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Soil microbial and nutrient responses to 7 years of seasonally altered precipitation in a Chihuahuan Desert grassland

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Abstract

Soil microbial communities in Chihuahuan Desert grasslands generally experience highly variable spatiotemporal rainfall patterns. Changes in precipitation regimes can affect belowground ecosystem processes such as decomposition and nutrient cycling by altering soil microbial community structure and function. The objective of this study was to determine if increased seasonal precipitation frequency and magnitude over a 7-year period would generate a persistent shift in microbial community characteristics and soil nutrient availability. We supplemented natural rainfall with large events (one/winter and three/summer) to simulate increased precipitation based on climate model predictions for this region. We observed a 2-year delay in microbial responses to supplemental precipitation treatments. In years 3–5, higher microbial biomass, arbuscular mycorrhizae abundance, and soil enzyme C and P acquisition activities were observed in the supplemental water plots even during extended drought periods. In years 5–7, available soil P was consistently lower in the watered plots compared to control plots. Shifts in soil P corresponded to higher fungal abundances, microbial C utilization activity, and soil pH. This study demonstrated that 25% shifts in seasonal rainfall can significantly influence soil microbial and nutrient properties, which in turn may have long-term effects on nutrient cycling and plant P uptake in this desert grassland.

Keywords: Big Bend National Park, desert ecosystems, extreme climate events, precipitation manipulation, soil microbial communities

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Introduction

Climate is the largest single factor that shapes ecosystems by regulating microbial community assemblages, plant production, and higher trophic dynamics (Noy-Meir, 1974; Breshears *et al.*, 2008; Kelly & Goulden, 2008). Global climate model scenarios suggest that most regions in the southwestern United States will experience substantial increases in temperature (Loarie, 2009; Solomon, 2009; IPCC 2011) and alterations to seasonal precipitation patterns by the end of the 21st century (Easterling *et al.*, 2000; Seager *et al.*, 2007; Min, 2011). However, belowground ecosystem responses to climate change remain highly uncertain (Davidson & Janssens, 2006; Solomon *et al.*, 2007; Borken & Matzner,

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2009a). Changes to historic precipitation patterns can strongly affect soil C storage and rates of soil microbial biogeochemical processes (Coe *et al.*, 2012; Evans & Wallenstein, 2012; Saiz *et al.*, 2012). Likewise, any change in seasonal precipitation patterns that alters soil microbial functional soil carbon or nutrient cycling characteristics can ultimately induce shifts in ecosystem functioning (Chapin *et al.*, 1997; Kardol *et al.*, 2010; Wallenstein & Hall, 2012).

Little is known about the effect of longer term changes in seasonal precipitation on soil microbial community structure and function, and subsequently on soil nutrient availability in arid ecosystems (Behan-Pelleteir & Newton, 1999; Collins *et al.*, 2008). The short-term impacts of precipitation pulse variability on soil microbial biogeochemical responses have been shown to briefly stimulate microbial decomposition rates (Jacobson & Jacobson, 1998; Sponseller, 2007) and

subsequent nutrient fluxes (Austin *et al.*, 2004; Borken & Matzner, 2009b; Placella *et al.*, 2012) in numerous studies. Likewise, precipitation has been suggested as a factor that regulates available soil C and N pools (Borken & Matzner, 2009b; Johnson *et al.*, 2012). Some studies have reported dramatic increases in fungal abundances with additional watering in a tall grass prairie (Williams & Rice, 2007), likely due to the rapid responses of fungi to increased soil moisture (Van Gestel *et al.*, 1993b). Several studies in the Chihuahuan Desert have suggested that seasonal precipitation variability can influence soil fungal community abundances and consequently alter microbial community functional dynamics (Collins *et al.*, 2008; Bell *et al.*, 2009; Cregger *et al.*, 2012). Nonetheless, soil microbial community responses to altered seasonal precipitation patterns for this extensive desert region remain unclear.

Persistent shifts in climate that span outside the range of existing microbial community tolerances may alter soil microbial assemblages toward a community better able to withstand the new environmental conditions (Schwinning & Sala, 2004; Owens *et al.*, 2012; Placella *et al.*, 2012; Sistla & Schimel, 2012). For example, saprophytic and arbuscular mycorrhizal (AM) fungi (in general) exhibit a wider range of heat and drought stress tolerance than bacteria (with the exception of *Actinomycetes* bacteria) (Van Gestel *et al.*, 1993a; Schimel *et al.*, 1999, 2007). Adaptive strategies allow soil fungi to regulate osmotic stress due to their extensive hyphal networks, allowing fungi to internally transfer moisture and nutrients across dry patches (De Boer *et al.*, 2005; Oren & Steinberger, 2008); thus, they are able to persist during drought and quickly respond to soil moisture following rainfall events (Zak *et al.*, 1995). Although many soil bacterial species have osmosensing and osmoregulation mechanisms (Wood *et al.*, 2001; Sleator & Hill, 2002), they are typically more susceptible to drought because they require localized water films on soil surfaces and within soil aggregates for dispersion and substrate diffusion (Carson *et al.*, 2010; Dechesne *et al.*, 2010). Likewise, limited soil diffusion and bacterial motility in dry and hot environments (low water potential) can constrain bacteria making them vulnerable to drought stress which can result in cell death (i.e., osmotic stress, desiccation, and nutrient limitations) (Soini *et al.*, 2002; Dechesne *et al.*, 2010; Kakumanu *et al.*, 2013).

