amendment nor limited irrigation had a significant effect on biomass of the soil biota groups. Modeled soil respiration and nitrogen mineralization fluxes were not different between treatments. A comparison of the structure and function of the agroecosystem soil food web and a nearby native grassland revealed that in this temperate system, the negative impact of long-term conventional agricultural management outweighed the impact of limited irrigation. One year of biochar amendment did not mitigate nor further contribute to the negative effects of historical agricultural management.

Keywords: biochar, corn, grassland, limited irrigation, maize, soil food web, water scarcity

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Introduction

Arid and semiarid regions are predicted to experience increased levels of drought (Seager et al., 2007) with increased temperatures and variability of rainfall associated with climate change (IPCC, 2014). Heightened drought will strain water resources in semiarid agricultural systems, where water availability is a major limiting factor for crop productivity. Given that the agricultural sector uses approximately 80% of consumptive water in the United States (NASS, 2014), pressure to reduce overall agricultural water use in response to water scarcity is likely to increase. As a result, there is a critical need for semiarid agriculture to find ways to manage water use in order to meet production demands for a growing population while simultaneously adapting to water scarcity.

Two such management strategies are amending soil with organic materials that increase the water-holding capacity of the soil and limiting irrigation inputs. When applied at strategic time points, limited irrigation reduces water use (Fereres & Auxiliadora Soriano, 2007; Dejonge et al., 2011) while still maintaining equivalent crop yields in some systems (Schneekloth et al., 2009). Research on limited irrigation has gained popularity in the face of climate variability and more frequent drought (Schneekloth et al., 2009), but the majority of these studies have focused on crop responses and often neglected the response of belowground communities which mediate nutrient availability for plants. The few studies that have investigated the impacts of limited
irrigation on soil biota are inconclusive but generally find a decrease in biomass and activity, although responses vary between different soil biota groups (Schnürer et al., 1986; Wang et al., 2008; Li et al., 2010).

Biochar, the recalcitrant product of pyrolysis of biomass under minimal oxygen conditions (Lehmann & Joseph, 2015), is of particular interest as a soil amendment because it is a coproduct of cellulosic bioenergy production that slowly degrades in soils (Lehmann et al., 2006). Biochar has the potential to mitigate C emissions from bioenergy production through long-term belowground C storage (Lehmann et al., 2006). Biochar has also been shown to have a positive effect on water storage and crop yields in agricultural systems, thereby mitigating water challenges in semiarid systems (Jeffery et al., 2011). Coupling biochar addition with limited irrigation strategies could therefore be a successful approach to reducing water consumption while sustaining crop productivity.

Previous research in temperate agricultural systems has focused on the effects of biochar on crop productivity (Jeffery et al., 2011; Crane-Droesch et al., 2013), while more recent investigations have highlighted the impacts of biochar amendment on soil biological communities (Liu et al., 2016), given their roles in regulating C and nutrient (e.g. nitrogen, phosphorous) cycling in soils (Paul, 2014). In general, agricultural conversion of native grasslands to cropland has shown detrimental impacts on belowground communities (Moore, 1994; Culman et al., 2010; DuPont et al., 2010). Such management-induced alterations to the structure of the soil food web can change the nature in which C and nutrients are processed in soils (Hendrix et al., 1986; Moore, 1994). For example, conventional agricultural management tends to support bacterially dominated soil food webs with increased C turnover, nutrient cycling rates, and losses, while less intensive practices create fungal dominated soil food webs with slower cycling rates resulting in greater C sequestration, nutrient use, and retention (Moore, 1994). Biochar addition may alter the soil environment through a number of different mechanisms that may favor fungal dominated soil food webs in agroecosystems: indirect effects on soil moisture and subsequent crop inputs (Atkinson et al., 2010; Spokas et al., 2012), changing bulk density and physical soil structure (Tryon, 1948; Atkinson et al., 2010; Laird et al., 2010; Abel et al., 2013), altering soil pH and nutrient dynamics (Cheng et al., 2008; Atkinson et al., 2010; Gaskin et al., 2010; Biederman & Harpole, 2013; Rogovska et al., 2014), and adding a recalcitrant C source that may or may not be utilized by the soil microbial community (Santos et al., 2012; Hammer et al., 2014; Jaafar et al., 2014; Gul et al., 2015). Soil biota are sensitive to physical and chemical changes to the soil environment such as soil structure (Young et al., 1998; Beylich et al., 2010), water dynamics (Schnürer et al., 1986; Williams, 2007; Wang et al., 2008), pH (Korthals et al., 1996; Pietri & Brookes, 2008; Rousk et al., 2010), and soil organic matter quantity and quality (Wardle, 1995). Given the complexity of biochar as a soil amendment, its potential utility in agriculture, and the multiple ways in which it can alter the soil environment, it is necessary to understand how biochar additions may change belowground functioning through direct and indirect effects on the soil biological community.

