



Grazing and edaphic properties mediate soil biotic response to altered precipitation patterns in a semiarid prairie



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ABSTRACT

Global circulation models predict shifts in precipitation patterns in arid and semiarid ecosystems worldwide. Independently of changes in total precipitation, rainfall is expected to concentrate in less frequent but larger events, and this redistribution will increase drought severity in biomes naturally controlled by precipitation pulses and water availability. This study focuses on effects of altering rainfall patterns on the soil food web of a semiarid prairie, and on impacts of grazing on soil food web resilience to climate change. We worked in a greenhouse with undisturbed soil monoliths extracted from grazed and ungrazed field plots. We planted all monoliths with *Bouteloua gracilis* that was the dominant grass at the experimental site, and let the grass establish before applying two different rainfall treatments over a five-month period, equivalent to the duration of the growing season in the area. We simulated the current rainfall pattern in the experimental area and also an altered rainfall pattern consisting of concentrating the total growing season precipitation in half the number of rain events. Rainfall concentration increased the amplitude of the water pulse and lengthened the dry periods between rain events thus worsening soil water stress. We analyzed the experimental monoliths for effects of rainfall pattern and grazing management C and N content, soil organic matter mineralization and soil biota abundance and functional diversity. Based on the experimental data, we simulated soil food web stability and mineralization capacity. We concluded that (a) rainfall concentration causes loss of key soil functional groups and reinforces soil food web stability, (b) organic matter mineralization by the soil food web decreases with rainfall concentration, (c) coarse-textured soils are more responsive than fine-textured soils to modifications of the rainfall regime, (d) soil food web responsiveness to changing rainfall regime is higher in grazed prairies than in prairies excluded from herbivores.

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1. Introduction

If meteorological model predictions hold true, precipitation extremes will become more common in the semiarid grasslands of the world, and summer droughts will rise in frequency, severity and duration in the near future (IPCC, 2013). Coupled with this, and

independent of changes in total annual precipitation, soil moisture is expected to decrease due to growing evapotranspiration associated to rising temperatures (Brewer and Mass, 2016).

As a rule, the climate of arid and semiarid grasslands is characterized by sporadic rainfall that imposes drastic fluctuations of soil water availability and pulsed patterns of biological activity (Loik et al., 2004) with important implications for belowground food webs and soil C and N cycling (Lundquist et al., 1999). Rain events are most often small, and their volume determines the depth to which moisture will trigger soil biological activity and its duration. During the dry interpulse periods, soil microbes are

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mostly inactive because most of their energy is allocated to accumulate the solutes required to overcome osmotic stress (Schimel et al., 2007). Soil rewetting causes a quick peak of microbial activity and growth accompanied by nutrient release (Fierer and Schimel, 2002). When water pulses are small, they stimulate soil surface dwelling microbes and promote mineralization in this zone, but may be insufficient to activate the roots of higher plants located below. When this happens, a part of the released nutrients can be lost (Schwinning and Sala, 2004). Although results are controversial, a number of studies suggest that C and N losses are greater in soils undergoing such drastic moisture oscillations than in soils under steady and favorable water conditions (Xiang et al., 2008).

It is thought that grassland soils are more responsive to alterations of the rainfall pattern than to changes in total rainfall amount (Bardgett and Wardle, 2010) and, particularly, to concentration of annual precipitation into fewer but larger rainfall events (Harper et al., 2005). The intensification of the rainfall pulses is predicted to affect soil functioning through direct modification of the activity and metabolic rates of the soil biota (Whitford, 1989) and through changes in the quantity and quality of resources provided by plants, with cascading effects on belowground food webs (Kardol et al., 2011).

Microbial abundance and functions are regulated by fungal and bacterial grazers (Ingham et al., 1985b). However, our knowledge on how these soil invertebrates respond to altered precipitation regimes, and on how their response influences soil C and N pools, is restricted to a few data on soil microfauna (Saetre and Stark, 2005). Microbes, protists and nematodes recover relatively rapidly from drying events (Fierer et al., 2003) compared to microarthropods that recover more slowly, if at all (Lindberg et al., 2002). This asynchrony poses a risk to the whole architecture of the soil food web, with potential broad impacts on ecosystem functioning in the long term (Darby et al., 2011).

Together with climate, land management is a powerful driver of changes in biodiversity, and intensification of agriculture and cattle ranching is estimated to cause larger impacts than climate change in the functioning of temperate grasslands (Sala et al., 2000). All prairies of the world are grazed by wild herbivores and, to varying degrees, by livestock (Suttie et al., 2005). Aboveground grazing impacts belowground chemistry through changes in plant communities, in patterns of C allocation to shoots and roots, in the quantity and quality of litter provided by plants, and in soil N concentration and spatial distribution (Bardgett et al., 1998). Besides, trampling by large herbivores modifies soil physical properties such as bulk density, soil pore size distribution and water film dynamics (Asner et al., 2004) which in turn determine predators' access to prey and foraging success (Hohberg and Traunspurger, 2005; Zahn et al., 2016). All these factors combined produce contrasting effects on soil organic matter pools depending on the environmental context (Milchunas and Lauenroth, 1993) and may trigger bottom-up alterations of the soil food web (Bardgett and Wardle, 2010).

Arid and semi-arid prairies are particularly vulnerable to livestock grazing (Mooney et al., 1995) whose negative impacts on soil physical properties may be detectable for decades after cessation of grazing (Daniel et al., 2002). There is also evidence of legacy effects of aboveground grazing on soil microbial communities that, in the long term, can condition soil chemistry and multitrophic interactions (Kostenko et al., 2012). Ultimately, these legacies will influence ecological patterns and define ecosystem resilience and vulnerability to climate change (Foster et al., 2003).

The purpose of this work was to study how grazing cessation influences the response of the soil food web to alterations of the local rainfall regime, in terms of structure, metabolism and stability. To do this, we extracted undisturbed soil monoliths from three

different sites of a semiarid prairie where grazed and ungrazed plots were available from a long-term manipulation. In a local greenhouse, and for five months, we exposed the monoliths to two different rainfall regimens: the current local rainfall regime and a modified rainfall regime resulting from concentrating the current total precipitation in half the number of rain events. We hypothesized that the architecture of the soil food web is sensitive to changes in rainfall patterns (a), that rainfall concentration in fewer but larger events has negative effects on soil food web stability (b) and slows down carbon and nitrogen mineralization (c), and that soil food web response to rainfall concentration is mediated by soil texture and land use (d).

2. Materials and methods

2.1. Experimental site

We obtained our experimental soils from a semiarid shortgrass steppe located at the westernmost part of the USA Great Plains (49°49'N, 104°46'W, 1600 m.a.s.l.) and dominated by the C4 grass *Bouteloua gracilis* (blue grama). Climate is semiarid, with 320 mm of average annual precipitation and mean annual temperature of 8.6 °C, with average monthly temperature ranging from −4 °C in winter to 22 °C in summer. The area was part of the USDA-ARS Central Plains Experimental Range (CPER) (sgsllter.colostate.edu).

