



Changes in variability of soil moisture alter microbial community C and N resource use

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ARTICLE INFO

Article history:

Received 3 January 2011

Received in revised form

22 April 2011

Accepted 26 April 2011

Available online 13 May 2011

Keywords:

Grassland

Soil carbon

Soil nitrogen

Carbon use efficiency

Soil moisture variability

Precipitation regime

Climate change

ABSTRACT

Grassland ecosystems contain ~12% of global soil organic carbon (C) stocks and are located in regions where global climate change will likely alter the timing and size of precipitation events, increasing soil moisture variability. In response to increased soil moisture variability and other forms of stress, microorganisms can induce ecosystem-scale alterations in C and N cycling processes through alterations in their function. We explored the influence of physiological stress on microbial communities by manipulating moisture variability in soils from four grassland sites in the Great Plains, representing a precipitation gradient of 485–1003 mm y⁻¹. Keeping water totals constant, we manipulated the frequency and size of water additions and dry down periods in these soils by applying water in two different, two-week long wetting–drying cycles in a 72-day laboratory incubation. To assess the effects of the treatments on microbial community function, we measured C mineralization, N dynamics, extracellular enzyme activities (EEA) and a proxy for substrate use efficiency. In soils from all four sites undergoing a long interval (LI) treatment for which added water was applied once at the beginning of each two-week cycle, 1.4–2.0 times more C was mineralized compared to soils undergoing a short interval (SI) treatment, for which four wetting events were evenly distributed over each two-week cycle. A proxy for carbon use efficiency (CUE) suggests declines in this parameter with the greater soil moisture stress imposed in LI soils from all four different native soil moisture regimes. A decline in CUE in LI soils may have been related to an increased effort by microbes to obtain N-rich organic substrates for use as protection against osmotic shock, consistent with EEA data. These results contrast with similar *in situ* studies of response to increased soil moisture variability and may indicate divergent autotrophic vs. heterotrophic responses to increased moisture variability. Increases in microbial N demand and decreases in microbial CUE with increased moisture variability observed in this study, regardless of the soils' site of origin, imply that these systems may experience enhanced heterotrophic CO₂ release and declines in plant-available N with climate change. This has particularly important implications for C budgets in these grasslands when coupled with the declines in net primary productivity reported in other studies as a result of increases in precipitation variability across the region.

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1. Introduction

Global climate models predict altered precipitation regimes across North America in this century associated with increases in average surface temperatures, consistent with recent observations (Karl and Trenberth, 2003). Across the US Central Plains, these alterations include an increase in the number and severity of droughts and larger rainfall events between drought periods, with little to no change in annual totals (Easterling et al., 2000; Knapp

et al., 2008). In this region, where annual potential evapotranspiration (PET) exceeds annual precipitation totals (Lauenroth and Burke, 1995), soil moisture is a limiting factor controlling both net primary productivity (NPP) and microbial processing of large pools of soil organic matter (SOM), and hence controlling soil carbon (C) and nitrogen (N) cycling (Harper et al., 2005; McCulley et al., 2005).

Investigators exploring how precipitation pulses drive ecosystem C and N cycling have typically focused on deserts and semi-arid grasslands, where the pulse-driven nature of the system is most evident (Austin et al., 2004). However, climate models predict that both relatively xeric and mesic grasslands of North America will experience enhanced precipitation variability in the future (Easterling et al., 2000; Knapp et al., 2008). Understanding these

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interactions is of particular importance in the grasslands of North America, where soil organic matter (SOM) concentrations are among the highest of all ecosystems (Scurlock and Hall, 1998), and where soils have the potential to serve as either a large C source or sink with climate change (Scurlock and Hall, 1998; Knapp et al., 2008). These systems, because of their capacity to store C and their predicted future exposure to enhanced precipitation variability have received significant research attention in recent years (Knapp et al., 2002, 2008; Fay et al., 2003; Harper et al., 2005; Fay et al., 2008; Heisler-White et al., 2008). However, most of these studies focus on the influence of altered soil moisture variability on aboveground system productivity, leaving many questions about the fate of that productivity, once incorporated into soil profiles, unaddressed. For example, increased variability of rainfall events can lead to decreased total soil respiration (Harper et al., 2005), but the mechanism by which any declines in heterotrophic soil activity occurs remain unclear.

Multiple studies demonstrate the influence of soil moisture availability and its variability as a key driver of grassland soil biogeochemistry. Rates of soil respiration decrease with mean annual precipitation (MAP) in grasslands of the Great Plains (McCulley et al., 2005), and the frequency and size of precipitation events can cause ecosystem-scale alterations in C and N cycling processes (Knapp et al., 2002; Schimel et al., 2007). Precipitation events, particularly those following periods of drought, can create large flushes of nutrients and soil organic carbon (SOC) by releasing, through diffusion, drought accumulated SOM, inorganic N and microbial necromass (Austin et al., 2004; Schimel et al., 2007; Iovieno and Baath, 2008; Butterly et al., 2009). This flush of fresh substrate can be followed closely by large pulses of microbial respiration (Fierer and Schimel, 2002; Iovieno and Baath, 2008; Butterly et al., 2009). In addition, soil moisture fluctuations can also be responsible for significant variation in N uptake and release via microbial function (Fierer and Schimel, 2002; Austin et al., 2004; Schimel et al., 2007). Soil microorganisms combat drought in several ways, all of which require an energetic investment and thus a drain on C resources (Borken and Matzner, 2009; Schimel et al., 2007). One source of drought protection is the manufacture of a layer of polysaccharide-rich mucilage that prevents desiccation (Borken and Matzner, 2009). Microorganisms also protect themselves against large, negative soil matric and osmotic potentials through the acquisition of protective osmolytes, generally N-rich substrates such as amino acids (Borken and Matzner, 2009; Schimel et al., 2007). Microorganisms can release these osmolytes quickly when the soil is re-wetted to protect themselves against osmotic pressure and cell lysis. This uptake and release of N-rich resources can affect N cycling on an ecosystem scale (Schimel et al., 2007).

We explored the influence of soil moisture variability on belowground grassland C and N dynamics by conducting incubations of soils obtained from research sites across the precipitation gradient of North America's Central Plains. These soils are relatively C-rich and experience significant variability in moisture content

across the growing season that is linked to variation in respiratory C losses (McCulley et al., 2005). Precipitation patterns on the eastern, mesic end of this precipitation gradient have historically exhibited relatively small but frequent rain events, while on the western end of this gradient soil moisture variability is much greater, with longer drought intervals and more extreme rainfall events (Lauenroth and Burke, 1995). As a first step towards understanding how climate change-driven alterations in soil moisture variability will alter C and N dynamics in these systems, we exposed these soils to simulated moisture regimes during a laboratory incubation. Throughout the incubation, we measured CO₂ released. We also assessed the net N mineralized, microbial biomass, and extracellular enzyme activities (EEA) associated with the degradation of C-, N-, and P-rich organic compounds, and calculated multiple proxies for microbial carbon use efficiency (CUE). We hypothesized that as variability in soil moisture increases, microbial C and N resource transformations will reflect a shift from biomass development to protection against moisture stress, and that this will be detectable via proxies for substrate use efficiency. We further hypothesized that soil microbial communities from the eastern, moister end of the precipitation gradient would experience relatively greater resource demands and lower substrate use efficiency with higher soil moisture variability than microbial communities adapted to life on the western end of the gradient, where ambient variation in precipitation is high and soil moisture levels are typically low.

Though data obtained from *ex situ* soil must be interpreted with caution, laboratory incubations permit us to investigate heterotrophic respiratory responses without the confounding inclusion of plants, and permit environmental control difficult to attain in intact systems. We intended this study to highlight the most important potential mechanisms affecting soil C and N resource use, for further exploration *in situ*. We sought to elucidate the mechanisms by which any changes in belowground microbial transformations of SOM may occur in these systems with changes in moisture variability, and to highlight those measures of SOM transformations likely to serve as bellwethers of changes in the future.

2. Materials and methods

2.1. Sites

We chose four sites along the east–west precipitation gradient across Kansas, USA, part of the North American Great Plains (Table 1). The eastern most site, part of the Kansas University Field Station lands (KUFS, W 95°14'35" N 38°10'21") is in an area where the average annual precipitation is 1003 mm. The soils are gravelly silt loams (smectitic, thermic, Typic paleudolls) and vegetation is dominated by the tallgrass prairie species *Andropogon gerardii* and *Sorghastrum nutans*. Our second site, located at the Konza Prairie Long Term Ecological Research site (KNZ, W 96°33'18" N 39°5'22"), has an average

Table 1
Characteristics of the four grassland study sites located across an east–west precipitation gradient in Kansas, USA. Clay, silt, SOM, pH and bulk density are means ± standard error ($n = 3$).