Few studies have investigated the impact of pyrolyzed materials including charcoal and ash on soil fauna (McCormack et al., 2013), and studies focused on the effects of human-made biochar on soil fauna are especially limited (e.g. Zhang et al., 2013; Marks et al., 2014; Domene et al., 2015; Soong et al., 2016). Fewer still have addressed the effects of biochar on the entire soil food web when applied in agricultural systems (McCormack et al., 2013). Many studies have considered the effect of biochar on the soil microbial community (bacteria and fungi; Lehmann et al., 2011; Liu et al., 2016), but responses are variable depending on biochar addition rate, biochar production conditions, and soil conditions (Acea & Carballas, 1999; Gomez et al., 2014; Jiang et al., 2015). Given the importance of both soil microbial and faunal communities for agroecosystem functioning (Brussaard et al., 2007), there is need to investigate how the entire soil food web responds to coupled biochar amendment and limited irrigation to determine whether or not substantial positive or negative side effects on belowground functioning will occur as a result. This study is the first to explicitly evaluate the interactive effects of biochar addition and limited irrigation on soil biological communities in the field.

Here, we address how biochar amendment and limited irrigation strategies, separately as well as in interaction (1) influence soil micro- and mesofauna biomass, and (2) alter structural and functional properties of the soil food web in an irrigated maize agroecosystem. We expect that the response of soil biota to biochar amendment and limited irrigation will vary between soil biota functional groups, given the differences in physiologies and water requirements of the different taxa. We hypothesize that limited irrigation will decrease the overall biomass and activity of soil biota, particularly those organisms that live in water films (i.e. nematodes, protozoa). By contrast, we expect soil biota biomass and activity (C and N mineralization rates) to increase in biochar-amended plots. We also hypothesize that biochar will favor fungi and their consumers relative to nonamended plots. Further, we hypothesize that biochar will mitigate the negative effects of limited irrigation by maintaining soil moisture. We expect a
significant interaction between biochar and limited irrigation, resulting in greater benefits of biochar to the soil food web by increasing soil biota biomass and function under limited irrigation relative to full irrigation.

To address these hypotheses, we sampled a maize agroecosystem that was amended with biochar 1 year prior to sampling and subjected to limited irrigation for one growing season. From these samples, we extracted soil organisms to estimate biomass for all soil food web functional groups. We then modeled structural and functional properties of the soil food web (Moore & de Ruiter, 2012) in the maize agroecosystem under the different management treatments.

We then aimed to evaluate the short-term effects of a change in agricultural management (limited irrigation and biochar amendment) within the broader context of land-use conversion from native grassland to agricultural system at our site. To do so, we compared the structural and functional properties of the soil food web in the maize agroecosystem to those of a soil food web from a nearby native grassland soil (Andrés et al., 2016) used as an uncultivated reference.

**Materials and methods**

*Site description and experimental design*

The study site is an experimental maize field located at the Agricultural Research Development and Education Center (ARDEC), Colorado State University, Fort Collins, Colorado. Founded in 1993, the field at ARDEC has been continuously used for experimentation under conventional agricultural management with irrigation, fertilizer, and herbicide inputs varying with experiment. The region is semiarid with an average high temperature of 16.7 °C and average low temperature of 1.1 °C, with 384 mm yearly average rainfall (Western Regional Climate Center, 2016). The soil is a Fort Collins Loam (Aridic Haplustolls in US Soil Taxonomy), with sandy loam texture (51% sand, 20% silt, 28% clay). The average soil bulk density is 1.3 g cm⁻³, with a total carbon (C) content of 1.5% and a total nitrogen (N) content of 0.1% (Abulobaida, 2014). The pH of the soil is 8.7 (Foster et al., 2016). Prior to 2005, the agroecosystem was managed under an irrigated plow-based tillage approach with wheat, corn, and dry bean production (Abulobaida, 2014). In 2005, the field was converted to conservation tillage under full and reduced irrigation with alfalfa-corn and dryland wheat–corn rotations (Abulobaida, 2014).

For this study, the field was prepared for planting in September 2013 by deep tilling to 30 cm and disk tilling to 12 cm. In November of that year, biochar was surface-applied at 30 Mg ha⁻¹ and disk tilled to 15 cm. The biochar was produced from virgin pine wood by Confluence Energy, LLC, Kremmling, CO, pyrolyzed beginning at 400 °C and ramping up to a maximum of 700 °C with 5 min of reaction time. Chemical and physical properties of the biochar are as follows: 71.9% total organic C, 0.60% total N, 9.4 pH (wet), and 0.326 g cm⁻³ bulk density (Control Laboratories, Watsonville, CA, USA). In early April 2014, fertilizer (200N-4P-1S-0.1Z g m⁻²) was applied followed by additional tilling to 10 cm. Maize varieties P8954 and P9305 (DuPont Pioneer, Johnston, IA, USA) were planted at 247 seeds km⁻² in late May 2014 and standard herbicide application occurred in June 2014. The experimental maize field was organized in a split-plot design with four replicate blocks. Primary plots were full (F) and limited (L) irrigation treatments that were split into two 4.5 × 4.5 m soil amendment treatment subplots, biochar (B) and nonamended control (C; n = 16, averaged across the two corn varieties). Limited irrigation was based on maize phenology to coincide with noncritical ear development phases. Full irrigation was calculated from evapotranspiration and precipitation rates and ranged from 1.52 and 2.54 cm applied once weekly. Over the season, the full irrigation plots received 22 cm and the limited irrigation plots received 15 cm from 3 May to 28 August 2014. The limited irrigation plots did not receive irrigation from 29 June to 28 July 2014, resulting in approximately a one-third reduction in irrigation applied relative to the full irrigation treatment. Volumetric soil moisture (%) and gravimetric soil moisture (g water g dry soil⁻¹) were measured to determine the effect of limited irrigation and biochar amendment on soil moisture content (see Foster et al., 2016 for gravimetric soil moisture data). To assess crop health status given the amendment and irrigation treatments described above, maize yield was determined at the end of the season as described in Foster et al. (2016).