2.2. Grazing treatment

In 1939, a long-term cattle grazing management experiment was started in the experimental area to determine effects of different grazing intensities. For our work, we chose a 62 ha area allocated to “moderate intensity” that had been grazed by heifers and wild herbivores for 75 years with a stocking density corresponding to 40% forage utilization. In 1996, three 30 m × 30 m plots had been fenced at three different sites (A, B and C) within this grazed area with the aim of excluding all large (heifers and wild ungulates) and small (lagomorphs and rodents) herbivores. The sites were less than 2 km away from each other and shared geological substrate, topography, climate and vegetation. A detailed description of the grazing management plots is available from Rebollo et al. (2013).

In April 2014, we visited the three fenced plots (ungrazed –UG– plots) and demarcated three new plots (grazed –G– plots) in the peripheral moderately grazed prairie. The new G plots were the same size and shape that the UG plots, and were located 20 m north of each.

We determined the soil physical and chemical properties of all plots from 60 (ten per plot) undisturbed samples taken with 25 cm² area and 15 cm deep soil corers. The samples were individually analyzed for soil texture, for total C and N by dry combustion of oven-dried and pulverized sub-samples (Leco TruSpec. LECO, St. Joseph, Michigan), and for inorganic C by the modified pressure-calculator method (*sensu* Sherrod et al., 2002). On the same date, ten additional soil samples per plot were cored out for soil biota characterization.

2.3. Rainfall treatment

In April 2014, immediately after soil thawing and before the start of the growing season, we pulled out four soil monoliths from each of the six field plots. To obtain monoliths containing undisturbed communities, we drove PVC pipes (35 cm long and 13 cm in diameter) into the ground to 3 cm from their upper rim, and retrieved the tubes filled with 32 cm high cylindrical soil monoliths. An additional set of undisturbed soil cores (5 cm in diameter and

15 cm deep) were extracted from the close vicinity of each monolith and were used to estimate soil bulk density, porosity and gravimetric water content at field capacity (–33 kPa) and at –2 MPa.

We transported the 24 pipes containing the soil monoliths to the greenhouse of the Plant Growth Facilities at Colorado State University. Once there, we removed the bottom 2 cm of soil to make room for perforated PVC plates coated with plastic mesh (1 mm size) that provided drainage and aeration. We saturated the monoliths with deionized water and allowed them to drain over 24 h to determine their water holding capacity (WHC) gravimetrically. Each monolith was then planted with 1 g of blue grama seeds (*Bouteloua gracilis* var. Hachita, provided by Pawnee Buttes Seed Inc., Greeley, Colorado). We let the grass establish under the greenhouse conditions (mean T: 20.5 °C; 37% air relative humidity; 35% blockage of the outdoors sunlight) for six months at 60% WHC.

For the rainfall treatment, we defined two different precipitation patterns. The “current rainfall” pattern (CR) was based on 2013–2014 rainfall registers provided by the CPER observatory after confirming that both years were representative of the climate of the region (Sala and Lauenroth, 1982; Sala et al., 1992). Mean annual precipitation is 326 mm (90% of which falls during the 6-month growing season), 67% of the rainfall events are less than 5 mm in volume and account for 12%–39% of the annual precipitation, and 17% of the events are 5–10 mm in volume. To define the “altered rainfall” pattern (AR), we adopted the climate models that predict that, in the coming decades, total annual precipitation will be redistributed in fewer but larger rain events. Accordingly, we concentrated the current growing season rainfall in half the current number of events. The detailed characteristics of the experimental rainfall patterns are summarized in Table 1.

In October 2014, when the grass had reached its maximum height, we randomly allocated two of the four monoliths extracted from each grazed or ungrazed plots to one of the two levels of the rainfall treatment (current or altered pattern). To simulate the current rainfall pattern, we wetted the allotted monoliths every Monday and Friday with deionized water following sequences of 70 ml, 70 ml and 100 ml, for a total of 4150 ml (equivalent to 316 mm) of cumulated rainfall at the end of the experiment. We simulated the altered rainfall pattern by wetting the allotted monoliths every Monday with 160 ml of deionized water for a total of 4160 ml (equivalent to 317 mm) of cumulated rainfall. We weighed the monoliths before watering in order to keep track of the evolution of the soil water content. The treatment lasted 152 days and, during this period, we rearranged the monoliths weekly to prevent possible effects of heterogeneous environmental conditions.

2.4. Monolith sampling

At the end of the rainfall treatment, we watered all monoliths to 60% field capacity for one week prior to sampling. We cut the grass at ground level and removed the soil monoliths from the PVC tubular containers. We cut each monolith horizontally in two

equivalent halves and took the top 15 cm thick undisturbed soil cylinder for analyses. We then split this cylinder vertically into four identical quarters that were allocated to, respectively, root biomass estimation (in dry weight after oven drying at 70 °C for four days), arthropod extraction, nematode extraction, and soil analyses plus microbe and protist enumeration.

2.5. Soil organic matter pools and C and N losses by respiration and leaching

From each monolith, 50 g of soil were air-dried, homogenized, sieved to 2 mm and then moistened to 60% WHC (previously determined after Colman, 1947) in 250 ml leaching cups equipped with 45 µm nylon mesh membranes (BPE4525 model, CellSmart Vacuum Filtration Systems, Argos Technologies Inc.). We placed 0.25 g of glass wool between the soil samples and the filters to prevent pore clogging as well as soil loss. We recorded the weights of the filters plus wet soils to facilitate water loss replacement by gravimetry. Each leaching cup was placed in a 2 L air-tight Mason jar with a lid fitted with a rubber septum to allow gas sampling. The jars were left to incubate in the dark at a constant temperature of 25 °C for 132 days.

On days 3, 6, 11, 22, 32, 52, 87 and 132 of the incubation, we measured CO₂ concentration in the headspace of the jars with an infrared gas analyzer (LI-COR, Lincoln, NE, USA). After gas sampling, we opened the jars, removed the cups and leached the soil samples with 100 ml of a nutrient-extract solution free from C or N. The solution consisted of 4.0 mmol.L⁻¹ CaCl₂, 2.0 mol.L⁻¹ KH₂PO₄, 1.0 mmol.L⁻¹ K₂SO₄, 1.0 mmol.L⁻¹ MgSO₄, 25 µmol.L⁻¹ H₃BO₃, 2.0 µmol.L⁻¹ MnSO₄, 2.0 µmol.L⁻¹ ZnSO₄, 0.5 µmol.L⁻¹ CuSO₄, and 0.5 µmol.L⁻¹ Na₂MoO₄ (Nadelhoffer, 1990) and was intended to replace nutrient loss over successive extractions (Zheng et al., 2012). We let samples equilibrate with the nutrient solution for 30 min prior to extract the leachates by vacuum filtration. Two 50 ml aliquots of the leachates were kept frozen in plastic vials until analyses for total dissolved C (TDC) and N (TDN), and nitrates and ammonium, respectively. After leaching, the soil samples were brought back to 60% WHC with distilled water before restarting the incubation.