Site	Kansas Field Station and Ecological Reserves	Konza Prairie LTER	K-State Western Kansas Agricultural Research Center	Nature Conservancy Smokey Valley Ranch
Abbreviation	KUFS	KNZ	HYS	SVR
Average annual precipitation	1003 mm	850 mm	579 mm	498 mm
Soil type	Typic paleudolls	Typic natrusolls	Cumulic haplustolls	Aridic haplustolls
% Clay	26.33 ± 0.66	27.63 ± 0.69	31.33 ± 0.84	24.98 ± 1.05
% Silt	61.93 ± 0.27	65.17 ± 1.18	58.38 ± 0.29	57.60 ± 0.87
% SOM	4.69 ± 0.22	5.86 ± 0.17	7.92 ± 0.55	3.13 ± 0.22
pH	5.99 ± 0.06	6.11 ± 0.11	6.59 ± 0.11	7.83 ± 0.06
Bulk density	1.30 ± 0.01	1.08 ± 0.06	1.07 ± 0.05	1.32 ± 0.03

annual precipitation of 835 mm and soils that are a mix of silty loams (smectitic, mesic, Typic natrusolls) and silty clay loams (fine, mixed, superactive, mesic, Pachic argiustolls). Dominant vegetation includes *A. gerardii*, *Andropogon scoparium*, *Panicum virgatum* and *S. nutans*. The third site, part of the Kansas State University's Western Kansas Agricultural Research Center (HYS, W 99°17'46" N 38°50'13"), receives an average of 578 mm of precipitation yearly and the soils are silt loams (fine-silty, mixed, superactive, mesic, Cumulic haplustolls). It is a mixed grass prairie that includes *A. gerardii*, *A. scoparium*, *Bouteloua gracilis*, *Hesperostipa comata*, *Bouteloua curtipendula* and *Pascopyrum smithii*. The westernmost site, The Nature Conservancy's Smokey Valley Ranch (SVR, W 100°58'55" N 38°51'50"), is a short grass prairie dominated by *B. gracilis* and *Buchloë dactyloides*. It receives an average of 485 mm of precipitation annually. The soils are silt loams (fine-silty, mixed, superactive, mesic, Aridic haplustolls). All sites, which are part of actively grazed rangeland, were fenced to exclude cattle and have been burned annually, with the exception of SVR, which is not burned.

2.2. Soil collection

Soils were collected from all four sites, from April 13 through April 15, 2008. We used 10 cm diameter by 10 cm beveled PVC to extract three soil cores from each site. The cores were placed in iced coolers and returned to the lab at the University of Kansas where they were stored at 4 °C until the start of the incubation. Soils were well-mixed and roots >2 mm in diameter were removed. A 15 g sub-sample from each core was placed in a 60 °C oven for 48 h to determine gravimetric soil moisture. This dried soil was then used to determine water holding capacity (WHC) by saturating 5 g dry soil placed in Whatman #4 filter paper fitted into funnels. These were covered to prevent evaporative loss and allowed to drain overnight (Fierer and Schimel, 2002). Before the start of the incubation, soils were conditioned for one week in an incubator set at 20 °C at field moisture, which was ~50% WHC for all soils.

2.3. Soil treatments

Soil from each of the three cores from each site was weighed into pre-weighed 5 cm diameter by 5 cm in length PVC cores fitted on the bottom with coarse filter paper (85 g dry weight). The cores were placed in 1 l Mason jars on top of a layer of glass beads to allow air circulation under the cores. The soils were separated into three treatment groups, with one sample from each core at each site per group so that there were a total of three replicates for each treatment for each site. The treatments consisted of a control, which was kept at 50% WHC throughout the incubation, and two different two-week long wetting–drying cycles that varied in both frequency and size of the water additions and dry down periods. Long interval (LI) soils were given enough water to bring them to 75% WHC, followed by dry down periods that were two weeks in duration. Short interval (SI) soils received water equal to one quarter of the LI treatment two times per week over each two-week cycle. Thus, for every two-week cycle, both treatments received the same total amount of water, differing only in the timing and size of the individual additions.

We conducted the experiment for a total of six, two-week cycles. Water was applied using a needle and syringe for even coverage. During the dry down periods, all soils were gently stirred regardless of treatment, at the same time, in order to turn them over and promote homogeneity of soil moisture throughout the PVC core. While we strove to keep aggregates intact during these soil turn-overs, we recognize that this may have influenced soil aggregation, C availability and the composition of the microbial communities, but we prioritized homogeneity of soil moisture within the soil cores. Because all soils were stirred regardless of water addition,

we assume that any effects due to stirring were equivalent among treatments. Control jars (maintained at 50% WHC) were covered with parafilm to allow gas exchange while preventing moisture loss during the incubation; all other treatment jars were left open to permit evaporation. All soils were weighed frequently to determine soil moisture content, and were maintained at 24 °C. Sub-samples of all soils were harvested on day 58, the beginning of the last two-week cycle, for analyses described below. At the end of the final cycle (day 72), all soils received one final wetting to bring them to ~75% WHC. This moistening event included the addition of leachate derived from leaf litter, described in detail below, to provide insight into the influence of variability of soil moisture regimes on microbial CUE.

2.4. Soil respiration

Soil respiration was measured four times per week for all samples, on the day prior to and 2 h after any water additions, regardless of whether or not the soils received water that day. On each sampling day we sampled headspace gas twice, once right after capping the jars and a second time after a period of 2–4 h. Gas samples were obtained through septa fitted into the jar lids via needle and syringe, and were analyzed for CO₂ concentration on a Varian gas chromatograph using a thermal conductivity detector (Varian, Walnut Creek, CA, USA). The difference between the two measures was used to calculate the rate of C respired. Total C respired over the course of the incubation was calculated by applying the average respiration rate between two sampling days to the time period between those two sampling events.

2.5. Extracellular enzyme activity

We analyzed the activity of ten extracellular enzymes in soils sampled 4 h after water addition on day 58 and on day 73, 25 h after leachate additions. We measured the activities of two cellulases and a hemi-cellulase (β -1,4-glucosidase (BG), cellobiohydrolase (CBH), β -1-4-xylosidase (BXYL)) as well as an enzyme responsible for the breakdown of starch, α -1-4-glucosidase (AG). We also measured a chitinase, β -1-4-N-acetylglucosaminidase (NAG), a peptidase, leucine amino peptidase (LAP), and the activity of phosphate-monoester phosphohydrolase (PHOS), which releases phosphates from organic matter. The activities of these enzymes were determined using corresponding substrates fluorescently labeled with methylumbelliferone (MUB) or methyl coumarin (MC) added to soil slurries in 96-well microplates as per Saiya-Cork et al. (2002). Soils from KUFS, KNZ and HYS, which were slightly acidic, were homogenized with 50 mM sodium acetate buffer at pH of 6.5, close to the pH_{H₂O} of these soils. Soils from SVR, which were slightly alkaline, were homogenized in sodium bicarbonate buffer at pH 7.8. Soils were incubated at 24 °C for ~18 h. Fluorescence was determined on a SpectraMax Gemini XS Fluorescence Platereader (Molecular Devices, Menlo Park, CA, USA) with 365 nm excitation and 460 nm emission filters.

We also measured the activities of peroxidase, phenol oxidase and urease using colorimetric assays set up in 96-well microplates (Saiya-Cork et al., 2002). Phenol oxidase and peroxidase activities were measured using the color change associated with the breakdown of the substrate 3,4-dihydroxy-L-phenylalanine (L-DOPA). Urease activity was assessed by measuring the ammonium accumulated through the incubation using the color change produced by the addition of ammonium cyanurate and ammonium salicylate. Plates were analyzed on a SpectraMax 340PC 384 Absorbance Platereader (Molecular Devices, Menlo Park, CA, USA) at 460 nm for phenol oxidase and peroxidase and 610 nm for urease.

We group these enzymes into four categories, each representing a critical feature of SOM breakdown. Labile C acquisition enzymes – BG, CBH, BXYL and AG – are responsible for the breakdown of C-rich

substrates that are relatively easy to access because of their polymeric, uniform structures. Relatively recalcitrant C acquisition enzymes – phenol oxidase and peroxidase – are associated with the breakdown of substrates with polymorphic structures such as lignin and humic acids. We group NAG, LAP, and urease as N acquisition enzymes, and consider PHOS, a phosphorus acquisition enzyme, separately.