We compared our agroecosystem soil food web data to that of a nearby native grassland that is considered an uncultivated reference. The grassland site is located at the Short Grass Steppe (SGS) Central Plains Experimental Range, just north of the agroecosystem site. Andrés et al. (2016) sampled soils from three sites within the SGS, each with one continuously grazed plot (30 × 30 m²). Further details regarding climate, dominant vegetation, and experimental design at SGS can be found in Andrés et al. (2016).

*Soil sampling*

Soil sampling occurred on 19 September 2014 after the final maize harvest of the season. In each subplot, four soil cores (5.5 cm diameter) were taken to a depth of 10 cm: two between and two within maize rows. Each between-row core was combined with one within-row for a total of two bulked samples from each subplot. One of the final bulked samples remained intact for microarthropod extraction, while the other sample was used for all other biological assays. Soils were collected in sealed plastic bags, stored in a cooler in the field, and transported immediately to the laboratory for soil fauna extraction. Subsamples for nematode extraction were taken from well-mixed nonmicroarthropod samples before these samples were 2-mm-sieved for further homogenization.

*Soil fauna extractions*

**Bacteria and fungi.** We estimated total bacterial and fungal biomass via direct counts using epifluorescent microscopy
techniques (Bloem, 1995). For both the bacteria and fungi assays, a 2-mm sieved subsample (5 g) from each field sample was blended with sterile deionized water and aliquoted (10 µl) onto a 10-well slide. Bacteria slides were stained with 5-(4,6-dichlorotriazin-2-yl) aminofluorescein, and fungi slides were stained with calcofluor fluorescent brightener following Frey et al. (1999). All direct counts were conducted using an Olympus Photomicrographic Microscope System with reflected light fluorescence attachment at 490 nm for bacteria and 334–365 nm for fungi. Total fungal biomass was scaled to active fungal biomass (10%) as described in Ingham & Klein (1984).

Protozoa. We estimated total protozoan biomass using the most probable number (MPN) technique (Darbyshire et al., 1974). A 2-mm sieved subsample (10 g) in 90 ml of sterile deionized water was serially diluted with tenfold dilutions to 10⁻⁶ ml. After each dilution in the series, four 0.5 ml subsamples were pipetted into four consecutive wells of a standard 24-well tissue culture plate. As a food source for the protozoan during incubation, Escherichia coli suspended in growth media was added to each well (50 µl). The plates were incubated at 14°C for 5 days. Thereafter, we observed each well under an inverted compound microscope (100× magnification) and recorded presence and absence of amoebae, flagellates, and ciliates. Total protozoan biomass was estimated using the MPN estimate program of the US Environmental Protection Agency (U.S. EPA, 2013).

Nematodes and arthropods. We extracted nematodes from subsamples (20 g) of each sample using the Baermann funnel method (Baermann, 1917). Soil samples were left in the extraction apparatus for 3 days, after which nematode samples were collected and preserved in formalin for taxonomic identification. Nematodes were sorted into functional groups based on their feeding morphology (Yeates & Coleman, 1982).

We heat-extracted microarthropods from bulked samples of intact cores using the Tullgren funnel method (Moore et al., 2000). Microarthropods were sorted into taxonomic functional groups (Moore et al., 1988).

### Soil food web modeling

We modeled structural and functional properties of the soil food webs for all the treatment combinations using a well-tested food web model (de Ruiter et al., 1994; Moore et al., 2005; Moore & de Ruiter, 2012). The model structure was based on functional groups of soil biota sensu Moore et al. (1988). To evaluate changes in food web structure (Moore & de Ruiter, 2012), we calculated the following metrics for all four treatment combinations (Table 1): number of basal resources (S_b; number of resources at the base of the food web, in this case detritus and roots); number of soil biota functional groups (S); connectance (C; amount of possible food chain links that are realized in the observed food web); linkage density (LD; number of functional groups multiplied by connectance); maximum food chain length (FCL_max); and mean food chain length (FCL mean).

