Ecology, 94(10), 2013, pp. 2334–2345

Altered precipitation regime affects the function and composition of soil microbial communities on multiple time scales

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Abstract. Climate change models predict that future precipitation patterns will entail lower-frequency but larger rainfall events, increasing the duration of dry soil conditions. Resulting shifts in microbial C cycling activity could affect soil C storage. Further, microbial response to rainfall events may be constrained by the physiological or nutrient limitation stress of extended drought periods; thus seasonal or multiannual precipitation regimes may influence microbial activity following soil wet-up. We quantified rainfall-driven dynamics of microbial processes that affect soil C loss and retention, and microbial community composition, in soils from a long-term (14-year) field experiment contrasting "Ambient" and "Altered" (extended intervals between rainfalls) precipitation regimes. We collected soil before, the day following, and five days following 2.5-cm rainfall events during both moist and dry periods (June and September 2011; soil water potential = −0.01 and −0.83 MPa, respectively), and measured microbial respiration, microbial biomass, organic matter decomposition potential (extracellular enzyme activities), and microbial community composition (phospholipid fatty acids). The equivalent rainfall events caused equivalent microbial respiration responses in both treatments. In contrast, microbial biomass was higher and increased after rainfall in the Altered treatment soils only, thus microbial C use efficiency (CUE) was higher in Altered than Ambient treatments ($0.70 \pm 0.03 > 0.46 \pm 0.10$). CUE was also higher in dry (September) soils. C-acquiring enzyme activities (β-glucosidase, cellobiohydrolase, and phenol oxidase) increased after rainfall in moist (June), but not dry (September) soils. Both microbial biomass C:N ratios and fungal : bacterial ratios were higher at lower soil water contents, suggesting a functional and/or population-level shift in the microbiota at low soil water contents, and microbial community composition also differed following wet-up and between seasons and treatments. Overall, microbial activity may directly (C respiration) and indirectly (enzyme potential) reduce soil organic matter pools less in drier soils, and soil C sequestration potential (CUE) may be higher in soils with a history of extended dry periods between rainfall events. The implications include that soil C loss may be reduced or compensated for via different mechanisms at varying time scales, and that microbial taxa with better stress tolerance or growth efficiency may be associated with these functional shifts.

Key words: microbial activity; microbial C use efficiency; microbial ecology; precipitation timing; soil biomass; soil C storage; soil respiration; soil water.

Introduction

Climate change is predicted to alter precipitation patterns globally, which may have major effects on the carbon (C) balance of grassland ecosystems (Knapp et al. 2002, Weltzin et al. 2003). Across the North American Great Plains, this altered precipitation timing is expected to be characterized by fewer, larger rainfall events with longer dry periods between storms, in effect causing a more droughty and more variable precipitation regime (IPCC 2007, Knapp et al. 2008). More broadly, both precipitation intensity and the frequency of dry days are projected to increase across the majority of Earth's terrestrial surface in coming decades (IPCC 2007). Even with no change in total annual precipitation, this altered hydrological regime can decrease plant photosynthetic rates and integrated aboveground productivity by increasing the duration of dry, physiologically stressful soil conditions (Fay et al. 2002, Knapp et al. 2002, Porporato et al. 2004).

Belowground C dynamics also respond to altered precipitation patterns. Soil wet-up is well known to stimulate soil respiration (Birch 1958), and soil microbial respiration is correlated with water content at

Manuscript received 19 November 2012; revised 21 February 2013; accepted 10 April 2013. Corresponding Editor: H. A. L. Henry.

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moderate moisture levels (Linn and Doran 1984) and when averaged across many soil types (Manzoni et al. 2012*a*). In a field experiment that imposed a more variable precipitation regime on an intact prairie (with no difference in total precipitation), average soil CO_2 efflux was lower, which was linked to the lower average soil water contents caused by the increased duration of dry-soil conditions (Harper et al. 2005, Fay et al. 2011). However, individual soil wetting events can cause higher respiration rates immediately following soil wetting when the between-event interval is longer (Fierer and Schimel 2002, Miller et al. 2005, Sponseller 2007). In other words, average rates and relationships may mask important event-driven dynamics that have larger than average effects on belowground C dynamics (Jentsch et al. 2007).

Although many studies measure net soil C loss (respiration) in response to changes in soil water, belowground C pools are also affected by plant litter and root exudate inputs, microbial growth and death, and organic matter decomposition (Schlesinger 1997). Microbial growth efficiency may vary independently of respiration under drying stress, such that a similar amount of C assimilation can occur with decreased microbial respiration rates at low water potential (Herron et al. 2009). Also, microbially produced enzymes in soil solution drive the decomposition of soil organic matter (SOM) and subsequent respiration or assimilation of C (Schimel and Weintraub 2003), and incorporating this mechanism into models of C dynamics during dry–wet cycles in soils improves their accuracy (Lawrence et al. 2009). To fully understand the mechanistic responses of belowground C dynamics to altered precipitation, microbial activity parameters beyond respiration must be considered.

A precipitation regime with higher variability might affect soil microbial C dynamics via direct correlations between soil water and microbial activity, or indirectly via the action of differential functional responses under contrasting conditions and/or shifts in microbial community structure. For instance, if there are physiological trade-offs between C assimilation and respiration under drought and re-wetting stress (Schimel et al. 2007), or taxa with different physiological traits are active under certain moisture conditions and not others (Aanderud and Lennon 2011), then soil water content and microbial activity may not be linearly related. Also, differential microbial response to soil water under different historical precipitation regimes might reflect a different community composition (Fierer et al. 2003). The study goal was to understand whether microbial C dynamics respond predictably to changes in soil water on daily, monthly, or multiannual time scales. Soils were sampled from a native tallgrass prairie ecosystem before and after rainfall events that occurred during periods of moderately high (June 2011) and low (September 2011, during a drought spell) soil water content. Within this framework, soils with a long-term history of normal precipitation timing ("Ambient" treatment) or experimentally extended intervals between rainfall events ("Altered" treatment) were sampled. Results revealed that microbial respiration, growth, and decomposition potential were affected by changes in soil water, all implying decreased C loss from drier soils but with different mechanisms in play at the rainfall event, seasonal drought, and long-term precipitation regime time scales.

Materials and Methods

Site description and experimental approach

The study was conducted in native tallgrass prairie located at the Konza Prairie Biological Station (39°05′ N, 96°35′ W), a Long-Term Ecological Research site in the Flint Hills of eastern Kansas, USA. The native grasses big bluestem (*Andropogon gerardii* Vitman), indiangrass (*Sorghastrum nutans* (L.) Nash.), little bluestem (*Schizachyrium scoparium* (Michx.) Nash.), switchgrass (*Panicum virgatum* L.), and forbs such as Canada goldenrod (*Solidago canadensis* L.) dominate the vegetation. The January mean temperature is −3°C (range −9° to 3°C) and the July mean is 27°C (range 20° to 33°C). Annual average precipitation totals 835 cm, 75% of which falls during the growing season (May–September) in rain shower or storm events. The landscape is characterized by shallow soils overlaying chert-bearing limestones and shales (Ransom et al. 1998), divided into upland plateaus with shallow soils, slopes with outcrops of limestone, and lowlands with deeper alluvial and colluvial soils. The study soils are lowland Irwin silty clay loams (fine, mixed mesic Pachic Argiustolls, USDA NRCS), and have a pH of 6.6 ± 0.05 (mean ± SE) in 1:2 soil : deionized water.