2.6. Soil microbial biomass and extractable soil N

We estimated microbial biomass C and N (MBC and MBN) using the fumigation–extraction method (Brookes et al., 1985; Doyle et al., 2004). In addition to analyses conducted on pre-incubation soils from each site, soil sub-samples from each treatment and each site were removed 4–6 h after wetting on day 58 and 25 h after leachate addition at the end of the incubation. We immediately extracted 2.5 g of this soil with 12.5 ml of 0.5 M K₂SO₄ while another 2.5 g was directly exposed to chloroform for 24 h. After venting, these soils were also extracted with 12.5 ml of 0.5 M K₂SO₄. Fumigated and un-fumigated extracts were subjected to persulfate digestion (Doyle et al., 2004). We used a NaOH concentration of 0.50 M to increase the final digest pH, ensuring the retention of dissolved inorganic carbon (DIC) in solution until analysis. We quantified the concentration of DIC in the digested extracts using a diffusion block (Doyle et al., 2004) on a Lachat auto-analyzer. Nitrate and nitrite concentrations in the extracts were determined via cadmium reduction. Microbial biomass C and N were calculated as fumigated extractable organic C (EOC) or total dissolved N (TDN) minus un-fumigated EOC and TDN, divided by an efficiency factor of 0.45 (Jenkinson et al., 2004). Glycine and nicotinamide standards were included in each analysis to test for digestion efficiency. Extractable organic C concentrations were corrected when necessary using the above standards. We determined soil inorganic nitrogen by quantifying nitrate and nitrite concentrations in un-fumigated soil extracts on a Lachat auto-analyzer using cadmium reduction, and ammonium concentrations on a diffusion block (Willason and Johnson, 1986; Doyle et al., 2004). Extractable organic N (EON) was calculated by subtracting total inorganic N from TDN.

2.7. Carbon use efficiency

At the end of the incubation, we wanted to determine how exposure to soil moisture variability treatments influenced the efficiency with which microbial communities used an added C substrate. To this end, we added water amended with C substrates from leaf litter leachate (LLC), bringing all soils to 75% WHC regardless of their previous moisture regime (LI or SI). The LLC was obtained by soaking deciduous tree litter, predominantly sugar maple and sweet gum trees, in de-ionized water for 14 days then filtering the leachate through a Whatman #4 filter. The resulting LLC contained 3164 mg l⁻¹ dissolved organic C and <0.1 mg l⁻¹ dissolved organic N. We diluted this LLC solution so that final C additions totaled 0.72 mg LLC g⁻¹ soil with a negligible amount of N (<0.04 µg g⁻¹ soil). This amount of added C was enough to replace approximately one-half the total C respired during the previous 72 days with relatively labile, C-rich compounds and enhanced our ability to track changes with previous LI vs. SI treatment in respiration rates and MBC. We consider these LLC additions to more accurately represent features of the complex substrate landscape experienced by soil microorganisms than addition of individual C compounds such as glucose, as is often used in incubation experiments (Rousk and Baath, 2007; Ziegler and Billings, 2011). After LLC addition at the end of the incubation, soil respiration rates were measured at intervals of 0.5, 1.5, 2.5, 4.5, 6.5, 8.5, 12.5, and 24.5 h while the jars remained capped.

We used three different methods to estimate microbial CUE after LLC additions. The first method is based on the amount of LLC utilized by microorganisms with the assumption that all leachate-derived substrate used, less what is respired, is incorporated into biomass (Frey et al., 2001; Thiet et al., 2006). This LLC based CUE is calculated as

$$CUE_{LLC} = \frac{(\Delta LLC - \sum CO_2 - C)}{\Delta LLC},$$

where ΔLLC is the change in the concentration of extractable organic C and $\sum CO_2 - C$ is the cumulative amount of C lost through respiration. Here, we assume that extractable organic C in these soils is dominated by the added LLC and that microbial activity after LLC addition is fueled primarily by the C contained in the leachate solution. In another approach, it is assumed that the sum of the MBC measured and the cumulative C lost via respiration is equal to the amount of substrate utilized (Frey et al., 2001; Thiet et al., 2006). This CUE measure is based on the change in MBC and is calculated as

$$CUE_{MBC} = \frac{\Delta MBC}{(\Delta MBC + \sum CO_2 - C)},$$

where ΔMBC is the amount of microbial biomass produced. Finally, we calculated a ratio of the amount of MBC produced per amount LLC consumed,

$$CUE_{ratio} = \frac{\Delta MBC}{\Delta LLC}.$$

Because we cannot estimate gross rates of microbial production without concerns about microbial recycling, a direct measure of microbial efficiency is not feasible. Though the absolute values of these proxies may be biased depending on the method of calculation (Thiet et al., 2006), the relative differences between treatments permit comparison between soils exposed to our experimental treatments, and provide informative measures of C use by these soils' microbial communities.

2.8. Statistical analyses

The analysis of soil water content (SWC) data measured multiple times over the course of the incubation required the use of 2-way repeated measures ANOVA in SAS PROC MIXED (SAS Institute, Cary, SC., USA) to determine day, treatment and day × treatment effects. Because the sampling dates were un-evenly spaced, we used the spatial power law to model the covariance structure. We used Differences of Least Squares Means (LSM) for pairwise comparisons with a Tukey–Kramer *P*-value adjustment (Tukey, 1953; Kramer, 1956) to control the maximum, experiment wise error rate (Hayter, 1989). In order to compare data between soils from different sites along the precipitation gradient where texture, pH and (SOM) differ, we normalized all LI and SI treatment data by dividing by the control treatment values. We present all data, except SWC, from LI and SI treatments as percentages of the control treatment. We used a 2-way ANOVA in SAS PROC GLM to determine the effects of treatment, soil origin (site) and treatment × site on total C respired, respiration rates, soil moisture variability (coefficient of variation, CV), EEA, inorganic N, MBC, MBN and measures of CUE. When data were non-normally distributed, we rank ordered the data and performed the same 2-way ANOVA as above on the ranks. This was required for day 58 measures of PHOS, phenol oxidase, peroxidase, MBC, MBN and inorganic N, and day 73 measures of LAP, urease, PHOS, phenol oxidase, peroxidase and CUE_{MBC}. All effects are considered significant at *P* < 0.05 unless noted.

When we harvested sub-samples on day 58, the beginning of the last wetting and drying cycle, SWC was greater in LI soils

compared to SI. Though this permitted us to assess differences in microbial function with varying SWC, we were primarily interested in the influence of legacy effects of soil moisture variability on microbial functioning, not SWC *per se*. We address this issue by analyzing for and focusing much of our discussion on treatment effects on day 73, when SWC was equivalent among treatments, and thus any differences in measures of microbial community function on this sampling date reflect legacy effects of months of imposed differences in soil moisture variability.

3. Results

3.1. Soil moisture content and variability

Long interval and short interval soils received the same amount of water in total over the course of the incubation, but the variation in timing and magnitude of these water additions resulted in significant differences in the coefficient of variation (CV) in soil moisture between treatments, and in soil water content (SWC, Fig. 1). The CV in soil moisture across the incubation was approximately twice as great in LI compared to SI soils for all sites (KUFS, 76.0 ± 3.5 vs. 38.2 ± 0.7 ,

$P = 0.0005$; KNZ, 63.2 ± 3.4 vs. 32.9 ± 2.5 , $P = 0.002$; HYS, 71.0 ± 2.1 vs. 36.1 ± 2.0 , $P = 0.0003$; SVR, 76.9 ± 3.4 vs. 39.5 ± 0.7 , $P = 0.0004$). Treatment, day and the interaction between treatment and day had significant effects on SWC. Within the interaction, SWC was not significantly different between LI and SI treatments on a majority of the dates measured, with the exception of the HYS soils. The number of days in which SWC in LI was significantly greater than in SI treatments was similar to the number of days in which SWC was significantly lower in LI compared to SI treatments.