Functional attributes of the food web were estimated using the model developed by Hunt et al. (1987) and Moore & de Ruiter (2012). Given the soil biota biomass estimates measured from soil samples, the model uses the untransformed field biomass estimates and published physiologies of the functional groups and the trophic interactions between them to estimate C and N mineralization rates through the web and their

### Table 1  Biomass estimates (mg C m⁻²) for all soil biota functional groups and taxonomic groups for all irrigation and amendment treatment combinations for the agroecosystem soils

| Treatment | Full | Biochar | Limited | Biochar |
|-----------|------|---------|---------|---------|
| Functional group | Control | Biochar | Control | Biochar |
| **Total microbial biomass** | 2109.63 ± 134.15 | 1831.99 ± 226.14 | 1835.96 ± 195.56 | 1776.52 ± 238.26 |
| Amoeba | 1.08 ± 0.41 | 0.41 ± 0.13 | 1.55 ± 0.96 | 12.09 ± 11.68 |
| Flagellates | 0.54 ± 0.21 | 0.13 ± 0.07 | 0.52 ± 0.26 | 0.46 ± 0.40 |
| Ciliates | 0 ± 0 | 0.01 ± 0.01 | 0.05 ± 0.05 | 0.03 ± 0.03 |
| **Total protozoa biomass** | 1.14 ± 0.41 | 0.52 ± 0.21 | 1.68 ± 0.96 | 6.51 ± 6.11 |
| Bacteriophagous nematodes | 3.00 ± 0.27 | 5.01 ± 2.91 | 4.67 ± 0.53 | 3.24 ± 0.29 |
| Fungivorous nematodes | 4.64 ± 2.23 | 3.53 ± 1.28 | 4.18 ± 0.77 | 4.33 ± 1.71 |
| Phytophagous nematodes | 4.72 ± 1.52 | 4.63 ± 1.43 | 2.53 ± 1.08 | 5.30 ± 3.30 |
| Omnivorous nematodes | 18.56 ± 3.94 | 16.49 ± 8.75 | 25.71 ± 8.15 | 11.06 ± 1.40 |
| Predaceous nematodes | 0 ± 0 | 0.30 ± 0.30 | 0 ± 0 | 0.16 ± 0.16 |
| **Total nematode biomass** | 19.22 ± 2.94 | 22.52 ± 11.03 | 24.51 ± 6.69 | 16.68 ± 4.70 |
| Collenbola | 0.02 ± 0.02 | 0 ± 0 | 0.03 ± 0.03 | 0 ± 0 |
| Cryptostigmatid mites | 0.89 ± 0.31 | 0.30 ± 0.22 | 0.64 ± 0.38 | 1.11 ± 0.63 |
| Noncryptostigmatid mites | 2.50 ± 0.90 | 0.48 ± 0.18 | 1.11 ± 0.36 | 2.02 ± 1.12 |
| Nematophagous mites | 0.91 ± 0.57 | 0.66 ± 0.30 | 0.72 ± 0.16 | 1.40 ± 0.41 |
| **Total microarthropod biomass** | 3.12 ± 0.89 | 1.10 ± 0.18 | 1.89 ± 0.41 | 2.90 ± 0.60 |

Estimates are mean ± SE (n = 4).
dynamic properties. Total biomass of all soil biota functional groups was determined based on our direct population counts and average estimates of organism size from Hunt et al. (1987), and was reported on a C basis, assuming 50% of biomass is C (Hunt et al., 1987). Bulk density was calculated from the intact cores used for microarthropod extraction and was used to convert biomass in mg C g dry soil\(^{-1}\) to mg C m\(^{-2}\). The model incorporates the field estimates of biomass, known natural death rates, feeding preferences, assimilation efficiencies, production efficiencies, and C : N ratios. Then, the model predicts feeding rates between functional groups and derives C and N mineralization rates for each functional group and for the entire soil food web (de Ruiter et al., 1994). We then compared these food web metrics and modeled C and N flux estimates to those of the soil food web at the Central Plains Experimental Range (Hunt et al., 1987; Andrés et al., 2016), a natural grassland used here as an uncultivated reference located just north of the agricultural site at ARDEC.

**Statistical approach**

Soil biota biomass estimates remained untransformed when serving as input for the food web model. Biomass estimates for all soil biota functional groups and modeled C and N mineralization rates were square-root-transformed prior to conducting further analysis. We fit a general linear mixed-effects model with soil biota biomass, modeled C flux, or N mineralization rates as the response variable, with irrigation, amendment, and functional group as the predictor variables, and block as a random effect prior to running ANOVA and Tukey-adjusted pairwise comparisons in R (R Core Team, 2015). To determine differences in food web structure between the treatment combinations, we conducted ANOVA and Tukey-adjusted pairwise comparisons on all standard soil food web metrics, and Fisher’s exact test to assess whether the presence of soil biota functional groups differed between the irrigation and amendment treatment combinations. To compare food web metrics and modeled C and N mineralization rates between the agroecosystem and grassland site, we fit a general linear model with food web metrics or mineralization rates as the response variable and site (agroecosystem or grassland) as the predictor variable and conducted ANOVA and Tukey-adjusted pairwise comparisons. It is important to note that the food web structure was identical for all three grassland sites because all soil organisms were present in all three food webs, thus resulting in the exact same food web metrics and standard errors of zero (Table 2).