A long-term precipitation modification experiment, the Rainfall Manipulation Plots (RaMPs), was established in 1997 and has been operational through every growing season since 1998. Twelve 14 × 9 m rainout shelters were built in three blocks of four shelters each over intact native prairie grassland. At each shelter, rainfall is excluded by a clear polyethylene roof, collected into two, 4-m^3 reservoirs that can store up to 10 cm of rainwater, then redistributed (at 2.5 cm/h) by an overhead irrigation system with 13 sprinkler heads to ensure uniform application. This design enables complete experimental control of the quantity, timing, and integrated variability in rainfall inputs (Fay et al. 2002). On control plots, rainfall is collected and redistributed immediately to follow ambient precipitation patterns ("Ambient" precipitation regime), while on extended-interval plots, the period between rain events is extended 50% over the ambient interval by collecting and holding all rainfall that occurs during that period, then reapplying all precipitation at once ("Altered" experimental precipitation regime). Thus, the cumulative growing-season rainfall amount is the same in both Ambient and Altered treatments, but moisture conditions in Altered soils are more variable. The median

rainfall event size is 15 mm in the Ambient treatment and 40 mm in the Altered treatment, and the median dry period is 3 days in the Ambient treatment and 20 days in the Altered treatment (Fay et al. 2011). Each plot ($n = 6$ per treatment) includes a 6×6 m sampling area surrounded by a 0.8-m buffer, and is isolated from surrounding soil by a 1.2 m deep subsurface barrier that limits lateral water movement.

In June and September 2011, 2.5 cm of accumulated natural rainwater was applied simultaneously to both Ambient and Altered treatment plots to simulate equivalent storm events on grasslands with control and extended-interval precipitation regime histories. In both treatments, the most recent preceding rainfall was approximately five days prior in June, and two weeks prior in September. We sampled soil during the afternoon preceding ("Pre" sampling) each rainfall event (rainwater was applied in the evening), the next day ("Pulse" sampling, allowing time for thorough infiltration through the top 20 cm of mineral soil), and five days following each rainfall event ("Post" sampling) after the soil water content had dried down. At each sampling plot, three 5.6 cm diameter cores of mineral soil were collected to 15 cm depth. These cores were combined to make a composite sample, and the soil was sieved (4 mm) and root material was removed before subsampling for various analyses. Soils were transported on wet ice to the laboratory, and one subsample was stored at 4°C for <2 days before soil water and microbial respiration analysis, while other subsamples were frozen immediately and stored at −20°C for all other analyses.

Soil water content and water potential

Soil water content was measured gravimetrically after drying overnight at 105°C. Gravimetric water content values were transformed to volumetric water content (VWC) by multiplying by bulk density (using BD = 1.2, as estimated by Blecker et al. (2006)). Water potential was calculated using a water release curve with parameters estimated using the RETC program (van Genuchten 1980, van Genuchten et al. 1991), $\alpha = 0.036$, $N = 1.368$, $\Theta_s = 0.496$, $M = 1 - 1/N$; and using $\Theta_r = 0.089$ for a typical silty clay loam soil (Carsel and Parrish 1988). The empirical values of water potential used for RETC program input were measured using a WP4 dewpoint potentiometer ($n = 5$, 5–25% VWC in increments of 5%) and a pressure chamber ($n = 5$, 0.4–0.5 MPa, ~35–40% VWC).

Microbial respiration

Fresh, root-free soils were used to measure microbial respiration rates. Approximately 10 g of field-moist soil was incubated at room temperature for three hours in a sealed serum bottle, and CO_2-C concentration in the headspace was measured 0, 1, 2 and 3 hours after the soil was added. Microbial respiration rate was calculated from the linear increase in CO_2-C over this time period as micrograms of C per gram dry soil per hour. CO_2-C concentration was quantified using a gas chromatograph (Model GC 8A; Shimadzu, Kyoto, Japan) equipped with a thermal conductivity detector and stainless steel Porapak column (2 m length, 5 mm outside diameter, 4 mm inside diameter).

DOC and TN, microbial biomass C and N, ammonium and nitrate, total C

Microbial biomass C (MBC) and N (MBN) were estimated as the extractable dissolved organic C (DOC) and total N (TN) liberated after a 24-hour chloroform fumigation (Brookes et al. 1985, Vance et al. 1987) of the field-moist soil. DOC and DON were extracted from fumigated and unfumigated soils by shaking in a 0.05 mol/L K_2SO_4 solution (~10 g soil in 40 mL extractant) for one hour, and quantified via combustion/chromatography analysis with a Shimadzu total C and N analyzer (Shimadzu Scientific Instruments, Columbia, Maryland, USA). Exchangeable DOC and TN of the unfumigated soils are reported as stand-alone variables, and the excess DOC or TN in fumigated soils was assumed to reflect MBC and MBN pool sizes. Final MBC and MBN values were calculated using the extraction efficiency coefficients $k_{EC} = 0.45$ and $k_{EN} = 0.56$ (Brookes et al. 1985, Vance et al. 1987). Also, an index of microbial C use efficiency (CUE) for the 24-hour period following each rainfall event was estimated for each replicate soil sample. Growth was estimated as the difference in MBC between "Pulse" and "Pre" sampling points (Δ micrograms MBC), cumulative respiration was calculated from the "Pulse" respiration rate (micrograms CO_2-C respired in 24 hours), and CUE was calculated as (Δ micrograms MBC/(Δ micrograms MBC + micrograms CO_2-C respired in 24 hours)). From the same K_2SO_4 extracts as DOC and TN, ammonium-N concentration was quantified using a modified indophenol method, and nitrate-N concentration was quantified using the VCl_3/Greiss method, using microplate spectrophotometry (Hood-Nowotny et al. 2010). Total soil C was measured on June pre-rainfall soils only using an isotope ratio mass spectrometer at the Oregon State University Stable Isotope Research Unit (Corvallis, Oregon, USA).

Extracellular enzyme activities (EEA)

Hydrolytic enzyme potential activities were measured using fluorometric substrates (methylumbelliferone [MUB] and methylcoumarin) and oxidative enzyme potential activities were measured using a colorimetric substrate (L-DOPA) in 96-well (6–8 technical replicates) plate assays (Saiya-Cork et al. 2002, Zeglin et al. 2007). Hydrolytic enzyme assays included phosphatase (Phos; EC 3.1.3.1, 4-MUB-phosphate), leucyl aminopeptidase (LAP; EC 3.4.11.1, L-leucine-7-amido-4-methylcoumarin), cellobiohydrolase (CBH; EC 3.2.1.91, 4-MUB-β-*D*-cellobioside), β-glucosidase (βG; EC 3.2.1.21, 4-MUB-β-*D*-glucoside), and β-*N*-acetylglucosaminidase (NAG; EC 3.2.1.14, 4-MUB-*N*-acetyl-β-*D*-glucosaminide), and

were run at a final substrate concentration of 40 μmol/L. Oxidative enzyme assays included peroxidase (Perox; EC 1.11.1.7, L-3,4-dihydroxyphenylalanine and H_2O_2) and phenol oxidase (Phenox; EC 1.10.3.2, L-3,4-dihydroxyphenylalanine) and were run at a final substrate concentration of 5 mmol/L. All assays were run at 24°C in 50 mmol/L sodium acetate buffer (pH 5) for 2 hours (βG and Phos), 4 hours (NAG and CBH), or 18 hours (LAP, Perox, and Phenox), with appropriate blank and quench controls, and final activities were standardized to nanomoles of substrate degraded (hydrolyzed or oxidized) per gram of dry soil per hour.