3.2. Respiration and microbial C use efficiency

Control soils, kept at a constant soil moisture level (50% WHC), released approximately 3–4 times more $\text{CO}_2\text{-C}$ than those undergoing wetting–drying treatments (Fig. 2). Total C mineralized in the LI soils was 1.4–2.0 times higher than soils undergoing the SI treatment. Normalizing respiration data by the control soils to permit comparison across sites, we observed that LI soils averaged across sites mineralized significantly more C ($39.0 \pm 2.7\%$ vs. $27.1 \pm 2.1\%$ of control soils; $P = 0.001$) and had higher respiration rates ($48.9 \pm 3.3\%$ vs. $29.9 \pm 1.8\%$ of control soils; $P < 0.0001$) than

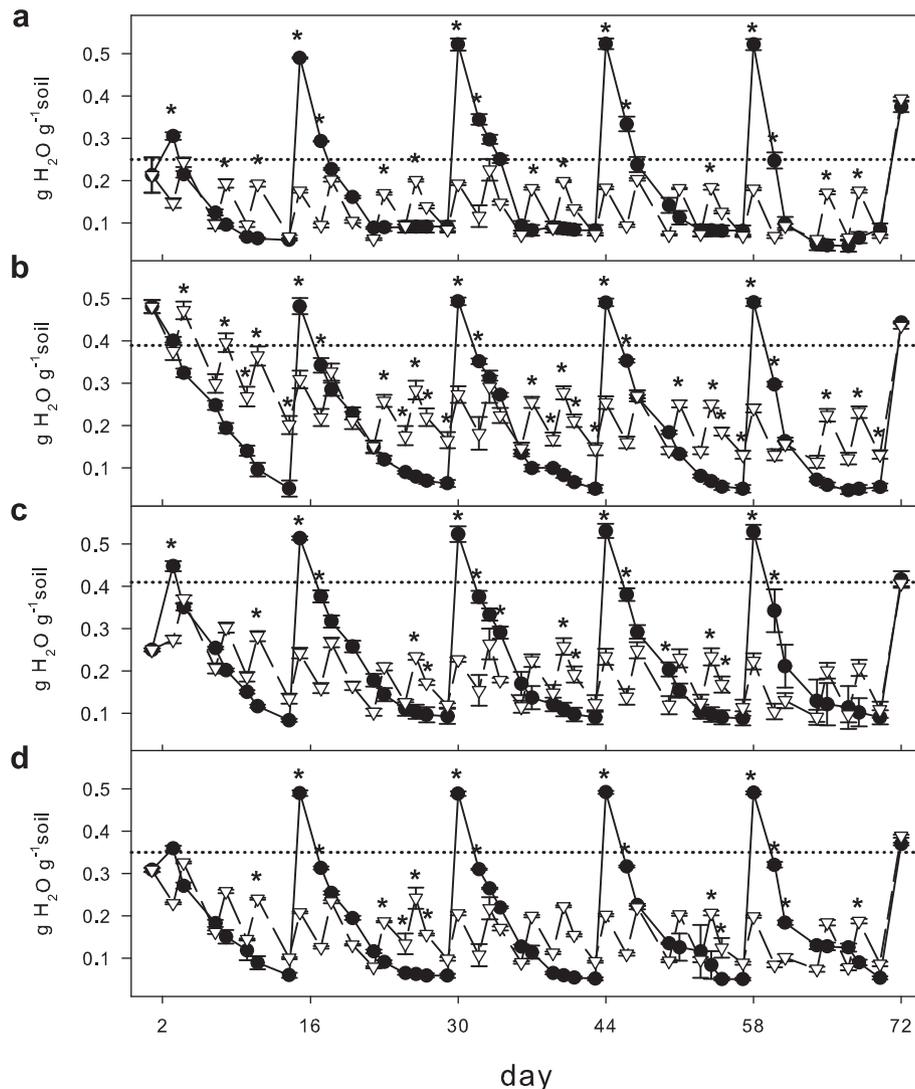


Fig. 1. Gravimetric soil water content throughout the incubation for LI (●) and SI (▽) treatments in soils from a) SVR, b) HYS, c) KNZ and d) KUFS. Horizontal, dashed line represents soil moisture level of control soils. Asterisks indicate significant treatment effects and error bars represent \pm SE of the mean.

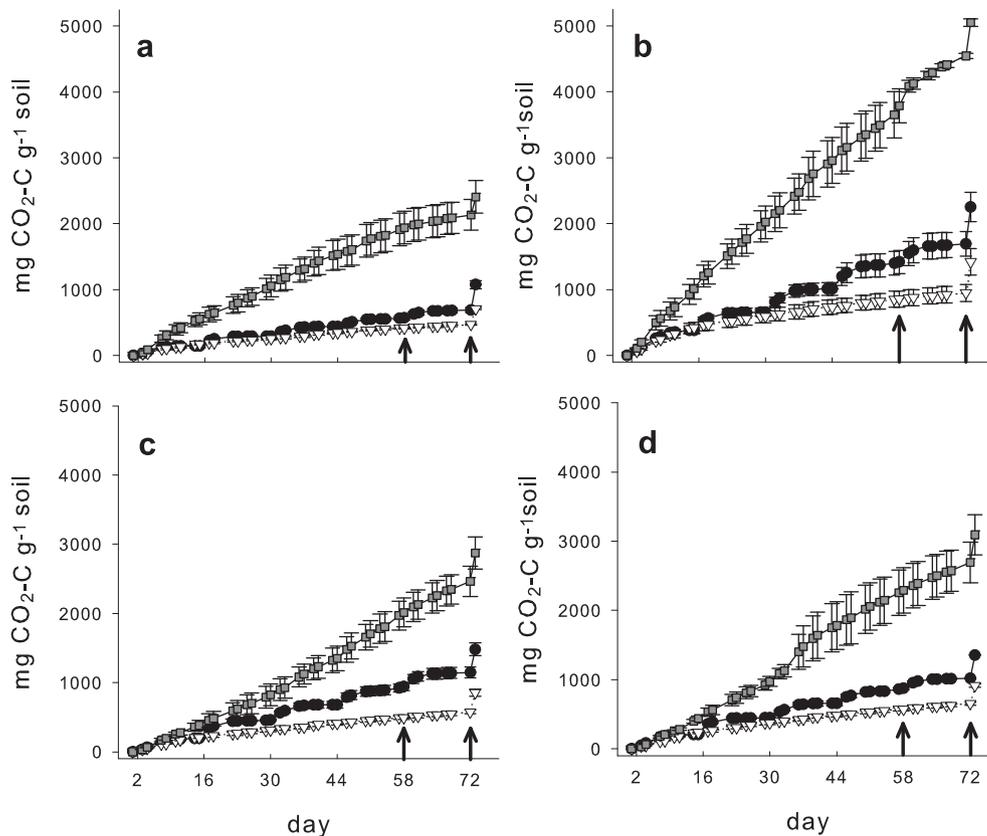


Fig. 2. Cumulative C respired over the entire 73 day incubation from control (■), LI (●) and SI (▽) soils from a) SVR, b) HYS, c) KNZ and d) KUFS. Arrows indicate soil analyses on day 58 and day 73 of the incubation. Error bars represent \pm SE of the mean.

microorganisms in SI soils through day 58. Soils from KUFS and KNZ mineralized more C and had higher respiration rates than soils from SVR ($P = 0.04$) through day 58.

Respiration rates in the LI and SI soils on day 72 were 1.2–4.6 times higher following the addition of LLC compared to the addition of water alone (Fig. 2). Total C mineralized between day 72 and 73 was equal to one-third to one-half of the total C mineralized in the previous 72 days, or 65–114% of the LLC added. LI soils had greater and SI soils had lower respiration rates and total C mineralized in the 24 h after LLC addition than control soils. Microorganisms in LI soils across sites mineralized significantly more C over the 24 h following LLC addition than in SI soils ($145.1 \pm 14.4\%$ vs. $96.3 \pm 9.4\%$), and LI soils had higher respiration rates than SI soils after LLC addition ($144.5 \pm 16.0\%$ vs. $94.8 \pm 9.9\%$), consistent with greater respiratory losses from LI soils throughout the incubation.

3.3. Extractable N and microbial biomass

By day 58, soils undergoing wetting–drying treatments had higher inorganic N availability relative to controls, with the exception of SVR LI soils (i.e. $>100\%$; Fig. 3a). In addition, by day 58, SI soils appeared to have accrued more inorganic N (NO_3^- plus NH_4^+) than LI soils ($P = 0.05$), and net N mineralization in SVR soils across treatments was lower than in soils from all other sites ($P = 0.05$; Fig. 3a). Similarly, on day 73, inorganic N availability was greater in SI than LI soils ($P = 0.0002$) and SVR soils had significantly less N relative to their controls when compared to HYS, KNZ and KUFS soils ($P = 0.002$; Fig. 3b). We were unable to detect EON in any of the soils sampled on either day 58 or day 73.

In all soils and treatments, absolute values of MBC ranged from 0.28 to 2.20 mg C g^{-1} soil while MBN ranged from 22.99 to

227.79 $\mu\text{g N g}^{-1}$ soil. Microbial biomass C on day 58 was not significantly different between treatments or sites (Fig. 3c). On day 73, after LLC additions, there were significant effects of treatment and site on MBC relative to control soils (Fig. 3d). SI soils had greater MBC than LI soils ($P = 0.05$), and MBC relative to control soils was higher in soils from SVR than in soils from HYS, which in turn was greater than MBC in soils from KNZ and KUFS ($P < 0.0001$; Fig. 3d).