**Results**

**Soil and crop empirical observations**

Extracted biomass of specific soil biota functional groups was not impacted by irrigation \(P = 0.94\), soil amendment \(P = 0.85\), or the interaction between irrigation and amendment \(P = 0.93\). Pairwise comparisons of soil amendment and irrigation treatments were not significant for any of the individual functional groups or total taxonomic groups (total microbial biomass, arthropods, nematodes, protozoa; Table 1). Similarly, we observed no differences in biomass estimates of aggregates of functional groups organized into different energetic pathways between the irrigation \(P = 0.97\), soil amendment \(P = 0.88\), nor their interaction \(P = 0.95\;\text{Fig. 1}\).

Across the entire season, full irrigation increased volumetric soil moisture by 22% relative to limited irrigation regardless of amendment \(P < 0.0001\) and a significant interaction between irrigation and soil amendment was observed \(P = 0.004;\text{Fig. S1}\). Additionally, biochar amendment increased volumetric soil moisture relative to the control under both limited \(P < 0.0001\) and full irrigation \(P < 0.0001\) across the entire season (Fig. S1). Two days prior to soil sampling (September 17, 2014), biochar-amended soils maintained higher volumetric soil moisture under both limited \(P = 0.005\) and full irrigation \(P = 0.02;\text{Fig. S1}\). Despite this increase in soil moisture, maize yield showed no response to irrigation treatment \(P = 0.21\) or biochar amendment \(P = 0.88\) at the end of the season (Foster et al., 2016).

| Table 2 Summary of food web metrics for all irrigation and amendment treatment combinations for the agroecosystem and the native grassland (Andrés et al., 2016) soils |
| Food web | \(S_r\) | \(S\) | \(C\) | LD | \(FCL_{\text{max}}\) | \(FCL_{\text{mean}}\) |
| --- | --- | --- | --- | --- | --- | --- |
| **Agroecosystem** | | | | | | |
| Full biochar | 2 ± 0 | 11.38 ± 0.53 | 0.479 ± 0.04 | 5.30 ± 0.25 | 2.88 ± 0.13 | 2.16 ± 0.05 |
| Limited biochar | 2 ± 0 | 12.13 ± 0.30 | 0.406 ± 0.02 | 4.88 ± 0.13 | 3.13 ± 0.13 | 2.19 ± 0.08 |
| Full control | 2 ± 0 | 12.25 ± 0.31 | 0.398 ± 0.02 | 4.83 ± 0.14 | 3.00 ± 0.00 | 2.09 ± 0.03 |
| Limited control | 2 ± 0 | 12.63 ± 0.46 | 0.381 ± 0.03 | 4.70 ± 0.21 | 2.88 ± 0.13 | 2.15 ± 0.04 |
| **Native grassland** | | | | | | |
| No treatment | 3 ± 0 | 18 ± 0 | 0.14 ± 0 | 2.52 ± 0 | 7 ± 0 | – |

Number of basal resources \(S_r\), number of functional groups \(S\), connectance \(C\); proportion of possible trophic links realized), linkage density (LD), maximum food chain length \(FCL_{\text{max}}\), and mean food chain length \(FCL_{\text{mean}}\) are mean of food webs ± SE \((n = 8)\), except for the native grassland \((n = 3)\).
Soil food web modeling

The irrigation treatment did not have a significant effect on food web connectance, the proportion of possible trophic links realized in a food web \( (P = 0.16; \text{Table 2}) \), or the number of functional groups \( (P = 0.18; \text{Table 2}) \). The soil amendment treatment only had a significant effect on connectance at the 0.10 level \( (P = 0.10; \text{Table 2}) \) but did not significantly affect the number of functional groups \( (P = 0.11; \text{Table 2}) \). Collembola were not present in biochar-amended plots, and predatory nematodes were not present in control plots, but these findings were not statistically significant under Fisher’s exact test \( (P = 0.48, 1, \text{respectively}) \). Although no significant differences in food web structure were observed for the different amendment and irrigation treatment combinations at the agroecosystem, marked structural changes became clear when comparing these agroecosystem soil food webs to the native grassland soil food webs presented in Andrés et al. (2016). Regardless of amendment and irrigation treatment combination, the agricultural soil food webs contain substantially fewer soil biota functional groups \( (S; P < 0.0001) \) and possessed higher connectance \( (P = 0.0001) \) compared to their natural

Fig. 1 Biomass (mg C m\(^{-2}\)) of soil food web energetic pathways: primary consumers (a) and secondary and tertiary consumers (b) in control (white bars) and biochar (gray bars)-amended plots under full and limited irrigation. Bars are mean ± SE of original (untransformed) data \( (n = 4) \).
grassland counterpart (Table 2; Andrés et al., 2016). More specifically, bacterial soil food web energetic pathways dominate the agroecosystem (Fig. 1). LD was much greater in agroecosystem soil food webs than in the native grassland soil food web (Table 2; \( P < 0.0001 \)). Also, the \( F_{CL_{\text{max}}} \) was much reduced in the agroecosystem soil food webs relative to the native grassland food web (Table 2; \( P < 0.0001 \)).