Phospholipid fatty acid analysis

Cell wall phospholipid fatty acids (PLFAs) were extracted from 5 g soil (Bligh and Dyer 1959, Frostegård et al. 1993). Soils were incubated in a 2:1:0.67 mixture of methanol : chloroform : citrate buffer, and phospholipids were isolated from the chloroform phase using 3 mL Supelco Supelclean LC-S1 SPE columns (Sigma-Aldrich, St. Louis, Missouri, USA), then saponified and methylated under mild alkaline conditions. The resulting fatty acid methyl esters (FAMEs) were analyzed using a Thermo Scientific Trace GC-ISQ mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) with a DB5-MS column gas chromatograph; soil extract FAME peaks were identified by comparison with the Bacterial acid methyl esters mix (BAME, Matreya 1114; Matreya LLC, Pleasant Gap, Pennsylvania, USA) and the internal standard methyl nonadecanoate was used to quantify the amount of C in each peak. The origins of selected soil PLFAs were classified as bacteria (15:0 iso, 15:0 anteiso, 15:0, 16:0 iso, 16:1ω7c, 16:0 10Me, 17:0 iso, 17:0 anteiso, 17:0 cyclo, 17:0, 18:1ω7c, 18:0 10Me, 19:0 cyclo) or fungi (18:2ω6,9). The relative micromoles of C per gram of dry soil in these PLFAs was used to calculate a fungal : bacterial biomass ratio (F:B), and the molar percentage C (relative abundance) of all PLFAs was compared among all samples using a community ordination analysis (Bååth and Anderson 2003, Brant et al. 2006).

Statistical analysis

The effect of month (June or September), time before/after rainfall (Pre, Pulse, or Post sampling times) and treatment (Ambient or Altered) on each response variable was compared using three-way analysis of variance (ANOVA). For CUE, time before/after rainfall was not a test factor, so two-way ANOVA was used. When necessary, dependent variable data were log-transformed to meet the assumption of normality (this was necessary for DOC, DOC:TN, MBC:N, and all enzyme activities). Linear regressions between soil water content and microbial response variables were evaluated using Pearson's R. The significance (rejection of null hypothesis, that the independent factor had no effect or that no linear correlation existed between variables) of all tests was evaluated using an α value of 0.05. Also, a nonparametric ordination analysis, nonmetric multidimensional scaling (NMS), of the relative abundance of microbial PLFAs was run using PC-ORD (McCune and Mefford 1999). Consistent solutions were reached for each of 10 individual analyses, and analysis included 50 independent runs each with real and standardized data. The effects of treatment, month, and rainfall on PLFA ordination axis scores and relative abundance were also evaluated using three-way ANOVA and correlation tests. ANOVA and correlation tests were run using R with R Commander (Fox 2005, R Development Core Team 2010).

Results

In June, pre-rainfall mean soil volumetric water content was 32.6% ± 0.7% (mean ± SE) and mean water potential was −0.011 ± 0.003 MPa (mean ± SE). Soils were considerably drier in September, with a pre-rainfall mean soil volumetric water content of 15.1% ± 0.5% and mean water potential of −0.83 ± 0.30 MPa (Appendix A: Fig. A2, Appendix B: Table B1). In both months, the 2.5-cm rainfall events caused an equivalent increase in soil water content across all field plots in both treatments (mean increase of 7.7% ± 0.7%). This wetting equated to a smaller mean water potential increase in June (0.007 ± 0.001 MPa) than in September (0.72 ± 0.28 MPa). Across all samples, soil water content ranged from 11.7% to 44.1% and estimated soil water potential ranged from −3.85 to −0.002 MPa. Soil temperatures were higher in September than June, except for the five-day post-rainfall soils in September, which were cooler due to cloudy conditions. Soil water and temperature conditions at the time of sampling were statistically equivalent in Ambient and Altered treatments ($P > 0.05$).

Pre-rainfall soil microbial respiration rates (micrograms CO_2-C per gram of dry soil per hour) were lower in September (0.58 ± 0.1 μg CO_2-C·g dry soil^{-1}·h^{-1}; mean ± SE) than in June (1.99 ± 0.2 μg CO_2-C·g dry soil^{-1}·h^{-1}), and the equivalent water pulse caused a larger absolute respiration response in June (change of 1.77 ± 0.29 μg CO_2-C·g dry soil^{-1}·h^{-1}, a 90% increase) than in September (change of 0.95 ± 0.2 μg CO_2-C·g dry soil^{-1}·h^{-1}, a 160% increase; (Fig. 1; Appendix B: Table B1). In June, respiration rates five days after the rainfall event were slightly lower than pre-rainfall, but in September, respiration rates five days after the rainfall event were closer to peak values than to pre-rainfall values. Soil microbial respiration responses to equivalent rainfall events were not affected by long-term experimental alteration of precipitation timing ($P = 0.29$). Respiration and water content were significantly positively correlated (Pearson's $R^2 = 0.77$, $P < 0.01$).

Soil MBC and MBN were also lower in September than in June (Fig. 2a, b; Appendix B: Table B1). MBC responded significantly to rainfall in the Altered treatment soils, increasing by 171 ± 28 and 147 ± 33 μg C/g dry soil (mean ± SE; a 19% and 18% change) and

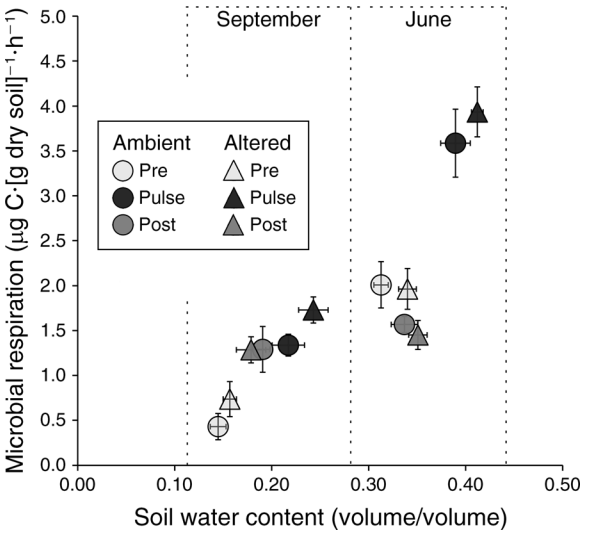

FIG. 1. The relationship between volumetric water content and microbial respiration of CO_2-C preceding (Pre), one day after (Pulse), and five days after (Post) a 2.5-cm rainfall event in Ambient and Altered treatment plots ($n = 6$) in June and September 2011 at the Konza Prairie Biological Station, a Long Term Ecological Research Site in the Flint Hills of eastern Kansas, USA. Values shown in all figures are means ± SE.

MBN responded by 25 ± 5 and 55 ± 10 μg N/g dry soil (a 25% and 142% change) in June and September. MBC pools did not change significantly in response to rainfall in Ambient treatment soils, but MBN increased significantly after rainfall in September in both treatments. By five days post-rainfall, however, MBC and MBN pools had decreased to near or equivalent to pre-rainfall conditions. Also, across all samples, MBC and MBN were higher in Altered treatment soils than in Ambient treatment soils (911 ± 18 > 814 ± 20 μg C/g dry soil and 89 ± 7 > 75 ± 7 μg N/g dry soil, $P < 0.02$). Rainfall-stimulated CUE was 51% higher in Altered than Ambient soils (0.70 ± 0.03 > 0.46 ± 0.10), and 54% higher in September than in June (0.71 ± 0.07 > 0.46 ± 0.07) (Fig. 2c; Appendix B: Table B1, $P < 0.03$); there were no significant interactive effects. While seasonal and post-rainfall dynamics were qualitatively similar in MBC and MBN, microbial biomass C:N was significantly higher in September than in June, and was highest in soils pre-rainfall in September (Fig. 2d; Appendix B: Table B1). MBC, MBN, and MBC:N were correlated with water content (Pearson's $R^2 = 0.62$, 0.86 and −0.67, respectively, $P < 0.01$).

Extractable DOC was higher in September than in June, whereas exchangeable TN was higher in June than September ($P < 0.05$; Appendix B: Table B1). Extractable DOC pools did not vary in June, but in September were significantly higher pre-rainfall and decreased by 33 μg C/g dry soil (a 24% change) after the moisture pulse. Extractable DOC pools did not vary significantly between Ambient and Altered treatment soils. Extractable TN did not respond to rainfall, but was significantly higher in Altered treatment soils than Ambient treatment soils across all samples (28 ± 1 > 26 ± 1 μg N/g dry soil). Nitrate-N levels were higher in September than June but did not vary by treatment or rainfall. Ammonium-N levels were higher in June than September, and showed a significant month by rainfall interaction, in that the ammonium pool decreased by 35–62% after the rainfall in September but did not differ by long-term precipitation treatment. Soil total C was 3.50% ± 0.08% in Ambient treatments and 3.44% ± 0.09% in Altered treatments, a nonsignificant difference.