Bacterial and fungal assemblages exhibit higher diversity in drought compared to wet conditions (Carson *et al.*, 2010; Hawkes *et al.*, 2011). Hence, drought may moderate intraspecific competition within bacterial and fungal communities, thereby promoting soil microbial species coexistence in arid ecosystems. During naturally dry conditions in this arid ecosystem,

as soil microbes experience shifts from historic seasonal precipitation patterns, bacterial and fungal abundances could shift in response to wetter soil conditions resulting from increased seasonal precipitation patterns (Carson *et al.*, 2010; Hawkes *et al.*, 2011; Johnson *et al.*, 2012). If a persistent shift in seasonal precipitation can initiate changes in soil microbial community abundances, then will persistent belowground changes in microbial community function and soil nutrient availability be observed?

In this study, our objective was to determine whether a chronic alteration in the timing and magnitude of precipitation over 7 years would induce a shift in soil microbial community structure, microbial functional dynamics, and subsequent soil nutrient and/or soil chemical properties (e.g., soil pH) in a Chihuahuan Desert grassland in Big Bend National Park, Texas, USA. We hypothesized that 25% increased supplemental seasonal rainfall, occurring as one large winter event and three large summer events, over a 7-year period would shift microbial assemblages toward increased fungal abundances (due to their abilities to quickly respond to available soil moisture) regardless of natural climate variability. We further hypothesized that bacterial abundances and functional characteristics would not strongly respond to supplemental seasonal watering treatments, but would instead track natural precipitation patterns by exhibiting higher activity during relatively wetter seasonal periods. In this research, we specifically addressed the following questions: (i) Does soil microbial community structure, microbial function, or soil nutrient characteristics shift in response to 7 years of increased seasonal precipitation? and (ii) If so, does microbial community structure or function correlate with soil nutrient pools? To test our hypotheses, we examined microbial community structure [fatty acid methyl ester (FAME) and microbial biomass C], function (via enzyme activities and carbon substrate utilization), and soil characteristics (soil organic matter, available inorganic N and soil P, and soil pH). To our knowledge, this is one of the first studies to examine long-term impacts of chronic increased seasonal precipitation on soil bacterial and fungal abundances, in association with altered biogeochemical cycling in a desert ecosystem.

Materials and methods

Study site

This research was conducted in a midelevation Chihuahuan Desert grassland (29°5′N, 103°10′W; 1526 m.a.s.l) in the Pine Canyon Watershed in Big Bend National Park (BIBE) located in southwest Texas. The soils are sandy loams (62% sand, 30%

silt, and 8% clay), with a minimal litter layer and an extremely rocky A-horizon that immediately overlays a fractured igneous bedrock foundation (Turner, 1997; Aide et al., 2003). Mean air temperatures range from 2 °C in the winter to 36 °C in the summer (Fig. 1a and b). Annual historic mean rainfall (HMR) is 365 mm (1976–2001; Fig. 2a and b). Most precipitation occurs during the summer monsoon (ca. 46% of annual rainfall) with substantially less rainfall in winter (ca. 10%; Fig. 2a). Over the 7-year study, we observed highly variable monthly and annual precipitation patterns ranging from 74 to 155% of the HMR recorded from 1976 to 2001 (Fig. 2a and b).

Experimental design

In April 2002, twelve 3 × 3 m plots were established in the Sotol Grassland to study soil microbial community, soil nutrient, and plant ecophysiology responses to changes in precipitation frequency and magnitude by manipulating rainfall patterns in the summer and winter months. Each plot contained three dominant perennial plant species native to the site: *Dasylirion leiophyllum* (sotol; Liliaceae); *Opuntia phaeacantha* (brown-spine prickly pear; Cactaceae), and *Bouteloua curtipendula* (side-oats grama; Poaceae). A more detailed site description can be found in Patrick et al. (2009), Bell et al. (2009), and Robertson et al. (2009).