On day 58, we found a significant effect of treatment on MBN with greater MBN in SI compared to LI soils, but there was also a significant treatment \times site interaction such that in HYS soils we saw the opposite effect, with greater MBN in LI than SI soils (Fig. 3e, $P = 0.03$). There were no differences in MBN between treatments or sites on day 73 (Fig. 3f).

3.4. Extracellular enzyme activities

Extracellular enzyme activities exhibited great variation between sites and with moisture treatment (Table 2). On day 58, EEA was generally greater in LI soils than in SI soils, as percent of controls (top panels, Figs. 4 and 5). As a percent of the controls, LI soils exhibited greater labile C acquisition EEA (BG, $P < 0.0001$; AG, $P = 0.0003$; CBH, $P < 0.0001$; BXYL, $P < 0.001$) relative to SI soils. In addition, LI soils also exhibited higher N acquisition (NAG, $P = 0.008$; LAP, $P < 0.0001$) and P acquisition (PHOS, $P < 0.0001$) enzyme activity compared to SI soils. Site of origin also governed EEA response to moisture treatment; activities of BG ($P < 0.0001$), CBH ($P < 0.0001$), BXYL ($P = 0.0001$), and LAP ($P = 0.02$) were higher in SVR LI and SI soils, relative to their controls, than in KUFS and KNZ soils relative to their control soils (top panels, Figs. 4 and 5). Recalcitrant C acquisition EEA (phenol oxidase and peroxidase) was not affected by site and the only detectable

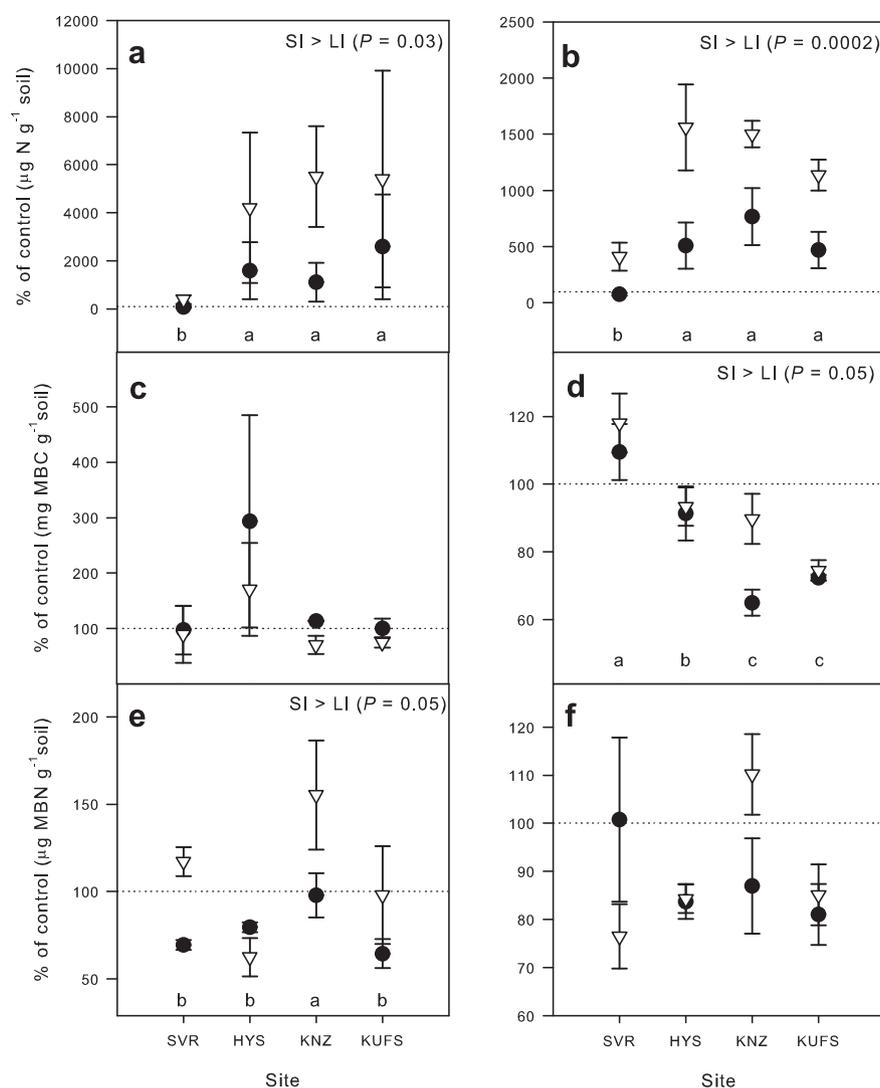


Fig. 3. Inorganic N availability in LI (●) and SI (▽) soils as a percentage of the control soils for day 58 (a) and day 73 (b), MBC in LI and SI soils as a percentage of the control soils for day 58 (c) and day 73 (d), and MBN in LI and SI soils as a percentage of the control soils for day 58 (e) and day 73 (f). Lower case letters indicate significant differences between sites. Dashed line is a reference for 0% difference between control and treatment soils and error bars represent \pm SE of the mean.

treatment effect was found in the HYS soils, in which peroxidase activity was higher in LI than SI soils ($P = 0.03$).

After LLC additions, when SWC was equivalent across treatments, labile C acquisition, NAG and PHOS activities in LI and SI soils were statistically equivalent (bottom panels in Figs. 4 and 5). LAP activity was greater in LI compared to SI soils ($P = 0.002$) and was affected by site such that activity in SVR soils was greater than that in KUFS soils, which was greater than activity in HYS and KNZ soils ($P < 0.0001$; Fig. 5f). PHOS, also affected by site, was greater in KUFS, KNZ and HYS soils than in SVR soils ($P = 0.0002$; Fig. 5h). Phenol oxidase activity was higher in the LI than the SI treatment for KUFS and KNZ soils ($P = 0.05$) and was undetectable in HYS and SVR soils for both treatments. Peroxidase activity did not vary between LI and SI treatments but was greater in SVR soils than in KUFS, KNZ and HYS soils (data not shown; $389.6 \pm 52.1\%$ vs. $0.28 \pm 0.07\%$; $P = 0.0003$).

3.5. Carbon use efficiency proxies

All three estimates of microbial CUE indicate that relative to control soils, SI soil microbial communities were generally more efficient at retaining C as biomass than those in LI soils, relative to C

lost as respiration (CUE_{LLC} , $P = 0.0004$; CUE_{MBC} , $P < 0.0001$; CUE_{ratio} , $P = 0.06$; Fig. 6). Differences by site depended on the method used to calculate CUE, but all proxies suggest that microbial communities in SVR soils had relatively high CUE. Calculation of CUE based on LLC accrual (CUE_{LLC}) indicates significant ($P = 0.01$) effects of site such that HYS soil communities show lower efficiency than SVR, KNZ and KUFS soil communities. There was a significant treatment \times site interaction for measures of CUE_{MBC} and within this interaction, SI soils had greater CUE than LI soils from all sites (SVR, $P = 0.09$; HYS, $P = 0.03$; KNZ, $P < 0.0001$; KUFS, $P = 0.08$; Fig. 6), while site differences were only evident in the LI soils. In the LI soils, CUE_{MBC} was greater in SVR soils compared to HYS and KNZ soils ($P = 0.05$; Fig. 6). CUE_{ratio} was marginally greater in SI than LI soils ($P = 0.06$) and was higher in SVR soils compared to KUFS soils ($P = 0.05$; Fig. 6).

4. Discussion

We sought to determine how the stress induced by soil moisture variability affects microbial C and N resource transformations. We hypothesized that increases in soil moisture variability would decrease substrate use efficiency. We found that regardless of the

Table 2
Minimum and maximum values of extracellular enzyme activities across all sites (SVR, HYS, KNZ, and KUFS) in control, long interval (LI), and short interval (SI) treatments (ng activity h⁻¹ g⁻¹ soil). See text for site and enzyme abbreviations.