The F:B ratio of PLFAs was not affected by long-term experimental alteration of precipitation timing ($P = 0.59$). However, F:B ratio was lower in June than September and decreased after rainfall, indicating a higher relative abundance of bacteria in June and in response to rainfall (Fig. 3a; Appendix B: Table B1). This rainfall response was briefer in June, as F:B ratio returned to the original range by five days post-rainfall, but the lower F:B in September was sustained for five days post-rainfall. F:B and water content were significantly negatively correlated (Pearson's $R^2 = -0.55$, $P < 0.01$). NMS of PLFA relative abundances resulted in a strong ordination model (stress = 14.50, instability < 0.000001, 74.6% of variability represented by two axes). There was no consistent effect of treatment, month, or rainfall on NMS Axis 1, but there was a treatment by month interaction ($P = 0.007$; Fig. 3b), indicating a different microbial community structure in the Ambient and Altered soils in September. PLFA NMS Axis 2 scores differed between June and September ($P = 0.006$), but not by precipitation treatment or rainfall timing ($P > 0.35$), although there was a month by rainfall interaction ($P = 0.02$; Fig. 3c), indicating a contrasting shift in microbial community structure following rainfall in June vs. September, as well as a different average microbial community structure in each month. There were many significant effects on individual PLFA relative abundances; the most highly significant differences ($P < 0.001$) included higher 18:1ω7 abundance in June, higher 16:1ω5, 18:2ω6,9 and 18:1ω9 abundance in September, and lower 18:2ω6,9 abundance in "Pulse" soils. PLFA NMS Axis 2 was correlated significantly ($P < 0.05$) with water content, ammonium, TOC:TN, microbial respiration, F:B and MBN, and PLFA NMS Axis 1 was correlated significantly with TOC, TN, ln NAG and ln Phenox (Appendix C: Table C1).

No EEA potentials were significantly affected by long-term experimental alteration of precipitation timing, but many EEA potentials changed in response to rainfall (Fig. 4; Appendix B: Table B2). Significant β-glucosidase, cellobiohydrolase, phenol oxidase, peroxidase, and L-aminopeptidase responses to rainfall were qualitatively different between June and September, generally increasing after rainfall in June and decreasing after rainfall in September. N-acetylglucosaminidase activities increased after rainfall in both seasons, and L-aminopeptidase activity increased briefly during the

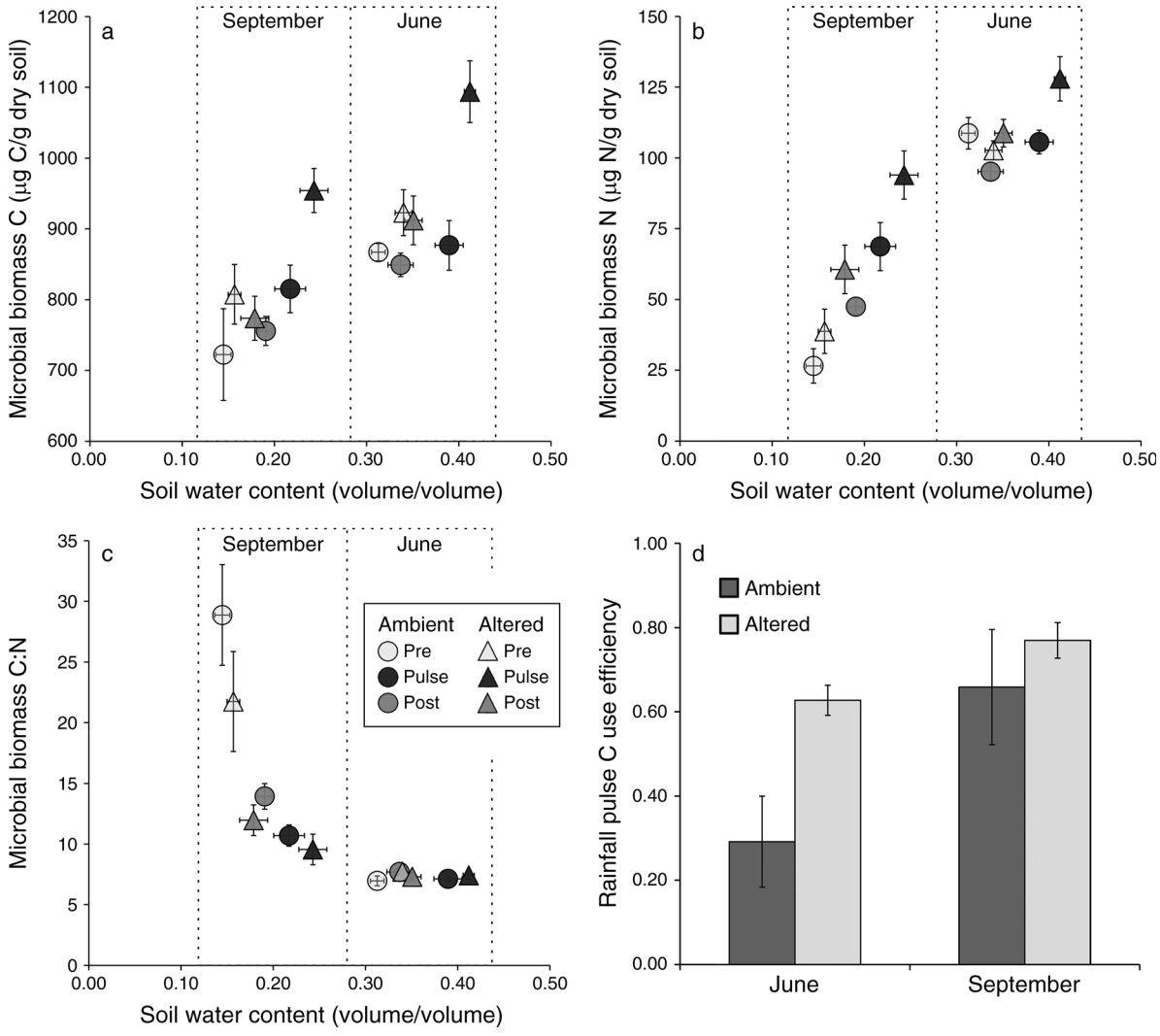

FIG. 2. The relationship between soil volumetric water content and (a) microbial biomass C, (b) microbial biomass N, and (c) microbial biomass C:N, preceding (Pre), one day after (Pulse), and five days after (Post) a 2.5-cm rainfall event in Ambient and Altered treatment plots ($n = 6$) in June and September 2011. (d) Microbial carbon use efficiency following soil wet-up in both months and treatments (Ambient, black bars; Altered, gray bars).

rainfall pulse in Altered soils in September. Phosphatase activity decreased after rainfall in both seasons. Following an initial increase, peroxidase activity decreased by five days after rainfall in June. Also, mean phosphatase, N-acetylglucosaminidase, and peroxidase activities were higher in June than September ($P < 0.05$), but the other activities did not differ by season.

DISCUSSION

Soil water contents and water potentials during the study period ranged from high, but not saturated (saturation estimated at 50% water volume/volume), to near the wilting point (estimated as 13% water volume/volume) (Appendix A: Fig. A2). Thus, the full data set reflects relationships between soil water content and microbial activity across a range of moderate to droughty soil water conditions in the context of a field-based, intact ecosystem experiment. All microbial C cycling parameters responded to changes in soil water, but not in similar ways. Microbial respiration and biomass were directly correlated with soil water content, but microbial biomass dynamics differed between months and between long-term Ambient and Altered precipitation regime treatments. Enzyme activities did not shift with soil water content or long-term precipitation regime, yet responded to rainfall differently in September than in June. Thus, the data suggest that the influence of soil water on microbial function may entail different mechanisms at varying time scales, from days (rainfall event) to months (seasonal drought) to years (precipitation regime alteration).