We measured TDC, TDN and total nitrate and ammonium produced by each soil sample from a unique composite sample resulting from pooling the eight leachates obtained during the incubation. TDC and TDN were measured in a Shimadzu TOC-L analyzer with a TN unit, and total nitrate and ammonium concentrations (EPA#353.2 and DIN#38406 methods, respectively) with an Alpkem Flow Solution IV auto analyzer (O.I. Analytical, College Station, Texas).

We intended to determine the size of the active and slow C pools by fitting the curve of CO₂-C evolved per unit of time to the two-pool first-order equation from André and Paustian (1987). However, some of the cumulative curves had not yet leveled off at the end of the incubation, and the respiration series did not fit the model. Therefore, in order to apply a unified criterion, we assumed that the cumulated CO₂-C evolved over the 132-day long incubation

Table 1
Main characteristics of the two rainfall patterns simulated in the experiment.

	Current rainfall pattern	Altered rainfall pattern
Total growing season rainfall (mm)	316	317
Number of rainfall events	44	22
Average time between events (day)	2.5	6
≤5 mm events (%)	68.2	0
7.5 mm events (%)	31.8	0
12 mm events (%)	0	100

was a proxy of the active C pool. The slow carbon pool was then obtained by subtracting the active C pool from total C.

2.6. Microbial functional diversity assessment

We assessed coarse microbial functional diversity by phospholipid fatty acid (PLFA) analyses. Despite some documented limitations (i.e. low specificity for fungi, or dependence of PLFA composition on environmental conditions), this method is useful to compare soil samples for differences in community structure (Leckie, 2005). We performed the analyses after Denef et al. (2007). Soil samples were sieved at 2 mm and all visible plant debris were removed. Total lipids were extracted from a 6 g freeze-dried soil sample per monolith with a 0.8:1:2 (in vol.) phosphate buffer: chloroform: methanol solution and partitioned by solid phase extraction into neutral lipids, glycolipids and phospholipids. Phospholipids were then methylated to fatty acid methyl esters (FAMES). FAMES were analyzed by gas chromatography-mass spectrometry with a Trace GC Ultra coupled to a Thermo ISQ (Thermo Scientific). We identified FAMES based on mass spectral and retention time matches to a 27 FAME mixture (Supelco), a bacterial acid methyl ester CP mixture (Matreya), and a GLC-110 Mixture (Matreya).

We identified a total of 13 PLFA microbial biomarkers. The saturated aC15:0, iC15:0, iC16:0 and iC17:0 were interpreted as biomarkers of gram-positive bacteria; cis-C16:1 ω 9, C18:1 ω 11c and cy-C19:0 were used as indicators of gram-negative bacteria; 10Me-C16:0 and 10Me-C17:0 represented actinomycetes, and C18:0 undetermined bacteria; C16:0 was regarded as an unspecific microbial marker, and C18:2 ω 9,12c represented fungi (Frostegård et al., 1993; Denef et al., 2007; Esperschütz et al., 2009). C18:1 ω 9c is a major cellular fatty acid in fungi (Ruess et al., 2002) but may also be found in bacteria (Haack et al., 1994). Our experimental sites had not been cultivated at least during the last century, and the structure of the soil microfauna did not indicate agricultural activity (i.e. high abundance of astigmatic mites in cultivated soils). Therefore, following Frostegård et al. (2011) who proposed that this fatty acid is mainly derived from bacteria in agricultural soils, but from fungi in forest soils, we accepted C18:1 ω 9c as a fungal marker.

2.7. Soil food web biomass, composition and structure

We calculated bacterial and fungal biomass from direct count of slides under epifluorescence microscope (Bloem, 1995). Since the method measures total (dead and living) fungal biomass, we divided the result per 10 to get an estimate of the live fraction (Hunt et al., 1987). This decision was based on evidence that, in the study area, and under soil moisture levels comparable to those simulated in our experiments, only 10% of the soil fungal biomass (in annual average) is active (Ingham et al., 1985a). Protist abundance (sorted into ciliates, amoebas and flagellates) was estimated by the most probable number method (Darbyshire et al., 1974) on 10 g soil samples. We extracted nematodes from 20 g soil samples with Baermann funnels for 3 days. Microarthropods were extracted from soil in Tullgren funnels for 6 days. We sorted all animals into functional trophic groups based on shared trophic position (similar predators and preys or resources) and life traits (Moore et al., 1988). All individuals included in a given functional group were attributed the same individual biomasses, metabolic rates and feeding preferences based on literature (Table S1 in Supporting Information). Biomass-C density was calculated for each group by multiplying its abundance (in individuals m⁻² in the soil top 15 cm) by half the individual body weight attributed to the group since we assumed that 50% of the dry weight of the soil living biomass is made of carbon.

We calculated some classic descriptors of the food webs under each of the experimental scenarios: number of functional groups (S), number of realized links between pairs of trophic groups (L) and link density L/S. Finally, we calculated the interactive connectance (C) as L/[S(S-1)/2], which excludes cannibalism and other cycles (Pascual and Dunne, 2006).

2.8. Carbon and nitrogen mineralization simulation

To simulate C and N mineralization by soil food webs, we followed Hunt et al. (1987). In the model, C mineralization rate (C_{ij}) in g C m⁻² yr⁻¹ is calculated for each consumer as follows:

$$C_{ij} = a_j (1 - p_j) F_{ij}$$

with a_j = assimilation rate, and p_j = production rate. F_{ij} represents the feeding rate of a consumer j on a resource i (for details, see Moore and de Ruiter, 2012). Nitrogen mineralization rate (g N m⁻² yr⁻¹) was derived from F_{ij} , based on the C to N ratio of prey (CN_i) and predator (CN_j) as follows:

$$N_{ij} = a_j \left(\frac{1}{CN_i} - \frac{P_j}{CN_j} \right) F_{ij}$$

We simulated C and N mineralization for each of the 12 experimental scenarios (all possible “site” x “grazing management” x “rainfall regime” combinations) based on the average biomass-C of each trophic group obtained from the experimental monoliths (with $n = 2$ monoliths per scenario). To allow for statistical testing, we performed ten Monte Carlo simulations per scenario based on the standard deviation of the means. We applied the same model to simulate biomass flow between the functional groups of the food web (in g C m⁻² yr⁻¹).

2.9. Food web stability estimation

Following May (1972), we considered that a food web is locally stable if all the eigenvalues of its interaction strength matrix (the Jacobian matrix) have negative real parts ($\lambda_{\max} < 0$). The Jacobian matrices were constructed based on the Lotka-Volterra differential equations that define the food web (Moore and de Ruiter, 2012).

We used the diagonal elements of the Jacobian matrices to get a quantitative measure of the food web stability. These elements were formulated as $\alpha_{ii} = -sd_i$ and are a measure of intragroup interferences as a function of the specific death rates of the individual functional group (de Ruiter et al., 1995). We specifically calculated the minimum value of s (S_{\min}) required to stabilize the food web (Neutel et al., 2002). The smaller S_{\min} , the more the food web will be stable.