We found no significant differences in modeled total soil respiration (irrigation: \( P = 0.32 \); amendment: \( P = 0.27 \)) and total N mineralization (irrigation: \( P = 0.71 \); amendment: \( P = 0.69 \)) between all irrigation and soil amendment treatment combinations (Table 3). However, in comparison with the native grassland soil food webs, modeled total soil respiration and N mineralization are lower in the agroecosystem soil food webs (Table 3). Modeled N mineralization is about four to eight times lower in the agroecosystem food webs than that of the grassland food webs (Table 3). Similarly, the agroecosystem food webs processed five to twelve times less C than the grassland food webs (Table 3). Both the total modeled N mineralization and C flux for the grassland food webs were significantly greater than for the agroecosystem food webs (N mineralization: \( P < 0.0001 \); C flux: \( P = 0.0008 \)). When C flux was partitioned by soil biota functional groups within the food web, lower C processing capacity was observed in the agroecosystem soil food webs relative to the native grassland system (Fig. 2). Carbon transferred from primary consumers (bacteria and fungi) to secondary consumers (bacterial consumers and fungal consumers) is much greater in the native grassland soil food webs (49 kg C ha\(^{-1}\) yr\(^{-1}\)), than in the agroecosystem soil food webs (6 kg C ha\(^{-1}\) yr\(^{-1}\); Fig. 2). Figure 2a shows modeled C flux data from the continuously grazed native grassland site B (GB in Andrés et al., 2016) because it has the lowest mineralization rates of all three sites (Table 3), and thus serves as a lower anchor for mineralization potential. Figure 2b displays modeled C flux data from the non-amended plot under full irrigation, which is the reference, ‘business-as-usual’ management scenario in our maize agroecosystem. These data are not significantly different than any of the other soil amendment and irrigation treatment combinations (Table 3).

### Table 3 Comparison of modeled total N mineralization (kg N ha\(^{-1}\) yr\(^{-1}\)) and CO\(_2\) efflux (kg C ha\(^{-1}\) yr\(^{-1}\)) from soil food webs in all treatment combinations at the agroecosystem and grazed native grassland sites (Andrés et al., 2016)

| Food web        | N mineralization (kg N ha\(^{-1}\) yr\(^{-1}\)) | CO\(_2\) efflux (kg C ha\(^{-1}\) yr\(^{-1}\)) |
|-----------------|---------------------------------------------|---------------------------------------------|
| **Agroecosystem** |                                             |                                             |
| Full biochar    | 4.55 ± 1.26                                 | 41.86 ± 11.40                               |
| Limited biochar | 4.34 ± 0.92                                 | 67.20 ± 12.75                               |
| Full control    | 4.38 ± 1.30                                 | 69.01 ± 9.02                                |
| Limited control | 5.69 ± 0.73                                 | 86.41 ± 9.81                                |
| **Native grassland** |                                         |                                             |
| Site A          | 34.66                                       | 911.8                                       |
| Site B          | 33.97                                       | 535.4                                       |
| Site C          | 35.27                                       | 839.5                                       |
| **Average**     | 34.63 ± 0.38                                | 762.23 ± 115.32                             |

Agroecosystem estimates are mean ± SE (\( n = 4 \)), and average native grassland estimates are mean ± SE (\( n = 3 \)).

**Discussion**

As climate variability increases the strain of water resources on agriculture, management strategies that help mitigate these stressors have become especially important. Biochar amendment is of particular interest for its potential to increase water availability in soils (Atkinson et al., 2010), while also serving as a long-term C management strategy in bioenergy production systems (Lehmann et al., 2006). However, the impacts of biochar on the soil biological community must first be critically evaluated before widespread application can be recommended. Overall, we did not see any effect of biochar amendment and irrigation treatment combinations on biomass of any soil biota functional groups; thus, we reject our hypothesis that responses would differ among soil biota groups.

Counter to our hypothesis that limited irrigation would decrease nematode and protozoan biomass, we found no measurable effects of limited irrigation on any soil biota groups, regardless of biochar amendment (Fig. 1). Likewise, our hypothesis that soil food webs would be dominated by different soil biota groups in biochar-amended and nonamended plots was not supported, as we found no differences in either the biomass of soil biota (Table 1, Fig. 1) or the structure and function of the soil food web (Tables 2 and 3). As hypothesized, biochar amendment did ameliorate the effect of limited irrigation on soil moisture by maintaining higher volumetric soil moisture relative to the control throughout the season and 2 days prior to sampling (Fig. S1). However, contrary to our expectation, the biochar-induced increase in soil moisture had no effect on the biomass and activity of the soil food web. Furthermore, the maize yield did not respond to either irrigation or amendment treatments (Foster et al., 2016).