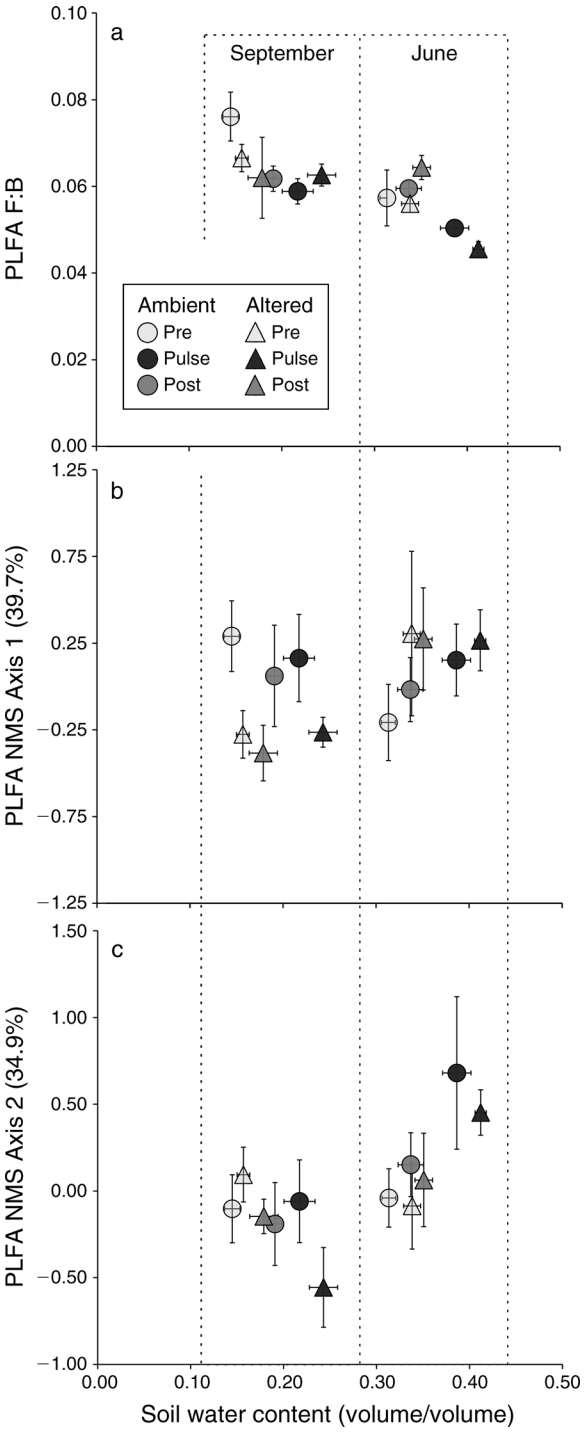

FIG. 3. The relationship between soil volumetric water content and (a) phospholipid fatty acid (PLFA) fungal : bacterial ratios (F:B), and (b) nonmetric multidimensional scaling (NMS) axis 1 values preceding (Pre), one day after (Pulse), and five days after (Post) a 2.5-cm rainfall event in Ambient and Altered treatment plots ($n = 6$) in June and September 2011. The percentage variation explained by each axis is shown following the axis label.

Soil microbial respiration responded to rainfall-driven and seasonal variation in soil water content similarly in both Ambient and Altered treatments (Fig. 1). It is generally understood that soil water content is positively correlated with microbial activity, even across contrasting soil types (Linn and Doran 1984, Manzoni et al. 2012*a*), and our data clearly show this pattern. The fact that respiration tracks soil water content so closely throughout the growing season has an interesting consequence when respiration data are integrated over longer temporal scales: the direct effect of modified hydrological regime becomes apparent. CO_2 efflux (total belowground respiration, both plant and microbial), collected every two weeks and averaged over an entire growing season, is significantly lower in the Altered precipitation treatment (Harper et al. 2005, Fay et al. 2011). The increased duration of low-moisture (and low-respiration) conditions imposed by the Altered precipitation treatment translates into lower CO_2 efflux when integrated over many months.

Microbial biomass was also correlated positively with soil water across the entire data set, but surprisingly, biomass C and N pools were significantly higher in Altered soils and increased consistently in response to rainfall in Altered treatment soils (Fig. 2a, b). In September, the biomass increase was stronger and more widespread (nearly significant in Ambient soils), and DOC and ammonium pools decreased significantly, possibly reflecting immobilization. This is a notable microbial functional shift in seasonally dry and long-term "droughtier" soils, particularly because it reflects greater microbial CUE following re-wet in these soils (Fig. 2c). Greater microbial investment of C to biosynthesis relative to maintenance following soil re-wet could explain the higher biomass pools in Altered soils; Evans and Wallenstein (2012) also saw this biomass difference. It has been noted that biomass accrual in response to wetting and drying may be indicative of soils with a long-term history of drought and infrequent wetting events (Fierer and Schimel 2002). Over extended periods of time, this mechanism could also facilitate a greater return of C to the SOM pool via microbial death (Appendix A: Fig. A1).

This differential microbial growth response following rainfall is an intriguing result, particularly because small shifts in microbial CUE can have significant consequences for soil C storage scenarios (Allison et al. 2010). However, the C source fueling microbial growth in this field study is unknown, our microbial CUE values are estimated (post-rainfall), not measured directly, and in general the influence of soil water on microbial CUE is not well understood (Manzoni et al. 2012*b*). Soil microbial biomass increase following soil re-wetting has been observed in the past (Lund and Goksoyr 1980, Bottner 1985), but the opposite has also been observed (van Gestel et al. 1993). Similarly, other studies have shown increased (Herron et al. 2009) or decreased (Tiemann and Billings 2011) microbial CUE under drier

conditions, and an earlier laboratory study using soils from the RaMPs experiment measured a biomass increase in Ambient, not Altered, soils following a re-wet event (Evans and Wallenstein 2012). The concurrent plant response to water variability is a clear difference between this field study and similar-themed laboratory studies, and is also a clear data gap in our "comprehensive" evaluation of the soil C cycle (Appendix A: Fig. A1). Root C deposition is challenging to measure (Kuzyakov and Domanski 2000), but can comprise a significant source of microbial C (Butler et al. 2004). In general, further experiments are necessary to define how microbial CUE changes with hydrological variability at rainfall event and seasonal scales in these soils. However, it is still possible to evaluate the possible factors affecting CUE as estimated from our field-based biomass and respiration data.

Three C sources could fuel microbial growth upon wet-up: soil DOC that has accumulated during the dry period via various mechanisms (Austin et al. 2004), microbial osmolytes that may be metabolized rapidly upon an increase in water potential (Halverson et al. 2000), or plant root exudates that are released to the soil as photosynthetic activity ramps up (Kuzyakov and Domanski 2000). Our data allow that either soil DOC or high-C osmolytes could have fueled microbial activity in September, as both soil DOC concentration and microbial C:N was highest before rainfall in September, and subsequently decreased upon re-wet (Fig. 2d; Appendix B: Table B2). These mechanisms may help explain the higher apparent CUE in September, in both Ambient and Altered treatments, but do not elucidate the difference in apparent CUE between Ambient and Altered treatments in June (Fig. 2c). While we do not know how root C exudation varies with water at this site, it is known that mean aboveground plant production is lower in Altered treatments, and that leaf photosynthetic rate increases with soil water potential (Nippert et al. 2009, Fay et al. 2011). It may be that extended intervals of low soil water in these field soils have, over 14 years, selected for microbial taxa with higher growth efficiency following rain events, better survival through dry periods, and/or greater ability to capitalize on the labile C exuded by roots immediately following rainfall.