These calculations were applied to the same 12 Jacobian matrices (one per experimental scenario) previously used for simulating mineralization (with $n = 2$ experimental replicates per scenario) and, as before, ten Monte Carlos simulations of S_{\min} based on the standard deviations of the means were performed per scenario to allow for statistical testing.

2.10. Statistical analyses

We first assessed the legacy of grazing cessation on soil physical, chemical and biological properties. The statistical design considered was a randomized complete block design, with sites as blocks and grazing as a fixed factor. Since previous work showed that soil texture had a crucial influence on the soil food web of the short-grass steppe (Andrés et al., 2016), we included texture (expressed

as percent sand content) as a covariate. After accounting for accidental deterioration of some sample containers, the final number of samples per block \times grazing management combination varied between five and ten.

The final design considered after applying the rainfall treatment was a randomized complete block split plot design, with two whole plots per block (site) representing the “grazed” and “ungrazed” levels of the grazing management treatment. Within each whole plot (grazing management level), two soil monoliths (subplots) had been randomly allocated to each level (“current” and “altered” pattern) of the rainfall treatment. As before, we used soil texture as a covariate. The number of replicates was $n = 2$ for soil parameters directly measured in the monoliths. For simulated parameters (simulated respired C, mineralized N and S_{min}), the number of replicates was $n = 10$ corresponding to the number of model runs.

The above analyses were performed with the *lmer* function of the *lme4* (Bates et al., 2015) package in R (R Development Core Team, 2016). Tests for fixed effects were obtained using the *lmerTest* package (Kuznetsova et al., 2016) with the Kenward-Roger's approximation for denominator degrees of freedom for the F statistics (Kenward and Roger, 1997). Tests for differences between treatment levels after fitting the linear models were evaluated from predicted marginal means using the *lsmeans* package (Lenth, 2016). Grazing management and rainfall regime were included in the analyses as categorical variables and soil texture as a continuous covariable. For the sake of clarity, factor level comparisons involving soil texture were classified as 40% and 80% sand content, standing for fine and coarse-textured soils, respectively (40% sand content was common at site A, and 80% sand content at sites B and C). After model fitting, residuals were used to check for normality and homogeneity of variance, and data were log-transformed when required.

Influence of site, grazing management and rainfall pattern on soil community functional diversity was examined by principal component analysis (PCA) with Canoco v.4.5 (Ter Braak and Smilauer, 2002) applied to the relative biomass-C of all soil functional groups, and to the mole percentages of the PLFA microbial biomarkers. Differences among treatments were examined for significance by similarity analyses (ANOSIM) with Primer v.6 (Clarke and Gorley, 2006). We tested the relationship between soil food web stability and connectance by means of an exponential regression with SPSS 19.0 (IBM, 2010).

3. Results

3.1. Site effect and effect of grazing cessation in the field plots

The three experimental sites significantly differed in soil physical, chemical and biological properties (Table 2). Plots at site A had fine-textured clay loam soil, while coarse-textured loamy soils predominated at sites B and C. Total C ($F_{1,1} = 725.5$, $p = 0.017$) and total N ($F_{1,1} = 823.58$, $p = 0.015$) significantly decreased with

increasing proportion of sand whatever the grazing management history. Soil acidity was independent of soil texture but affected by management, with lower pH in the grazed than in the ungrazed soils ($F_{1,47} = 4.959$, $p = 0.031$). C/N and total soil biomass-C were not affected by texture or grazing. Since soil properties linked to soil hydrological behavior were measured using unique soil columns per plot, statistical analyses were not applicable, but data suggested that soil bulk density was higher and soil porosity lower in grazed than in the ungrazed plots (Table 3) at sites A and B.

3.2. Soil hydrological behavior under the simulated rainfall patterns

The experimental soils showed disparate responses to changes in rainfall pattern (Table 3) depending on their site of origin. Throughout the experimental period, the percentage of registrations showing matric potentials below -2 MPa (out of 46 measurement dates) was greater under the altered than under the current rainfall pattern, particularly in soils from sites B and C. The amplitude of the fluctuation in soil water content (difference between the maximum and minimum water content measured during the experimental period) was wider under the altered than under the current rainfall pattern for all monoliths.

3.3. Effect of treatments on total soil C and N

Effects of rainfall pattern on total soil C and N content depended on soil texture and grazing management, as suggested by significant two-way interactions. No significant three-way interaction between rainfall pattern, grazing management and soil texture was detected. Total soil C and N were greater under the altered than under the current rainfall pattern in fine-textured soils, but no effect of rainfall was observed in coarse-textured soils (sand \times rainfall interaction: $F_{1,15} = 5.819$, $p = 0.029$ for C and $F_{1,15} = 5.207$, $p = 0.037$ for N), (Fig. 1a and b). Grazing management influenced soil response to changes in rainfall pattern with regard to total C and N (management \times rainfall interaction: $F_{1,15} = 5.377$, $p = 0.035$ for C and $F_{1,15} = 5.466$, $p = 0.034$ for N). In grazed soils, shifting from the current to the altered pattern led to higher concentrations of C and N but no effect was found in ungrazed soils (Fig. 1c and d).

3.4. Effect of treatments on respired C and net loss of dissolved C and N

Total CO_2 -C evolved during the 131-day long soil incubation amounted to $680 \pm 294 \mu\text{g CO}_2\text{-C g soil}^{-1}$ (equivalent to $7.2 \pm 0.3\%$ of total organic C) regardless of treatments or soil texture. Total amounts of dissolved C and N collected in leachates depended on rainfall patterns, but only in fine-textured soils (sand \times rainfall interaction: $F_{1,16} = 5.451$, $p = 0.033$ for C and $F_{1,16} = 6.587$, $p = 0.021$ for N), with more C and N collected under the altered than under the current rainfall pattern (Fig. 1e and f). No effect of grazing management was detected.

Table 2

Main soil properties in the grazed (G) and ungrazed (UG) field plots at sites A, B and C. Data correspond to the top 15 cm of the soil. Mean \pm standard deviation with $n = 10$ samples per plot.

	Site A		Site B		Site C	
	G	UG	G	UG	G	UG
Sand (%)	36.6 \pm 3.2	37.0 \pm 2.2	81.8 \pm 2.5	75.0 \pm 2.5	80.2 \pm 1.3	80.0 \pm 1.6
pH (0.01 M CaCl_2)	6.7 \pm 0.1	6.6 \pm 0.1	6.3 \pm 0.2	6.6 \pm 0.2	6.4 \pm 0.3	6.6 \pm 0.3
C/N	9.1 \pm 0.6	9.1 \pm 0.2	8.5 \pm 0.3	8.8 \pm 0.7	8.4 \pm 0.3	8.6 \pm 0.5
Total C (%)	1.7 \pm 0.2	1.8 \pm 0.2	0.6 \pm 0.1	0.9 \pm 0.2	0.7 \pm 0.1	0.7 \pm 0.1
Total N (%)	0.20	0.22	0.08	0.11	0.09	0.09
Total biomass-C (kg C ha^{-1})	225 \pm 12	255 \pm 110	152 \pm 16	89 \pm 11	247 \pm 7	347 \pm 15

Table 3

Some soil properties linked to the hydrological behavior of the experimental monoliths depending on origin (sites A, B and C), land management history (UG: ungrazed plots; G: grazed plots) and rainfall pattern (current or altered). Percentage of registers showing matric potentials below -2 MPa over the rainfall experiment. Water pulse amplitude is the difference (in ml water) between the maximum and minimum water content for each type of monolith measured during the experiment. Soil properties were measured in a unique soil sample per plot. Values for gravimetric water content, % registers and water pulse amplitude are averaged from $n = 2$ monoliths.