Enzyme	Sample day	Control soils (ng activity g ⁻¹ soil h ⁻¹)		LI soils (ng activity g ⁻¹ soil h ⁻¹)		SI soils (ng activity g ⁻¹ soil h ⁻¹)	
		min	max	min	max	min	max
BG	58	88.05	300.08	126.53	333.53	88.75	274.56
	73	65.52	264.51	80.71	282.37	80.06	276.27
CBH	58	22.72	187.40	37.21	182.18	26.88	161.16
	73	14.06	157.12	20.31	177.41	18.19	141.13
BXYL	58	39.97	124.12	57.41	123.77	40.71	113.84
	73	22.33	88.93	24.46	100.62	29.61	89.54
AG	58	17.30	64.53	30.90	89.69	26.53	84.94
	73	11.86	54.12	22.47	74.09	19.20	57.05
NAG	58	22.07	226.14	23.38	187.40	16.10	140.24
	73	20.66	221.35	12.07	200.31	12.23	137.82
LAP	58	88.22	235.06	123.35	349.38	47.57	215.60
	73	78.13	180.05	56.11	214.50	42.44	255.68
Urease	58	353.91	1233.05	0	1802.52	0	1120.04
	73	124.33	2128.18	0	775.0	0	821.94
PHOS	58	189.76	355.69	219.36	440.24	151.73	309.12
	73	161.98	296.83	142.30	321.84	104.17	317.53
Phenol oxidase	58	0	25299.49	0	30608.80	0	49788.48
	73	0	67735.33	0	3497.40	0	1490.01
Peroxidase	58	171.61	84276.33	0	70477.52	0	81061.84
	73	0	42692.05	701.10	177870.67	1144.22	176348.20

native soil moisture regime, the activities of soil microbial communities were similarly influenced by increased soil moisture variability, with apparent decreases in CUE. In addition, we wanted to investigate how microbial communities, adapted to different *in situ* soil moisture regimes, would respond to similar patterns of soil moisture variability. We hypothesized that in order to cope with high soil moisture variability stress, we would see microbes shift their patterns of resource allocation, and that these shifts would be of higher magnitude in soils from the mesic end of the gradient than in soils from the western end of the precipitation gradient,

where microorganisms are adapted to higher soil moisture variability stress. We found patterns of C use in response to treatment that were similar across sites, but differences in N acquisition and use between sites.

4.1. Effects of soil moisture variability on microbial function

Respiration rates and the total amount of C mineralized after LLC additions varied with treatment, suggestive of changes in microbial community functioning induced by altered SWC variability.

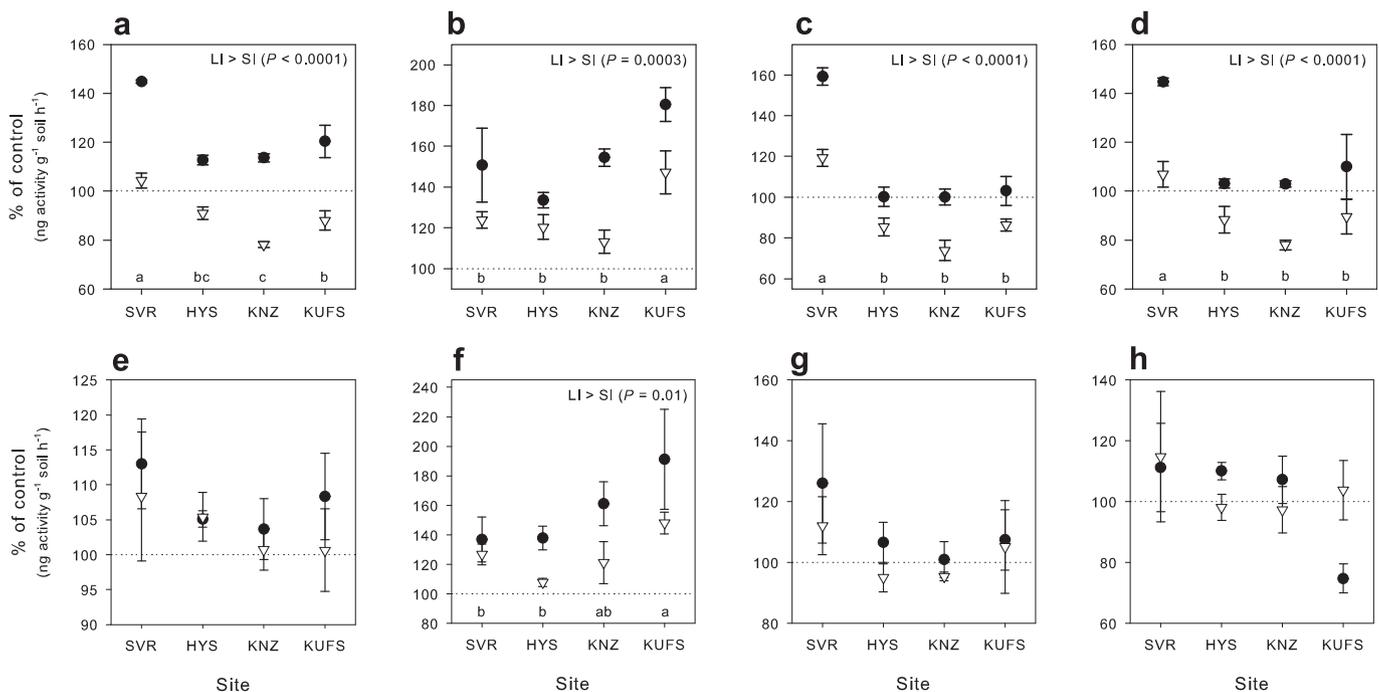


Fig. 4. Extracellular enzyme activities targeting labile C substrates for LI (●) and SI (▽) treatments as a percentage of the control soils from assays on day 58 a) BG, b) CBH, c) BXYL, and d) AG and on day 73 e) BG, f) CBH, g) BXYL and h) AG. Lower case letters indicate significant differences between sites. Dashed line is a reference for 0% difference between control and treatment soils error bars represent ± SE of the mean.

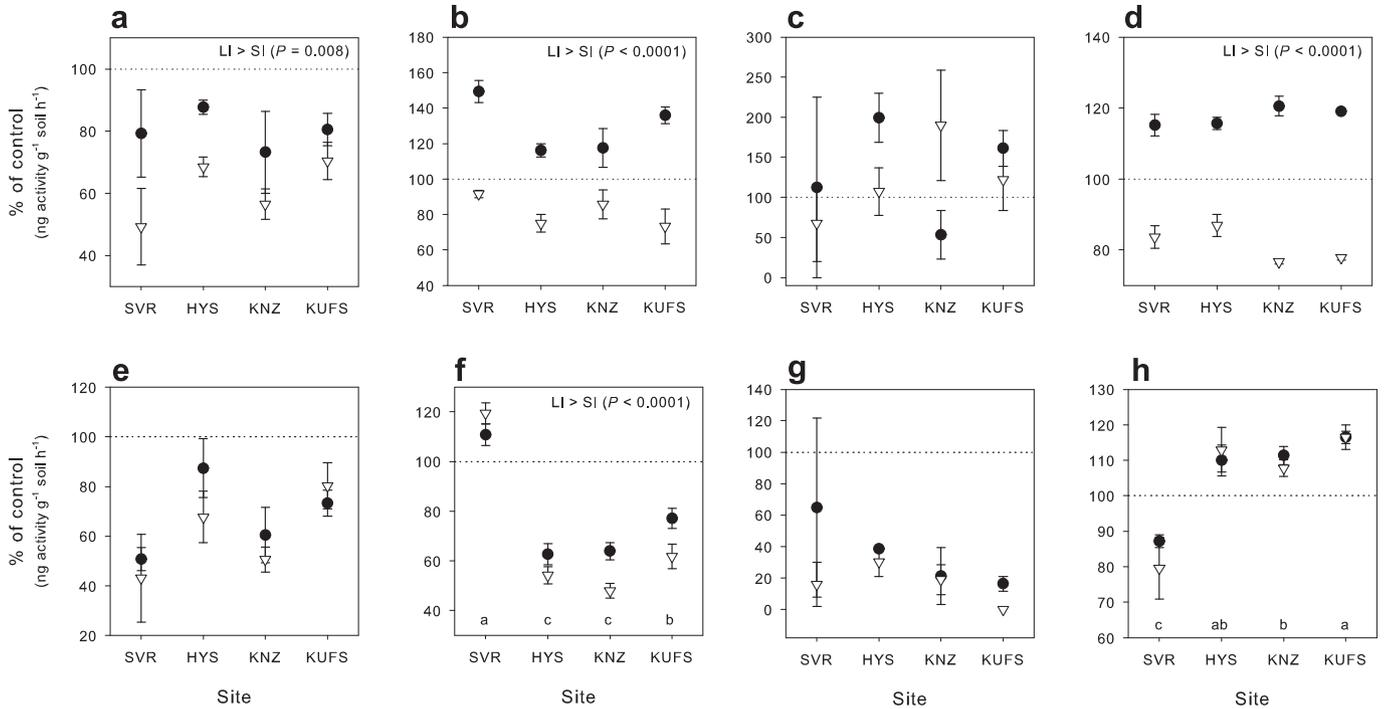


Fig. 5. Extracellular enzyme activities targeting N and P-rich substrates, for LI (●) and SI (▽) treatments as a percentage of the control soils from assays on day 58 a) NAG, b) LAP, c) urease, and d) PHOS and on day 73 e) NAG, f) LAP, g) urease and h) PHOS. Lower case letters indicate significant differences between sites. Dashed line is a reference for 0% difference between control and treatment soils and error bars represent ± SE of the mean.