The absence of significant effects of irrigation treatments on the soil biota and food webs is surprising considering that previous studies have shown positive responses of soil biota to increased soil moisture in irrigated agroecosystems (Schnirrer et al., 1986; Wang et al., 2008; Li et al., 2010), although responses vary by functional group. Many natural and agricultural systems are...
water-limited and soil biota (Schnürer et al., 1986; Hunt et al., 1987), enzymatic activity (Li et al., 2010), and microbial biomass (Wang et al., 2008; Li et al., 2010) often show differential responses to water additions. In a barley agroecosystem, soils that received just one water addition (limited irrigation) were compared to fully irrigated plots and had reduced fungal hyphal length, decreased bacterial numbers, and negligible changes in protozoan abundance (Schnürer et al., 1986). However, an increase in nematode abundance was observed in both limited and fully irrigated plots (Schnürer et al., 1986). This suggests that the response to irrigation treatments differs between soil biota groups, a trend we did not observe in our study likely because limited irrigation maintained adequate soil moisture for soil biota functioning. The fact that the crop showed no response to either treatment may explain our results given the tight coupling of soil biota to water and plant production in semiarid regions (Collins et al., 2008).

Our results contribute to the unequivocal conclusions about the impacts of biochar on soil biota. While biochar has previously been observed to have substantial effects on soil biological community dynamics (Lehmann et al., 2011; Liu et al., 2016), numerous studies on the effects of biochar on soil microbial biomass have reported responses ranging from positive (Anderson et al., 2011; Luo et al., 2013; Domene et al., 2014; Gomez et al., 2014; Zhang et al., 2014b), to negative (Dempster et al., 2012), to neutral effects (Castaldi et al., 2011; Chen et al., 2013; Ameloot et al., 2014; Rutigliano et al., 2014; Zhang et al., 2014a; Noyce et al., 2015). Although not observed in our study, biochar has been shown to differentially affect primary consumers. Several studies suggest that pyrolyzed materials are preferentially consumed by bacteria, particularly gram-positive bacteria (Santos et al., 2012; Gomez et al., 2014; Jiang et al., 2015; Soong et al., 2016), but biochar has also been observed to serve as suitable habitat for fungi (Hammer et al., 2014; Jaafar et al., 2014).

Studies that investigate the response of protozoa and soil fauna to biochar addition are particularly sparse (Lehmann et al., 2011). Our study is the first to explicitly examine the response of protozoan communities (amoebae, ciliates, and flagellates) to biochar addition. We did not observe an effect of biochar amendment on protozoan biomass or activity in this agroecosystem. Experimental investigations of microarthropod and nematode responses to biochar are limited, and available results are contradicting. Microarthropods have been observed to both tolerate and avoid biochar in field and laboratory conditions (Bunting & Lundberg, 1987; Phillips et al., 2000; Salem et al., 2013; Domene et al., 2015). Although the absence of collembolan in our biochar-amended plots was not statistically significant, such biochar avoidance behavior has been observed (Domene et al., 2015). Our finding that soil nematode abundance and biomass did not respond to biochar addition after 1 year matches results from studies in a temperate grassland soil (Soong et al., 2016), a survey of forest soils (Matlack, 2001), and with the exception of

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increased fungivore abundance and decreased phytophagous nematode abundance, a report from a wheat agroecosystem (Zhang et al., 2013). While caution must be taken when comparing man-made biochar with naturally occurring charcoal as the processes by which they are created can substantially alter the way they function in soil and interact with soil biota (Lehmann & Joseph, 2015), the impacts of these materials on protozoan and soil invertebrates have largely been benign.

The soil food web model suggests little difference in food web structure (number of functional groups, connectance, and trophic links; Table 2) and no difference in function (C and N mineralization; Table 3) between biochar and irrigation treatments. Although connectance was slightly higher in the biochar-amended plots when averaging over irrigation treatment, this result was only significant at the \( P = 0.10 \) level. This effect is likely due to the lack of collembolan in the biochar plots, resulting in lower number of functional groups, but this is not statistically significant (Table 2). Given the lack of significant differences in other structural and functional metrics of the soil food web, this marginally significant difference in connectance presence remains inconclusive. The similarity of modeled soil respiration levels between biochar-amended and nonamended plots suggests that biochar C was not mineralized to CO\(_2\), indicating long-term C storage in these soils. Together, our results indicate that the soil biological community was not affected, structurally or functionally, by biochar addition through its indirect effect on soil pH (McCormack et al., 2013). However, the soil in our agricultural study site is basic (pH 8.7) and no increase in pH was observed after biochar addition (Foster et al., 2016). The maintenance of soil pH after biochar addition may explain why we did not observe any effect of biochar amendment on soil biota biomass or food web structural and functional properties.

We propose two additional explanations for rejecting our initial hypotheses. First, our study duration (1 year) and timing may have been too short to observe significant effects of coupled limited irrigation and biochar amendment. Our sampling occurred at the end of the growing season and may not have captured seasonal variability in responses of soil biota biomass to the treatments. Given the variability of field conditions, climate, and soil biota biomass estimates, assessing soil biological response to biochar at one time point just 1 year after application may not have allowed for enough treatment-response time. For example, only after 3 years after biochar addition, in a temperate maize field in northern New York, Domene et al. (2014) found an increase in microbial abundance. However, in agreement with our results, no significant differences in mesofaunal activity were observed (Domene et al., 2014), suggesting that the stimulating effects of biochar addition were not transferred from microbial trophic levels through the rest of the soil food web even after 3 years. Moreover, field biomass estimates of soil biota were quite variable in our study (Table 1), resulting in greater difficulty detecting minor effect of the treatments at one time point.