Soil properties		Gravimetric water content (g H ₂ O g dry soil ⁻¹)		% Registers below -2 MPa		Water pulse amplitude (ml)			
Site	Land management	Bulk density (g cm ⁻³)	Estimated porosity (cm ⁻³)	-0.03 MPa	-2 MPa	Current rainfall pattern	Altered rainfall pattern	Current rainfall pattern	Altered rainfall pattern
A	UG	1.01	0.62	0.32	0.148	100	100	430	585
	G	1.08	0.59	0.27	0.12	100	100	492	591
B	UG	1.3	0.51	0.11	0.05	71	98	525	725
	G	1.56	0.41	0.11	0.05	91	100	623	778
C	UG	1.48	0.44	0.13	0.05	92	100	562	769
	G	1.45	0.45	0.12	0.05	88	100	600	785

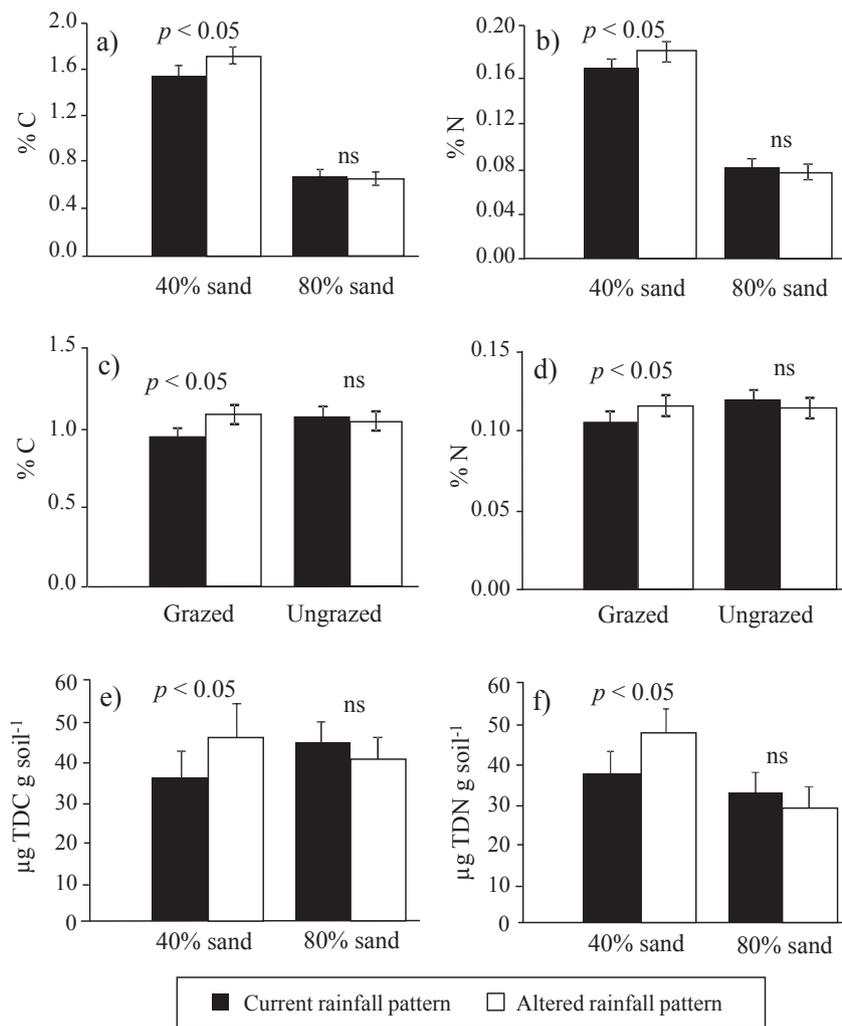


Fig. 1. Effect of rainfall patterns (current or altered) on (a) total soil C and (b) total N depending on soil texture expressed as percent sand content. Effect of rainfall patterns on (c) total soil C and (d) total soil N depending on grazing management (grazed or ungrazed soils). Effect of rainfall patterns on total dissolved C (e) and total dissolved N (f) collected in soil leachates, depending on soil texture. Vertical bars denote standard errors ($n = 6$ monoliths). Significant differences ($p < 0.05$) and no significant effects (ns) are shown.

3.5. Effect of treatments on soil biota biomass and diversity

Root biomass was the same in all monoliths (4.6 ± 1.9 g d.w. in the top 15 cm) regardless of soil texture and treatments. Most soil biota biomass-C was provided by microbes ($35.3 \pm 13.3\%$ was accountable to bacteria and $59.7 \pm 15.1\%$ to fungi). Neither total

biomass-C (10.3 ± 6.3 g C m⁻²) nor microbial-C (9.9 ± 6.3 g C m⁻²) were affected by treatments or soil texture. The biomass-C provided by the soil invertebrate groups (Table S2, Supporting Information) was not sensitive to treatments or soil texture, with the exception of nematodes (Fig. 2) whose biomass-C was lower under the altered than under the current rainfall pattern, but only in

sandy soils (sand \times rainfall interaction: $F_{1,16} = 9.571$, $p = 0.007$).

The PCA seemed to suggest differences in microbial diversity at site A relative to sites B and C (Fig. 3a), but neither site nor grazing management, nor rainfall pattern had significant effect on microbial biodiversity when estimated by PLFA markers (ANOSIM: $R = 0.722$, $p = 0.133$). Neither could we find significant effects of site or treatment on soil biota diversity when considering the relative biomass-C of the soil trophic groups (Fig. 3b) at the resolution level adopted in this work (ANOSIM: $R = 0.278$, $p = 0.133$).

3.6. Food web structure, metabolism and stability

The structure of the soil food web in each of our 12 experimental scenarios is shown in Figs. S1–S6 (Supporting Information). All food webs included two decomposer energy channels (based on SOM exploitation by bacteria and fungi, respectively) and one herbivore energy channel (based on root grazing by phytophagous invertebrates). The three channels (*sensu* Moore and Hunt, 1988) were connected to one another by nematophagous mites that were the top predators in all soils, and also by predaceous nematodes when present. We did not find any predator able to feed on fungivorous microarthropods (collembolans and cryptostigmatic and prostigmatic mites) and then, with the exception of the fungivorous nematodes, the fungal channel was not top-controlled. The basic parameters of the food webs are shown in Table 4.