Although we have no direct measure of the amount of LLC incorporated in biomass or mineralized, the relatively large differences in MBC accrual and respiration rates after LLC addition compared to the addition of water alone supports our assumption that the EOC in these soils was dominated by the added LLC and that microbial activity after LLC addition was fueled by the sudden availability of EOC, though some priming of indigenous SOC may have also occurred (Kuzakov, 2010). The greater release of CO₂ from LI soils, in concert with equivalent labile C acquisition enzyme activities in LI and SI soils at the end of the incubation, implies that increasing SWC variability can induce declines in microbial CUE. This is consistent with our calculated estimates of CUE. Lower CUE in LI soils may result from higher levels of physiological stress experienced by all LI microorganisms, regardless of their native precipitation regime. Both longer drought intervals and larger pulses of water such as those imposed by the LI treatment are associated with a higher potential for cell lysis due to osmotic pressure (Iovieno and Baath, 2008; Schimel et al., 2007; Fierer and Schimel, 2002). Microorganisms undergoing the LI treatment thus

may have devoted more of their C resources to survival mechanisms such as mucilage production, membrane transport proteins and protective osmolyte production and the respiratory costs associated with these functions rather than to biomass accrual (Borken and Matzner, 2009; Schimel et al., 2007; Fierer and Schimel, 2002).

Several observations of N cycling in these soils are consistent with microbial communities in LI soils, regardless of native precipitation regime, experiencing greater physiological stress. Soil N dynamics can be an important indicator of the physiological status of a microbial community because the protective osmolytes used by microorganisms to combat physiological stress tend to be N-rich organic compounds such as amino acids (Schimel et al., 2007). Both SI and LI soils from HYS, KNZ and KUFS accrued inorganic N relative to control soils (385–550%, Fig. 3a and b), which were kept at a constant soil moisture level over the course of the incubation. This suggests that N demand was greater with wetting and drying regardless of treatment. There were also differences in N dynamics between the treatments; microbes in SI soils appeared to have

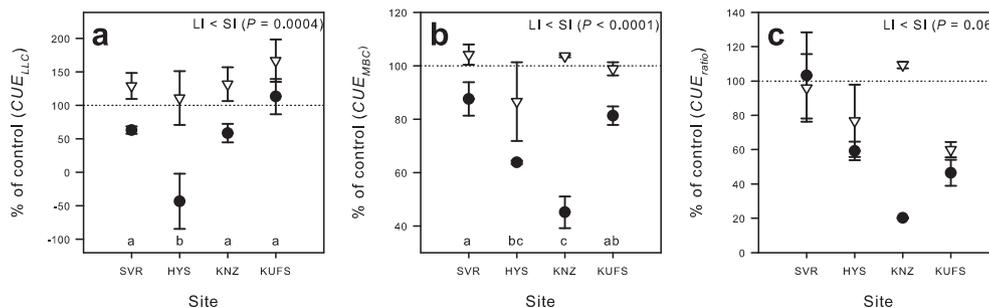


Fig. 6. Estimates of CUE based on a) LLC utilization, CUE_{LLC} b) MBC accrual, CUE_{MBC} and c) a ratio between the two, CUE_{ratio} in LI (●) and SI (▽) soils from all four study sites presented as a percentage of the efficiency in control soils. Dashed line is a reference for no difference between control and treatment soils and error bars represent ± SE of the mean.

greater net N mineralization than microbes in LI soils throughout the incubation as inorganic N levels were higher in SI soils than in LI soils on both day 58 and 78 (Fig. 3a and b). Further, when SWC was similar N acquisition enzyme activity (LAP) was greater in LI soils relative to SI soils (Fig. 5f), while PHOS activity was not different suggesting that LI microorganisms invested more resources in obtaining organic N in excess of basic nutrient requirements. This in turn is consistent with studies suggesting that N-rich osmolytes are an important means of protection from moisture stress for microbes (Borken and Matzner, 2009; Schimel et al., 2007). When soils were sub-sampled after water addition on day 58, we found no differences in MBC but greater MBN in SI than in LI soil microorganisms (with the exception of HYS soils). This is also consistent with the idea that with a larger pulse of water and thus higher osmotic potential stress, LI microorganisms released more organic N-rich osmolytes in order to prevent cell lysis. We were unable to detect a concurrent increase in EON that would support this, but it is unclear whether or not microorganisms release protective osmolytes to the extracellular environment or mineralize them (Williams and Xia, 2009; Fierer and Schimel, 2003; Halverson et al., 2000; Kempf and Bremer, 1998). The lower level of MBN right after wetting coupled with the higher N acquisition EEA in LI soils suggests that LI soil microbial communities may be less efficient in their use of N because of their use of N-rich osmolytes, while their SI counterparts appear to have more readily been able to perform net N mineralization as well as N immobilization.

Obtaining and then employing N-rich organic compounds to protect against the stresses imposed by high moisture variability also incur a C cost. Enhanced LAP activity in LI soils, for example, can only occur with a greater C investment in LAP production. In addition, transporting soluble N osmolytes such as amino acids to and from the cytoplasm incurs a respiratory cost of C that otherwise could be used for growth (Schimel et al., 2007; Iovieno and Baath, 2008). All else equal, the enhanced C costs and higher respiratory C losses that must accompany greater N acquisition efforts by a microbial community would impose a lower CUE on that community. These concepts are consistent with LI soils in the current experiment experiencing greater respiratory losses and exhibiting associated lower CUE as estimated by three different proxies.

4.2. Effects of soil origin on response to soil moisture variability

Soil microbial community structure varies with precipitation across the Great Plains of North America (McCulley and Burke, 2004). In spite of these apparent differences in community composition, we observed a convergence in microbial community response to water treatment, regardless of soil origin. We observed very few differences between sites in the direction of microbial response of respiration and enzymatic activity to LI and SI treatments. The only significant interactions between treatment and site were in MBN on day 58 and LAP activity after LLC additions. In all other analyses, treatment effects shared the same directionality, if not the same magnitude, between sites. In other stressful situations, such as heat shock, starvation or N limitation, bacteria appear to respond universally through global regulation of multiple operons and regulons (Kim and Gadd, 2008). Such a universal microbial response to shock or stress may have masked any inherent differences in community composition existing in soils in the current study.

Even though responses of microorganisms to varying levels of soils moisture stress seem to follow similar patterns between treatments, the magnitude of these responses varies, though not in the manner we hypothesized. Instead of finding a greater magnitude of response in soils from the mesic end of the gradient, differences were found between the westernmost site,

SVR, and all other sites. For example, we found that on day 58 inorganic N in SVR soils was more similar to controls than inorganic N in soils from other sites. In addition, we saw higher EEA (BG, CBH, BXYL, LAP) in SVR soils compared to all other sites, as well as relative to control soils averaged across treatments. This higher activity, relative to control soils in particular, suggests that SVR soil communities are extremely well adapted to highly variable and low levels of soil moisture. This level of adaptation allows them to carry out higher levels of these activities under more variable and limiting conditions than when presented with constant, non-limiting soil moisture conditions. If SVR soils are representative of other semi-arid grassland soils, it suggests that these soils may be better able to maintain function under higher levels of precipitation variability than soils native to more mesic areas.

Although our laboratory manipulations are difficult to compare to *in situ* studies, it is interesting to note that the convergence of microbial community responses to imposed changes in moisture variability observed in this study, regardless of native precipitation regimes, is not consistent with field studies (Knapp et al., 2002, 2008; Harper et al., 2005; Heisler-White et al., 2008). Rainfall manipulations in relatively xeric grasslands that simulate larger precipitation events with lengthened intervals between them report generally increasing aboveground NPP (Heisler-White et al., 2008), while at more mesic sites similar treatments can result in decreased aboveground NPP (Knapp et al., 2002, 2008; Harper et al., 2005; Heisler-White et al., 2008). Based on these studies, we anticipated that the precipitation regime at a soil's site of origin – xeric or mesic – would be a key determinant of microbial community response to changes in moisture variability. Instead, we found that soils from all sites mineralized significantly more C with LI treatment. These studies suggest that the importance of native precipitation regime as a driver of ecosystem C flux responses to altered rainfall timing in North American grasslands, and highlights the potential for divergent autotrophic vs. heterotrophic responses to increased moisture variability.