Biomass C:N and PLFA data may reflect shifts in microbial community composition related to tolerance to low soil water. MBN dynamics were greater in drier soils, resulting in a large increase in microbial C:N at the lowest soil water contents (Fig. 2b, d). Microbial cells that remain intact at lower soil water conditions might have higher C:N due to osmoadaptation strategies (West et al. 1988). Fungi are expected to both withstand drought conditions better than bacteria, and to have a higher mean C:N than bacteria ($\sim 10 > \sim 4$) (Harris 1981, Strickland and Rousk 2010). In addition, certain fungi may accumulate higher levels of intracellular C than N as compatible solutes under water potential stress conditions (Schimel et al. 1989, 2007). The ratio of fungal to bacterial (F:B) PLFAs was higher in drier soils (Fig. 3a), so both of these explanations are viable. While our data did not show a precipitation regime effect on F:B ratio, F:B was higher in the Altered than Ambient treatment in soils collected in the wintertime (December) from the same plots (Evans and Wallenstein 2012). In our data set, PLFA composition differed between June and September along an axis correlated with seasonally varying soil conditions including water content (Fig. 3c, Appendix C: Table C), and fungal PLFAs (16:1ω5, 18:2ω6,9 and 18:1ω9) were relatively more abundant in September. In addition, PLFA composition shifted differently after rainfall in June than in September, and diverged between Ambient and Altered soils in September independently of fungal abundance or soil moisture (Fig. 3b, c), suggesting real but complex microbial community structure responses to seasonal drought and altered long-term precipitation timing. Physiological differences among bacterial taxa may contribute to differences in total microbial cell abundance during seasonally to annually variable moisture conditions, or bacterial microhabitat within the complex soil pore space may affect the relative abundance of taxa during periods of low soil moisture when only small pores are filled with water (e.g., the September sampling period). It is also conceivable that certain phylotypes have adapted to the more droughty conditions over the 15-year experimental time period, but more data or targeted experiments are necessary to address that mechanism directly.

Unlike microbial respiration, biomass, and PLFAs, EEA potentials were not correlated with soil moisture, suggesting that water content did not directly influence enzyme activity. However, these data do indicate that the enzymatic potential for SOM degradation was higher in June, particularly following the rainfall event. Phosphatase, N-acetylglucosaminidase, and peroxidase activities were higher in June than September; more strikingly, the activity of C-acquiring β-glucosidase, cellobiohydrolase, and oxidative enzymes tended to increase after rainfall in June but decrease after rainfall in September, and N-acquiring L-aminopeptidase activity also increased considerably after rainfall in June (Fig. 4). An increase in enzyme activity implies production of new enzymes, while a decrease in enzyme activity implies degradation of enzyme (generally assumed to be a constant process) and a lack of microbial investment to replenish that pool (Schimel and Weintraub 2003). Overall, the high microbial respiration rates following rainfall in June (Fig. 1) may have been associated with enough microbial C demand to induce the production of extracellular enzymes to degrade SOM (Allison and Vitousek 2005), in contrast to September when C demand was lower. N demand was also induced in June, yet was still evident in September. Competition between plants and microbes for N occurs in these soils (Dell and Rice 2005), and enzyme activity ratios in these

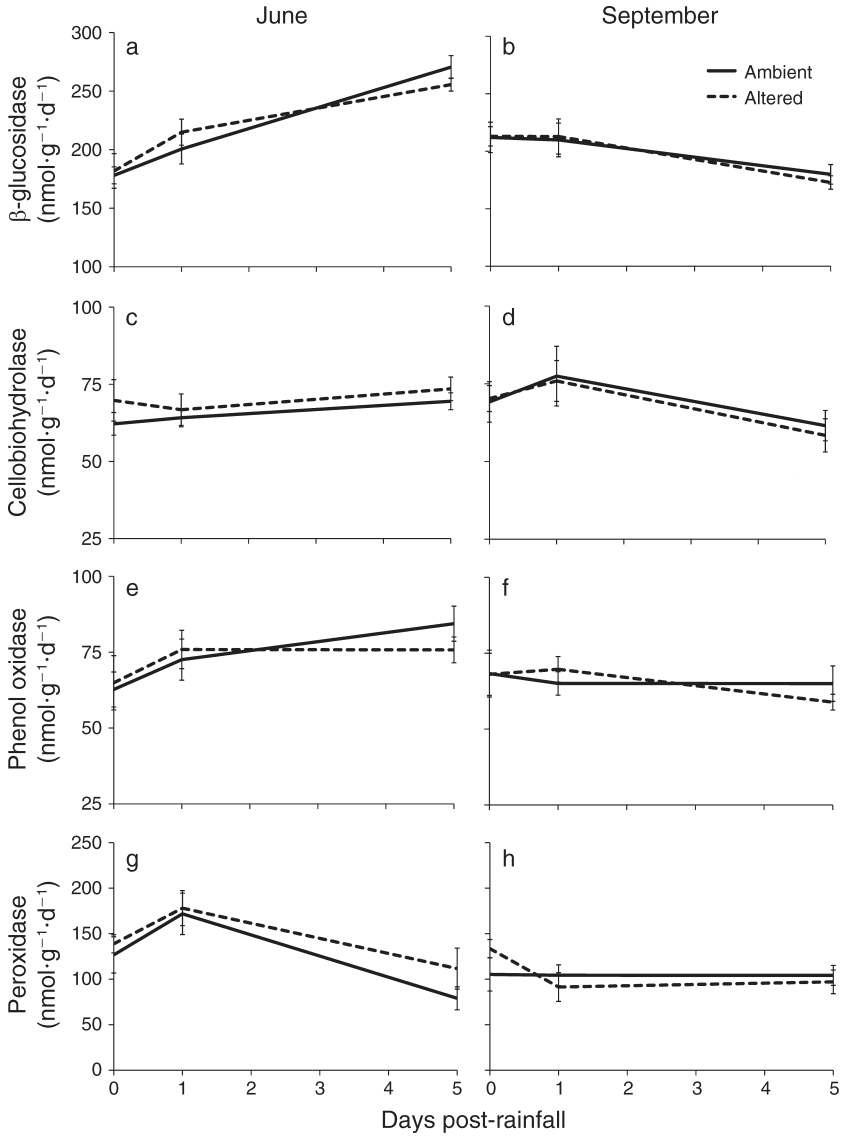

FIG. 4. C-acquiring extracellular enzyme activity potentials preceding (0 days), one day after, and five days after a 2.5-cm rainfall event in Ambient (solid lines) and Altered (dashed lines) rainfall treatment soils ($n = 6$) in June (left panels) and September (right panels) 2011: the cellulolytic enzymes (a, b) β-glucosidase and (c, d) cellobiohydrolase; the oxidative enzymes (e, f) phenol oxidase and (g, h) peroxidase; the N-acquiring enzymes (i, j) L-aminopeptidase and (k, l) N-acetylglucosaminidase; and (m, n) the P-acquiring enzyme phosphatase.

soils compared to soils globally (Sinsabaugh et al. 2009) suggest greater investment toward N acquisition relative to C acquisition ($\beta g : (LAP + NAG) = 1.18 \pm 0.03 < 1.41$) and P acquisition ($(LAP + NAG) : Phos = 0.58 \pm 0.02 > 0.44$), all implying primary microbial N limitation. Also, the energy cost associated with C- and N-acquiring extracellular enzyme production (Schimel and Weintraub 2003) could help to explain the lower microbial CUE observed following the rainfall event in June (Fig. 2c).