Second, the biochar application rate used in our study (30 Mg ha\(^{-1}\)) may not have been enough to significantly alter directly the soil biota or the soil environment within which soil organisms interact and function. Our application rate was comparable to or greater than other field trials in temperate maize agroecosystems (Jones et al., 2012; Domene et al., 2014; Brantley et al., 2015), and larger application rates are not currently foreseen as feasible given high costs of biochar application (Field et al., 2013; Shackley et al., 2015).

Our results may be explained by the state of the agroecosystem food web before biochar and limited irrigation were applied. The agricultural field has been intensively managed with conventional techniques including frequent tillage and pesticide application, all of which have been shown to adversely affect the soil food web increasing soil organic matter decomposition with subsequent losses of soil fertility (Fig. 2; Tables 2 and 3; Moore et al., 1984; Hendrix et al., 1986; Moore, 1994; Neher, 1999; Kladivko, 2001). Additionally, agricultural conversion in general has been observed to negatively impact soil biota (Culman et al., 2010; DuPont et al., 2010). Therefore, we hypothesized that past agricultural practices may have degraded the soil food web to a point that biochar application and limited irrigation did not have significant effects. To examine this, we put our results in a larger agricultural context by comparing the soil food web structural and functional properties from the agricultural site in this study to the soil food webs of an adjacent native grassland that served as an uncultivated reference (Andrés et al., 2016).

Clear structural differences were observed between the agroecosystem and native grassland soil food webs. The agroecosystem soil food webs had fewer functional groups present, and higher connectance values than that of the native grassland soil food webs (Table 3), suggesting a loss in functional diversity and trophic interactions among soil biota functional groups with the onset of agriculture. Several treatment combinations in the agroecosystem lacked ciliates, predatory nematodes and

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Collembola (Table 1) which were present in the grassland sites (Andrés et al., 2016), further confirming the loss of soil biota functional groups due to intensive agriculture. Additionally, the native grassland system supported greater food chain lengths, decreasing LD across the web (Table 2), suggesting greater food web stability than in the agroecosystem. The decrease in $F_{CL_{max}}$ in the agroecosystem relative to the native grassland food webs (Table 2) again points to a degradation of trophic structure during the agricultural conversion.

Functionally, the food webs within the agroecosystem also showed signs of being altered in all treatment combinations relative to those from the native grassland. Modeled total C and N mineralization are markedly reduced in the agroecosystem relative to the native grassland (Table 3). This suggests a loss of C and N cycling capacity in the agricultural soils, relative to their native grassland reference. In fact, the amount of C transferred from primary consumers (bacteria and fungi) to secondary consumers (bacterial and fungal consumers) is approximately eight times greater in the native grassland than in the agroecosystem soil food webs (Fig. 2), providing evidence that belowground C and N cycling has been altered as a result of agricultural conversion. We propose that, in this system and others (see Castracani et al., 2015), agricultural management impacts greatly outweigh any potential effects of biochar amendment and limited irrigation. For intensively managed agricultural systems, biochar addition and its possible effects on soil water and nutrient dynamics are not significant drivers of soil food web productivity and the ecosystem processes they regulate in the short term.

Our study is the first to take a comprehensive, field-based approach to assessing the impacts of coupled biochar amendment and limited irrigation on soil food web structure and function. Our findings suggest that, in the short term, biochar may not impact the entire soil food web in temperate agroecosystems, as has previously been observed for soil microorganisms alone (Lehmann et al., 2011). When considering the potential benefits of biochar as a C storage mechanism to close the C loop in bioenergy production, our results support continued research of biochar additions, as there were no negative effects on belowground food web structure and function. However, our comparison with the native grassland points to a bigger issue: the degradation of soil food web structure and function due to traditional agricultural management strategies. We contend that biochar amendment and limited irrigation likely did not have an effect on the soil food web because the soil biological community in our system is already degraded, lacks complexity, and functions at a reduced rate relative to its native grassland equivalent. In this setting, after 1 year, coupled limited irrigation and biochar amendment did not mitigate, nor further contribute to the negative effects of agricultural management in this semiarid maize agroecosystem.

Given our findings and the short duration of our study, long-term studies are needed to gain insights on the ecological effects of biochar and limited irrigation on the soil food web. In addition to conventional agricultural settings, investigations of soil food web responses to biochar in alternative agricultural management systems, such as no till and organic, are important lines of inquiry. Examining how biochar may affect these soil food webs will lead to meaningful insights and management implications for key ecosystem services provided by soil biota.

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### Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

**Figure S1.** Volumetric water content (%) of soils for control and biochar plots under full and limited irrigation across the 2014 growing season.