5. Conclusions

Increases in soil moisture variability impose increased physiological stress on soil microorganisms that can create an increase in N demand, concurrently lowering CUE and increasing C losses through respiration. This decrease in CUE regardless of the soils' native precipitation regime, particularly when coupled with decreases in NPP as variability in precipitation patterns increase across the region (Knapp et al., 2002, 2008; Heisler-White et al., 2008), may lead to increased C losses from these grassland systems. In this study, we found that an increase in soil moisture variability could increase heterotrophic C losses by up to 200% while also decreasing net N mineralization by up to 49%, a feature that ultimately would limit the N available for NPP if realized *in situ*.

Though several studies are consistent in their conclusions about productivity responses to altered rainfall timing, these studies do not explore heterotrophic responses in isolation (Knapp et al., 2002, 2008; Heisler-White et al., 2008). Plant communities tend not to respond as quickly to resource pulses as microbial communities (Austin et al., 2004) and this phenomenon can be especially prevalent after dry periods (Harper et al., 2005). The findings in the current study indicate that increased moisture variability enhances heterotrophic soil respiratory losses regardless of those soils' native precipitation regime. As such, our results point to divergent patterns in autotrophic vs. heterotrophic responses to increased variability in soil moisture regimes, and highlight the need for more belowground studies that explore the mechanisms driving these differences.

Acknowledgments

We thank D. Kettle, B. Johanning, and V. Salisbury of the University of Kansas Field Station, R. Manes and R. Martin of the Nature Conservancy, K. Harmony of the Kansas State University Western Agricultural Research Center, and J. Briggs and J. Blair of the Konza Prairie LTER for logistic support and access to field sites. We also thank R. Rastok and A. Reed for help in the field and the lab, and three anonymous reviewers whose comments helped improve this manuscript. We acknowledge funding from NSF EPS-0553722, the Kansas Technology Enterprise Corporation, and University of Kansas General Research Fund allocation #2301257. This represents University of Kansas Field Station publication number 907.

References

- Austin, A.T., Yahdjian, L., Stark, J.M., Belnap, J., Porporato, A., Norton, U., Ravetta, D.A., Schaeffer, S.M., 2004. Water pulses and biogeochemical cycles in arid and semiarid ecosystems. *Oecologia* 141, 221–235.
- Borken, W., Matzner, E., 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. *Global Change Biology* 15, 808–824.
- Brookes, P.C., Kragt, J.F., Powlson, D.S., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil-nitrogen – the effects of fumigation time and temperature. *Soil Biology & Biochemistry* 17, 831–835.
- Butterly, C.R., Bunemann, E.K., McNeil, A.M., Baldock, J.A., Marschner, P., 2009. Carbon pulses but not phosphorus pulses are related to decreases in microbial biomass during repeated drying and rewetting of soils. *Soil Biology & Biochemistry* 41, 1406–1416.
- Doyle, A., Weintraub, M.N., Schimel, J.P., 2004. Persulfate digestion and simultaneous colorimetric analysis of carbon and nitrogen in soil extracts. *Soil Science Society of America Journal* 68, 669–676.
- Easterling, D.R., Meehl, G.A., Parmesan, C., Changnon, S.A., Karl, T.R., Mearns, L.O., 2000. Climate extremes: observations, modeling, and impacts. *Science* 289, 2068–2074.
- Fay, P.A., Carlisle, J.D., Knapp, A.K., Blair, J.M., Collins, S.L., 2003. Productivity responses to altered rainfall patterns in a C-4-dominated grassland. *Oecologia* 137, 245–251.
- Fay, P.A., Kaufman, D.M., Nippert, J.B., Carlisle, J.D., Harper, C.W., 2008. Changes in grassland ecosystem function due to extreme rainfall events: implications for responses to climate change. *Global Change Biology* 14, 1600–1608.
- Fierer, N., Schimel, J.P., 2002. Effects of drying–rewetting frequency on soil carbon and nitrogen transformations. *Soil Biology & Biochemistry* 34, 777–787.
- Fierer, N., Schimel, J.P., 2003. A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. *Soil Science Society of America Journal* 67, 798–805.
- Frey, S.D., Gupta, V., Elliott, E.T., Paustian, K., 2001. Protozoan grazing affects estimates of carbon utilization efficiency of the soil microbial community. *Soil Biology & Biochemistry* 33, 1759–1768.
- Harper, C.W., Blair, J.M., Fay, P.A., Knapp, A.K., Carlisle, J.D., 2005. Increased rainfall variability and reduced rainfall amount decreases soil CO₂ flux in a grassland ecosystem. *Global Change Biology* 11, 322–334.
- Halverson, L.J., Jones, T.M., Firestone, M.K., 2000. Release of intracellular solutes by four soil bacteria exposed to dilution stress. *Soil Science Society of America Journal* 64, 1630–1637.
- Hayter, A.J., 1989. Pairwise comparisons of generally correlated means. *Journal of the American Statistical Association* 84, 208–213.
- Heisler-White, J.L., Knapp, A.K., Kelly, E.F., 2008. Increasing precipitation event size increases aboveground net primary productivity in a semi-arid grassland. *Oecologia* 158, 129–140.
- Iovieno, P., Baath, E., 2008. Effect of drying and rewetting on bacterial growth rates in soil. *FEMS Microbiology Ecology* 65, 400–407.
- Jenkinson, D.S., Brookes, P.C., Powlson, D.S., 2004. Measuring soil microbial biomass. *Soil Biology & Biochemistry* 36, 5–7.
- Karl, T.R., Trenberth, K.E., 2003. Modern global climate change. *Science* 302, 1719–1723.
- Kempf, B., Bremer, E., 1998. Uptake and synthesis of compatible solutes as microbial stress responses to high-osmolality environments. *Archives of Microbiology* 170, 319–330.
- Kim, B.H., Gadd, G.M., 2008. *Bacterial Physiology and Metabolism*. Cambridge University Press, Cambridge.
- Knapp, A.K., Fay, P.A., Blair, J.M., Collins, S.L., Smith, M.D., Carlisle, J.D., Harper, C.W., Danner, B.T., Lett, M.S., McCarron, J.K., 2002. Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. *Science* 298, 2202–2205.
- Knapp, A.K., Beier, C., Briske, D.D., Classen, A.T., Luo, Y., Reichstein, M., Smith, M.D., Smith, S.D., Bell, J.E., Fay, P.A., Heisler, J.L., Leavitt, S.W., Sherry, R., Smith, B., Weng, E., 2008. Consequences of more extreme precipitation regimes for terrestrial ecosystems. *Bioscience* 58, 811–821.
- Kramer, C.Y., 1956. Extension of multiple range tests to group means with unequal numbers of replications. *Biometrics* 12, 307–310.
- Kuzayakov, Y., 2010. Priming effects: interactions between living and dead organic matter. *Soil Biology and Biochemistry* 42, 1363–1371.
- Lauenroth, W.K., Burke, I.C., 1995. Great Plains, climate variability. In: *Encyclopedia of Environmental Biology*. Academic Press, New York, NY, pp. 237–249.
- McCulley, R.L., Burke, I.C., 2004. Microbial community composition across the Great Plains: landscape versus regional variability. *Soil Science Society of America Journal* 68, 106–115.
- McCulley, R.L., Burke, I.C., Nelson, J.A., Lauenroth, W.K., Knapp, A.K., Kelly, E.F., 2005. Regional patterns in carbon cycling across the Great Plains of North America. *Ecosystems* 8, 106–121.
- Rousk, J., Baath, E., 2007. Fungal and bacterial growth in soil with plant materials of different C/N ratios. *FEMS Microbiology Ecology* 62, 258–267.
- Saiya-Cork, K.R., Sinsabaugh, R.L., Zak, D.R., 2002. The effects of long-term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. *Soil Biology & Biochemistry* 34, 1309–1315.
- Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function. *Ecology* 88, 1386–1394.
- Scurlock, J.M.O., Hall, D.O., 1998. The global carbon sink: a grassland perspective. *Global Change Biology* 4, 229–233.
- Thiet, R.K., Frey, S.D., Six, J., 2006. Do growth yield efficiencies differ between soil microbial communities differing in fungal: bacterial ratios? Reality check and methodological issues. *Soil Biology & Biochemistry* 38, 837–844.
- Tukey, J., 1953. Multiple comparisons. *Journal of the American Statistical Association* 48, 624–625.
- Willason, S.W., Johnson, K.S., 1986. A rapid, highly sensitive technique for the determination of ammonia in seawater. *Marine Biology* 91, 285–290.
- Williams, M.A., Xia, K., 2009. Characterization of the water soluble soil organic pool following the rewetting of a dry soil in a drought-prone tallgrass prairie. *Soil Biology and Biochemistry* 41, 21–28.
- Ziegler, S., Billings, S.A., 2011. Interactive effects of elevated CO₂ and nitrogen fertilization on substrate carbon flow through forest soil microbial communities. *Journal of Geophysical Research – Biogeosciences* 116, G01011, 14.