The data on extracellular enzyme activity, microbial biomass, and respiration during variable soil water conditions in this native prairie ecosystem soil all indicated that drier conditions might have negative feedbacks on soil C loss, supporting the conclusions made in earlier studies from this long-term field experiment (Harper et al. 2005). While microbial biomass C has increased by 12%, 14 years of altered precipitation treatment has not changed the large total soil C pool (3.5% total C); effects of altered turnover in the total C pool may only become apparent after multi-decadal or longer time scales (Parton et al. 1987). The mechanistic hypotheses presented by this study offer further insight into how soil microbial C dynamics might vary on different temporal scales under future precipitation regimes. Microbial C use efficiency after rainfall

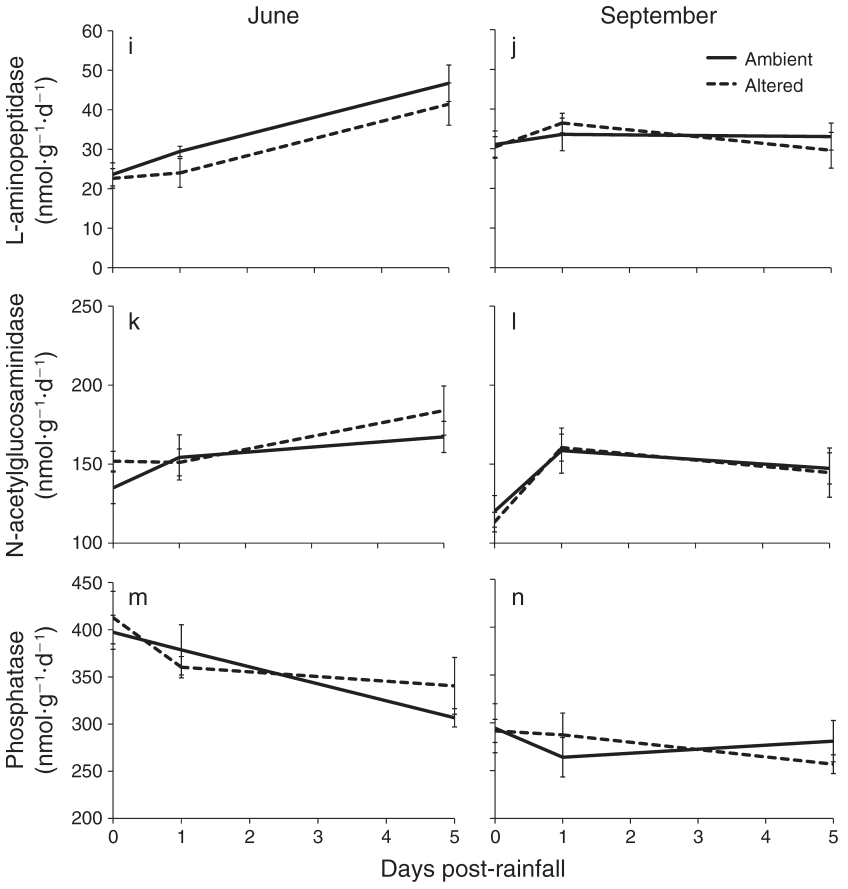

FIG. 4. Continued.

events was greater in drier soils and soils with a history of extended dry intervals between rainfall events, and the potential for SOM decomposition after rainfall events was lower in drier soils. Microbial activity in dry soils might be driven by cells that survive or grow more efficiently during periods of low nutrient supply or water potential stress, and soil microbial function may be linked with plant function in drought-prone field soils. The importance of the timing of precipitation, particularly individual rainfall events and soil dry-down/re-wet periodicity, should be taken into account when evaluating or projecting the biogeochemical consequences of climate change. Elucidating the C allocation physiology of the microbial cells active during variable soil moisture conditions, and linking aboveground and belowground C cycle responses to an altered precipitation regime will contribute to the understanding of soil C dynamics under future climate scenarios.

ACKNOWLEDGMENTS

Thanks to all RaMPs and Konza Biological Station and Long Term Ecological Research program infrastructure and personnel, particularly, John Blair, Patrick O'Neal, and Jeff Taylor. Thanks to Maria Dragila and Nathaniel Tisdell for assistance with the soil water release curve, and to Shawn P. Brown, Spencer Lickteig, Anne Rigdon, and Patricia Zarate for their assistance in collecting and processing the field samples. Thanks to John Blair for constructive feedback on the manuscript, and to two anonymous reviewers for their thoughtful and substantive comments. This is contribution no. 13-332-J from the Kansas Agricultural Experiment Station. The RaMPs project is supported in part by an NSF grant to the Konza Prairie Long-Term Ecological Research program. This research was funded by DOE Contract No. DE-SC0004953.

LITERATURE CITED

Aanderud, Z. T., and J. T. Lennon. 2011. Validation of heavy-water stable isotope probing for the characterization of rapidly responding soil bacteria. Applied and Environmental Microbiology 77:4589–4596.

Allison, S. D., and P. M. Vitousek. 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biology and Biochemistry 37:937–944.

Allison, S. D., M. D. Wallenstein, and M. A. Bradford. 2010. Soil-carbon response to warming dependent on microbial physiology. Nature Geoscience 3:336–340.

Austin, A. T., L. Yahdjian, J. M. Stark, J. Belnap, A. Porporato, U. Norton, D. A. Ravetta, and S. M. Schaeffer. 2004. Water pulses and biogeochemical cycles in arid and semiarid ecosystems. Oecologia 141:221–235.

Bååth, E., and T. H. Anderson. 2003. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. Soil Biology and Biochemistry 35:955–963.

Birch, H. F. 1958. The effect of soil drying on humus decomposition and nitrogen availability. Plant and Soil 10: 9–31.

Blecker, S. W., R. L. McCulley, O. A. Chadwick, and E. F. Kelly. 2006. Biologic cycling of silica across a grassland bioclimosequence. Global Biogeochemical Cycles 20: GB3023.

Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37:911–917.

Bottner, P. 1985. Response of microbial biomass to alternate moist and dry conditions in a soil incubated with C-15 labeled and N-15 labeled plant material. Soil Biology and Biochemistry 17:329–337.

Brant, J. B., E. W. Sulzman, and D. D. Myrold. 2006. Microbial community utilization of added carbon substrates in response to long-term carbon input manipulation. Soil Biology and Biochemistry 38:2219–2232.

Brookes, P. C., A. Landman, G. Pruden, and D. S. Jenkinson. 1985. Chloroform fumigation and the release of soil nitrogen–a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry 17:837–842.

Butler, J. L., P. J. Bottomley, S. M. Griffith, and D. D. Myrold. 2004. Distribution and turnover of recently fixed photosynthate in ryegrass rhizospheres. Soil Biology and Biochemistry 36:371–382.

Carsel, R. F., and R. S. Parrish. 1988. Developing joint probability distributions of soil water retention characteristics. Water Resources Research 24:755–769.

Dell, C. J., and C. W. Rice. 2005. Short-term competition for ammonium and nitrate in tallgrass prairie. Soil Science Society of America Journal 69:371–377.

Evans, S. E., and M. D. Wallenstein. 2012. Soil microbial community response to drying and rewetting stress: does historical precipitation regime matter? Biogeochemistry 109: 101–116.

Fay, P. A., J. M. Blair, M. D. Smith, J. B. Nippert, J. D. Carlisle, and A. K. Knapp. 2011. Relative effects of precipitation variability and warming on tallgrass prairie ecosystem function. Biogeosciences 8:3053–3068.

Fay, P. A., J. D. Carlisle, B. T. Danner, M. S. Lett, J. K. McCarron, C. Stewart, A. K. Knapp, J. M. Blair, and S. L. Collins. 2002. Altered rainfall patterns, gas exchange, and growth in grasses and forbs. International Journal of Plant Sciences 163:549–557.

Fierer, N., and J. P. Schimel. 2002. Effects of drying–rewetting frequency on soil carbon and nitrogen transformations. Soil Biology and Biochemistry 34:777–787.

Fierer, N., J. P. Schimel, and P. A. Holden. 2003. Influence of drying–rewetting frequency on soil bacterial community structure. Microbial Ecology 45:63–71.