Based on our simulations, we found marginally significant effects of rainfall pattern, grazing management and soil texture on the amount of carbon respired and the amount of nitrogen mineralized by the food web (sand \times rainfall \times management interaction: $F_{1,109} = 3.725$, $p = 0.056$ for C, and $F_{1,109} = 3.549$, $p = 0.062$ for N). In fine-textured soils, C respiration and N mineralization were insensitive to treatments. Conversely, in coarse-textured soils both rates were significantly lower under the altered than under the current rainfall pattern, with this effect being more evident in grazed than in ungrazed soils (Fig. 4a and b).

All soil food webs were asymptotically stable. Stability as estimated by S_{min} , was sensitive to changes in rainfall pattern only in coarse-textured soils (sand \times rainfall interaction: $F_{1,110} = 319.9$, $p < 0.001$) with S_{min} being lower (and the stability higher) under the altered than under the current rainfall pattern. Grazing management also influenced S_{min} response to changes in rainfall regime (management \times rainfall interaction: $F_{1,110} = 15.49$, $p < 0.001$). S_{min} was always lower (and the stability higher) under the altered than

under the current rainfall regime, and this effect was greater for the grazed than for the ungrazed soils (Fig. 4c and d).

We found a positive exponential relationship between interactive connectance and S_{min} ($r^2 = 0.703$; $F = 25.04$; $p = 0.001$), i.e., a significant negative relationship between food web connectance and stability (Fig. 5).

4. Discussion

In the near future, the dry-wet cycles that typically control belowground processes in arid and semiarid ecosystems are expected to decrease in frequency and to gain in volume. In this work, reducing by half the number of rain events during the growing season while doubling their volume resulted in increased water stress in the soil of the experimental monoliths, meaning more prolonged dry interpulse periods and greater amplitude of the fluctuation in soil water content. These effects were more intense in the fine-textured than in the coarse-textured soils because of higher capacity for water retention of clay relative to sand (Rawls et al., 1982).

Based on the findings of other researchers, we had expected rainfall concentration and the resulting reinforcement of soil water stress to result in decreased soil biomass and to cause changes in the relative biomass abundance of the various soil food web groups. Particularly, we had expected the dominance of fungi over bacteria and of gram-positive over gram-negative bacteria to increase with soil water stress (Allen, 2007; Yuste et al., 2011), and the same trend was posited for the dominance of drought-resistant invertebrates over water-dependent invertebrates (Tsiafouli et al., 2005; Geisen et al., 2014). But, unexpectedly, none of these effects could be confirmed in this work.

In search for an explanation of the lack of observed effects, we checked our experimental design for the adequacy of the soil stress levels resulting from the rainfall treatments. In our monoliths, soil water potential fluctuated over time while staying below -2 MPa most of the time, and these experimental conditions seem very appropriate to test our hypotheses given the thresholds for water stress of various soil biota. *Bouteloua gracilis* starts reallocating carbon to roots between -1.5 and -3 MPa (Chung and Trlica, 1980), microbes' tolerance to drought is species-specific and ranges from -0.1 to -15 MPa (Lavelle and Spain, 2003), and almost all protists and about half of all nematodes are inactive below -0.4 MPa (Whitford, 1989). Among belowground arthropods, springtail tolerance to drought spans from less than -1.5 MPa to more than -2.5 MPa, while some cryptostigmatic mites remain active below -9.8 MPa (Holmstrup et al., 2015). Along the same methodological line, a possible explanation to this general lack of response may be the short duration of the experiment, since previous works in arid ecosystems indicate that effects of shifts in rainfall patterns on soil microbial biomass require at least three years to materialize (Bell et al., 2014). On the other hand, several authors support our results and sustain that the functional composition of the soil bacterial consortia is resilient to changes in rainfall patterns (Fierer and Jackson, 2006; Cruz-Martinez et al., 2009), with soils of semiarid ecosystems being particularly insensitive to water stress due to historical selection of stress-tolerant microbes and fauna (Fierer et al., 2003).

Nematodes provided an exception to the general lack of rainfall pattern effect on soil biota abundance. Nematodes' negative response to increasing water stress has already been highlighted by other researchers (Kardol et al., 2010). Nematodes can survive extreme drought in anhydrobiosis (Treonis et al., 2000), but their populations may be reduced due to increasing difficulties to get access to their prey as soil water films become thinner, which is more likely to occur in coarse-textured soils than in fine-textured

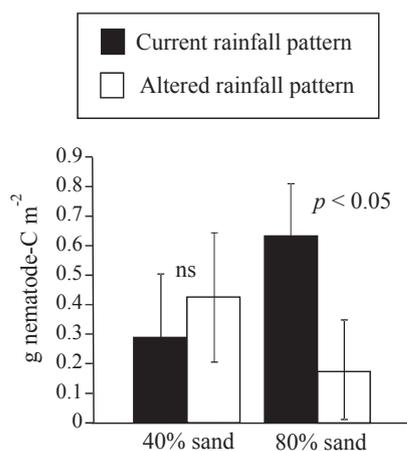


Fig. 2. Effect of rainfall patterns (current or altered) on nematode biomass-C depending on soil texture expressed as percent sand content. Vertical bars denote standard errors ($n = 6$ monoliths). Significant differences ($p < 0.05$) and no significant effects (ns) are shown.