Fox, J. 2005. The R Commander: a basic-statistics graphic user interface to R. Journal of Statistical Software 19:1–42.

Frostegård, A., A. Tunlid, and E. Bååth. 1993. Phospholipid fatty-acid composition, biomass and activity of microbial communities from 2 soil types experimentally exposed to different heavy metals. Applied and Environmental Microbiology 59:3605–3617.

Halverson, L. J., T. M. Jones, and M. K. Firestone. 2000. The release of intracellular solutes by four soil bacteria exposed to dilution stress. Soil Science Society of America Journal 64: 1630–1637.

Harper, C. W., J. M. Blair, P. A. Fay, A. K. Knapp, and J. D. Carlisle. 2005. Increased rainfall variability and reduced rainfall amount decreases soil CO_2 flux in a grassland ecosystem. Global Change Biology 11:322–334.

Harris, R. F. 1981. Effect of water potential on microbial growth and activity. Pages 23–95 *in* J. F. Parr, W. R. Gardener, and L. F. Elliott, editors. Water potential relations in soil microbiology. Soil Science Society of America, Madison, Wisconsin, USA.

Herron, P. M., J. M. Stark, C. Holt, T. Hooker, and Z. G. Cardon. 2009. Microbial growth efficiencies across a soil moisture gradient assessed using C-13 acetic acid vapor and N-15 ammonia gas. Soil Biology and Biochemistry 41:1262–1269.

Hood-Nowotny, R., N. Hinko-Najera Umana, E. Inselbacher, P. Oswald-Lachouani, and W. Wanek. 2010. Alternative methods for measuring inorganic, organic, and total dissolved nitrogen in soil. Soil Science Society of America Journal 74:1018–1027.

IPCC (Intergovernmental Panel on Climate Change). 2007. Climate change: the physical science basis. Summary for policymakers. Cambridge University Press, New York, New York, USA.

Jentsch, A., J. Kreyling, and C. Beierkuhnlein. 2007. A new generation of climate-change experiments: events, not trends. Frontiers in Ecology and the Environment 5:365–374.

Knapp, A. K., et al. 2008. Consequences of more extreme precipitation regimes for terrestrial ecosystems. BioScience 58:811–821.

Knapp, A. K., P. A. Fay, J. M. Blair, S. L. Collins, M. D. Smith, J. D. Carlisle, C. W. Harper, B. T. Danner, M. S. Lett, and J. K. McCarron. 2002. Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. Science 298:2202–2205.

Kuzyakov, Y., and G. Domanski. 2000. Carbon input by plants into the soil. Journal of Plant Nutrition and Soil Science 163: 421–431.

Lawrence, C. R., J. C. Neff, and J. P. Schimel. 2009. Does adding microbial mechanisms of decomposition improve soil organic matter models? A comparison of four models using data from a pulsed rewetting experiment. Soil Biology and Biochemistry 41:1923–1934.

Linn, D. M., and J. W. Doran. 1984. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Science Society of America Journal 48:1267–1272.

Lund, V., and J. Goksoyr. 1980. Effects of water fluctuations on microbial mass and activity in soil. Microbial Ecology 6: 115–123.

Manzoni, S., J. P. Schimel, and A. Porporato. 2012*a*. Reponses of soil microbial communities to water-stress: results from a meta-analysis. Ecology 93:930–938.

Manzoni, S., P. Taylor, A. Richter, A. Porporato, and G. I. Agren. 2012*b*. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytologist 196: 79–91.

McCune, B., and M. J. Mefford. 1999. PC-ORD. Multivariate analysis of ecological data. Version 4. MjM Software, Gleneden Beach, Oregon, USA.

Miller, A. E., J. P. Schimel, T. Meixner, J. O. Sickman, and J. M. Melack. 2005. Episodic rewetting enhances carbon and nitrogen release from chaparral soils. Soil Biology and Biochemistry 37:2195–2204.

Nippert, J. B., P. A. Fay, J. D. Carlisle, A. K. Knapp, and M. D. Smith. 2009. Ecophysiological responses of two dominant grasses to altered temperature and precipitation regimes. Acta Oecologica 35:400–408.

Parton, W. J., D. S. Schimel, C. V. Cole, and D. S. Ojima. 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. Soil Science Society of America Journal 51:1173–1179.

Porporato, A., E. Daly, and I. Rodriguez-Iturbe. 2004. Soil water balance and ecosystem response to climate change. American Naturalist 164:625–632.

R Development Core Team. 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ransom, M. D., C. W. Rice, T. C. Todd, and W. A. Wehmueller. 1998. Soils and soil biota. Pages 48–66 *in* A. K. Knapp, J. M. Briggs, D. C. Hartnett, and S. C. Collins, editors. Grassland dynamics: long-term ecological research in tallgrass prairie. Oxford University Press, New York, New York, USA.

Saiya-Cork, K. R., R. L. Sinsabaugh, and D. R. Zak. 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. Soil Biology and Biochemistry 34:1309–1315.

Schimel, J., T. C. Balser, and M. Wallenstein. 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88:1386–1394.

Schimel, J. P., W. J. Scott, and K. Killham. 1989. Changes in cytoplasmic carbon and nitrogen pools in a soil bacterium and a fungus in response to salt stress. Applied and Environmental Microbiology 55:1635–1637.

Schimel, J. P., and M. N. Weintraub. 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biology and Biochemistry 35:549–563.

Schlesinger, W. H. 1997. Biogeochemistry: an analysis of global change. Second edition. Academic Press, London, UK.

Sinsabaugh, R. L., B. H. Hill, and J. J. F. Shah. 2009. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462:795–798.

Sponseller, R. A. 2007. Precipitation pulses and soil CO_2 flux in a Sonoran Desert ecosystem. Global Change Biology 13: 426–436.

Strickland, M. S., and J. Rousk. 2010. Considering fungal:bacterial dominance in soils–methods, controls, and ecosystem implications. Soil Biology and Biochemistry 42:1385–1395.

Tiemann, L. K., and S. A. Billings. 2011. Changes in variability of soil moisture alter microbial community C and N resource use. Soil Biology and Biochemistry 43:1837–1847.

Vance, E. D., P. C. Brookes, and D. S. Jenkinson. 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19:703–707.

van Genuchten, M. T. 1980. A closed-form equation for predicting the hydraulic conductivity of unsaturated soils. Soil Science Society of America Journal 44:892–898.

van Genuchten, M. T., F. J. Leij, and S. R. Yates. 1991. The RETC code for quantifying the hydraulic functions of unsaturated soils. Version 1.0, EPA Report 600/2-91/065. U.S. Salinity Laboratory, USDA, ARS, Riverside, California, USA.

van Gestel, M., R. Merckx, and K. Vlassak. 1993. Microbial biomass and activity in soils with fluctuating water contents. Geoderma 56:617–626.

Weltzin, J. F., et al. 2003. Assessing the response of terrestrial ecosystems to potential changes in precipitation. BioScience 53:941–952.

West, A. W., G. P. Sparling, T. W. Speir, and J. M. Wood. 1988. Comparison of microbial C, N-flush and ATP, and certain enzyme activities of different textured soils subject to gradual drying. Australian Journal of Soil Research 26:217–229.

Zeglin, L. H., M. Stursova, R. L. Sinsabaugh, and S. L. Collins. 2007. Microbial responses to nitrogen addition in three contrasting grassland ecosystems. Oecologia 154:349–359.

Supplemental Material

Appendix A

Figures showing the generalized ecosystem C cycle and estimated soil water release curve for the study site (*Ecological Archives* E094-215-A1).

Appendix B

Tables showing all soil and microbial data with statistical significance noted (*Ecological Archives* (E094-215-A2).

Appendix C

Table showing correlations between PLFA NMS axes and microbial and soil characteristics (*Ecological Archives* E094-215-A3).