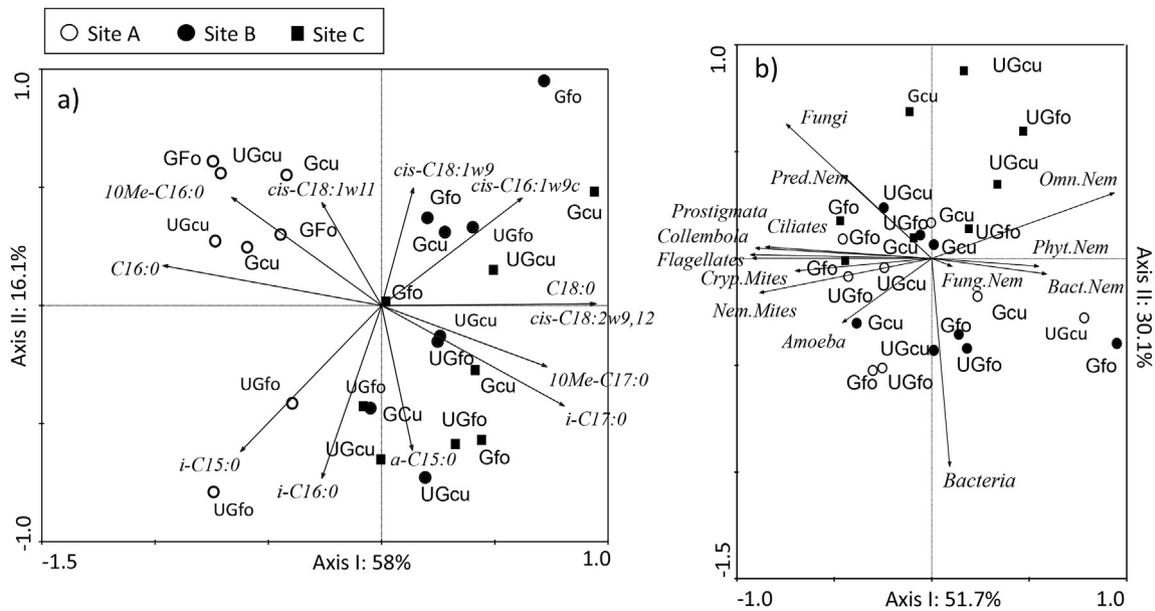


Fig. 3. PCA ordination of our 24 soil monoliths (two per experimental scenario) based (a) on mole percent PLFA-C of the PLFA microbial markers, and (b) on percent biomass-C of the functional groups of the soil biota. The percentage of variance explained by each principal component is shown next to the correspondent axis. G: grazed soils; UG: ungrazed soils; cu: current rainfall regime; fo: altered rainfall regime. Microbial PLFA markers and functional soil groups in italics.

Table 4

Basic parameters of the soil food webs for all possible combinations of rainfall pattern (current or altered), grazing management (grazed –G– or ungrazed –UG–) and origin of the soil monoliths (sites A, B or C). *Basal resources (SOM fast and slow pools and roots) are included in the counting.

	Site A				Site B				Site C			
	Current pattern		Altered pattern		Current pattern		Altered pattern		Current pattern		Altered pattern	
	G	UG										
Number of functional groups (**)	15	16	13	16	15	17	15	14	15	16	15	14
Number of links	23	24	21	24	23	33	19	17	23	32	19	22
Linkage density	1.53	1.50	1.62	1.50	1.53	1.94	1.27	1.21	1.53	2.00	1.27	1.57
Connectance	0.22	0.20	0.27	0.20	0.22	0.24	0.18	0.19	0.22	0.27	0.18	0.24

soils (Landesman et al., 2011).

In semiarid ecosystems, soil drying–rewetting cycle frequency regulates all aspects of carbon and nutrient turnover (Austin et al., 2004), and another hypothesis of this study was that rainfall concentration will slow down soil organic matter mineralization. Our results showed that rainfall concentration in fewer but larger rain events contributed to total soil C and N build up which, in the absence of changes in plant biomass in our experimental monoliths, may be attributable to a decline in mineralization. To go deeper in this issue, we simulated the potential ability of the soil food web to mineralize soil organic matter under the various experimental rainfall scenarios. Simulated mineralization decreased with decreasing number of rainfall events which is fully in line with the aforementioned experimental results, confirms our hypothesis and emphasizes the importance of taking into account the entire soil food web when modeling soil organic matter mineralization. Our results are in accordance with the finding of Knapp et al. (2002, 2008) that fewer and larger rain events slows mineralization, and are consistent with other experiments that have shown that repeated drying and rewetting increase soil microbial biomass and activity and stimulate mineralization (Xiang et al., 2008; Fierer and Schimel, 2002).

In our food web model, the metabolic parameters attributed to the soil biota were kept constant in all simulations, and microbial biomass was not affected by treatments. Therefore, reductions in organic matter mineralization following rainfall concentration

must be attributed to changes in the abundance of groups of the soil food web other than microbes. As noted above, nematodes were the only invertebrates whose abundance declined in our experimental monoliths following rainfall concentration, and their omnivorous and predaceous forms totally disappeared under the most severe water stress conditions. Therefore, nematodes are potentially responsible for the observed decrease in mineralization, which would be consistent with the present knowledge on the chief role this group plays in the nutrient cycle belowground (Ingham et al., 1985b; Ferris et al., 1998).

We had posited that rainfall concentration will modify the energetic architecture of the soil food web thus leading to reduced stability. Contrary to expectations, our model suggested that the opposite is true, and that increasing water stress reinforced soil food web stability. Concentrating total precipitation in fewer and larger events resulted in the removal of omnivorous or predatory nematodes, or both, with detrimental consequences for connectance and interaction strengths in the food web. In the treatment under current rainfall pattern, omnivorous nematodes channelized up to 93% of the energy flow from bacteria to top predators (Figs. S1–S6, Supporting Information), and their disappearance caused not only a decrease in connectance but also a decline in the proportion of strong links, which is thought to reinforce the stability of the food web (Garlaschelli et al., 2003; McCann et al., 1998). By contrast, trophic links between predaceous nematodes and their prey were weak (as they accounted for

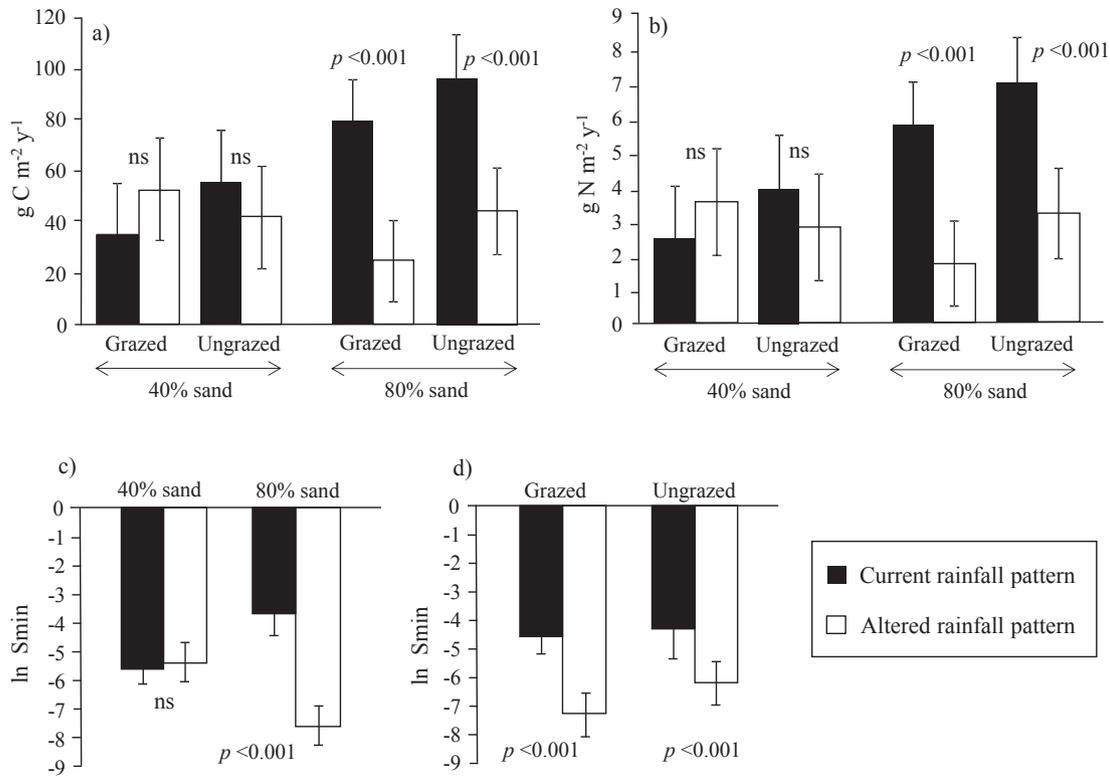


Fig. 4. Effect of rainfall patterns (current or altered) on (a) total C and (b) total N mineralized by the soil food web, depending on grazing management (grazed and ungrazed soils) and soil texture, as predicted by the energy flux model. Effect of rainfall patterns (current or altered) on soil food web stability depending on (c) soil texture and (d) grazing management (grazed or ungrazed). The greater S_{min} the lower the stability of the web. Soil texture is expressed as percent sand content. Vertical bars denote standard errors ($n = 10$ simulations).

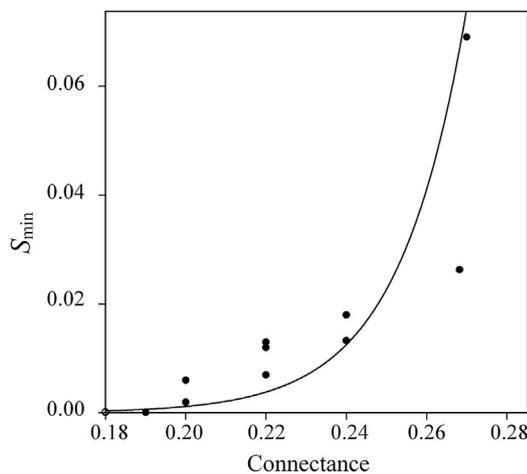


Fig. 5. Relationship between the estimator of food web stability S_{min} and the connectance (C) of the soil food web. S_{min} is inversely related to food web stability and, therefore, stability exponentially decreases with increasing connectance.

less than 1% of the total energy flow). Yet, predaceous nematodes constitute a strategic node of the web since they connect the bacterial and fungal detrital channels between them and with the herbivore channel. Because of the disappearance of the predaceous nematodes, the connection between all (fungal, bacterial and herbivore) energy channels became very weak or even disappeared, bringing the fungal channel to isolation. These findings add to the increasing recognition of the key role of nematodes for soil food web architecture and functioning (Ferris et al., 2001).

The debate about which properties underlie food web stability is long-standing (May, 1973; Pimm, 1984) with diversity and connectance at the center. In this work, food web stability negatively correlated with connectance and, more specifically, with unweighted connectance though some authors insist that connectance is not related with stability unless it is “weighted”, i.e. unless link weights (informing about the amount of energy channeled per link) are included in the calculation (Zhao et al., 2016).

As we had conjectured, effects of rainfall concentration on the soil food web were affected by soil texture, with decreasing capacity for mineralization and increasing food web stability occurring only in coarse-textured soils. We could not find other studies about effects of rainfall patterns on the stability of the soil food webs, but our results are in accordance with previous findings that mineralization is more responsive to water regime in coarse-textured soils than in fine-textured soils (Austin et al., 2004; Cable et al., 2008), at least in semiarid zones. Soil water retention capacity negatively correlates with the proportion of coarse particles (Gupta and Larson, 1979), and water drains significantly faster in sandy soils than in clayey soils. Therefore, under similar rainfall regimes, the inhabitants of sandy soils are likely to be exposed to longer and harsher periods of water stress than those inhabiting fine-textured soils. As mentioned before, the observed reinforcement of the stability of the soil food web following rainfall concentration may be attributable to negative effects of increasing water deficit on nematode populations. Nematodes are aquatic animals that require water films to get around and reach their prey. As water drains and water films on the soil surfaces become thinner, nematodes are increasingly restricted in their movement (Elliott et al., 1980), and starvation and population decay are likely to be the rule in sandy soils during dry periods between rain events.

Together with soil texture, we had expected grazing cessation to influence soil response to changes in rainfall patterns. To test this hypothesis, we had worked with soils from grazed field plots and with soils from plots that had been fenced against herbivores for 18 years. Unexpectedly, the grazed and ungrazed soils only differed by pH and compaction (that were lowest and highest, respectively, in the grazed soils) and were comparable in terms of C and N content, total biomass and relative abundance of soil biota. In semiarid grassland, soil compaction is a well-known consequence of trampling by livestock (McCalla et al., 1984), although grazing may or may not modify soil pH (Lopez-Sangil et al., 2011; Pei et al., 2008). Following grazing cessation, both acidity and compaction progressively recover, but differences between excluded and grazed soils may be observed for up to decades after exclusion (Jeddi and Chaieb, 2010). Based on previous works in semiarid nutrient-poor ecosystems (Sankaran and Augustine, 2004; Steffens et al., 2008), we would have expected total carbon and microbial biomass to be lower in the grazed than in the excluded soils, with bottom-up implications for the soil food webs. The absence of significant differences may be explained by very slow recovery of the pre-grazing soil condition. However, this explanation is not robust, since effects of grazing on soil microbial communities are short-lasting, and differences in microbial and invertebrate abundance between grazed and excluded soils can be observed as soon as two years after grazing cessation (Bardgett et al., 1993). A more plausible explanation may be that grazing had little effect on the soils of the shortgrass steppe. It has been suggested that the soil community of this ecosystem is unresponsive to grazing (Leetham and Milchunas, 1985; Milchunas et al., 2008) due to very long-lasting coevolution of the steppe with large herbivores. Nevertheless, and in accordance with our expectations, even being apparently negligible, grazing legacy influenced the response of our soils to changes in the precipitation pattern. On the one hand, benefits of rainfall concentration on total soil C and N content were only evident in the grazed soils and, on the other, the reduction in mineralization and the increase in the stability of the soil food web resulting from rainfall concentration were more apparent in the grazed soils than in soils long excluded from grazing. Our data suggest that, as previously stated by Belnap (1995), in arid and semiarid grasslands trampling by large herbivores may have important and long-lasting implications on soil food web dynamics.

5. Conclusions

Establishing effects of land management on the resilience of soil functions to climate change is among the main questions to solve for soil ecologists. The evidence from this study indicated that, in semiarid grasslands, soil food webs are sensitive to changes in rainfall patterns, and that the extent and direction of their response are strongly modulated by soil texture and, to a lesser extent, by grazing management. Total precipitation redistribution in fewer but larger rain events increases soil water stress (i.e. longer dry periods) and produces larger fluctuations of the soil water potential. Even if rainfall concentration has no effect on soil microbial biomass, the relative abundance of invertebrate groups that play key roles in the architecture of the soil food web may be affected and so will the energy flow through the decomposer and herbivore channels. The results from this study suggest that in semiarid environments, where soil water is limiting, lengthening the dry periods between rainfall events increases soil food web stability and reduces its ability to mineralize soil organic matter, thus contributing to carbon sequestration in soils. These conclusions ensue from modeling soil food web stability and mineralization ability based on results of experimental trials. Model ecosystems, however, are simplifications of the real world and are necessarily based

on assumptions that need to be updated in the light of new knowledge.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2017.06.022>.

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