The twelve plots were randomly assigned to four different treatments, including: control (C) = natural precipitation; summer (S) = natural precipitation plus 25% supplemental summer precipitation; winter (W) = natural precipitation plus 25% supplemental winter precipitation; and summer + winter (S + W) = natural precipitation plus supplemental 25% in both summer and winter. Watering treatments were applied to simulate fewer, but larger magnitude storm events based on climate model scenarios for this region (Johns et al., 1997;

National Assessment Synthesis Team UGCRP, 2001; Seager et al., 2007). The initial watering (using BIBE groundwater) events in 2002 were calculated as 25% of historic seasonal mean precipitation and applied in August and September (Table 1; Fig. 2). For all subsequent watering events, water was added to the experimental plots as a single large magnitude event in the winter (applied to W and S + W plots in February or early March), and as three distinct large magnitude events in the summer (applied to S and S + W plots in June, July, and August; Table 1). Additional watering amounts for 2003–2008 were determined as 25% of precipitation recorded 3 months prior to the single winter addition, and 1 month prior to each of the three summer additions. Watering occurred at approximately the same dates across the study period (Table 1). Plots were slowly hand watered (with a watering can) to minimize surface runoff. Soil and air temperatures were monitored (daily) across the 7-year study to verify that soil temperatures remained relatively similar among the treatment and control plots.

Soil samples were collected biannually in winter and summer, approximately 1 month after the watering event(s) in 2002–2006, and 1 day prior to watering in Feb and Aug 2007–2008 to better isolate soil responses attributed to additional watering from past years. The intention of our sampling strategy throughout the study was to avoid the initial surge in soil microbial activity known to occur immediately after rain events (Jacobson & Jacobson, 1998; Austin et al., 2004; Collins et al., 2008), thereby allowing us to assess longer term responses of the microbial community. Soil samples were randomly collected in each plot to equally represent soil beneath the dominant species, as well as interplant spaces (soil areas between the basal plant structures). Composite soil samples (consisting of four subsamples) were collected within each plot from 0 to 15 cm depths, sieved through a 2 mm sieve in
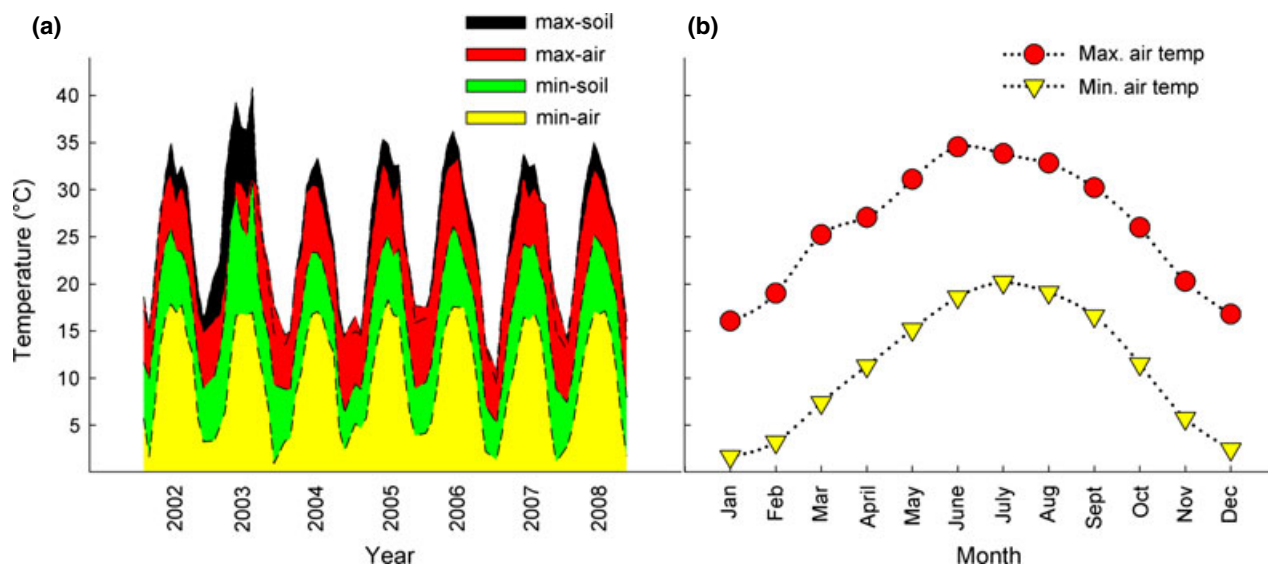

Fig. 1 (a) Average soil and air temperatures (15 cm depth and 1 m above soil surface, respectively) at the Sotol Grassland (1550 masl) in Big Bend National Park (2002–2008). Temperatures were recorded every 36 min. (b) Average monthly air temperature recorded for Panther Junction Visitor Center (1143 masl), Big Bend National Park between 1976 and 2000.
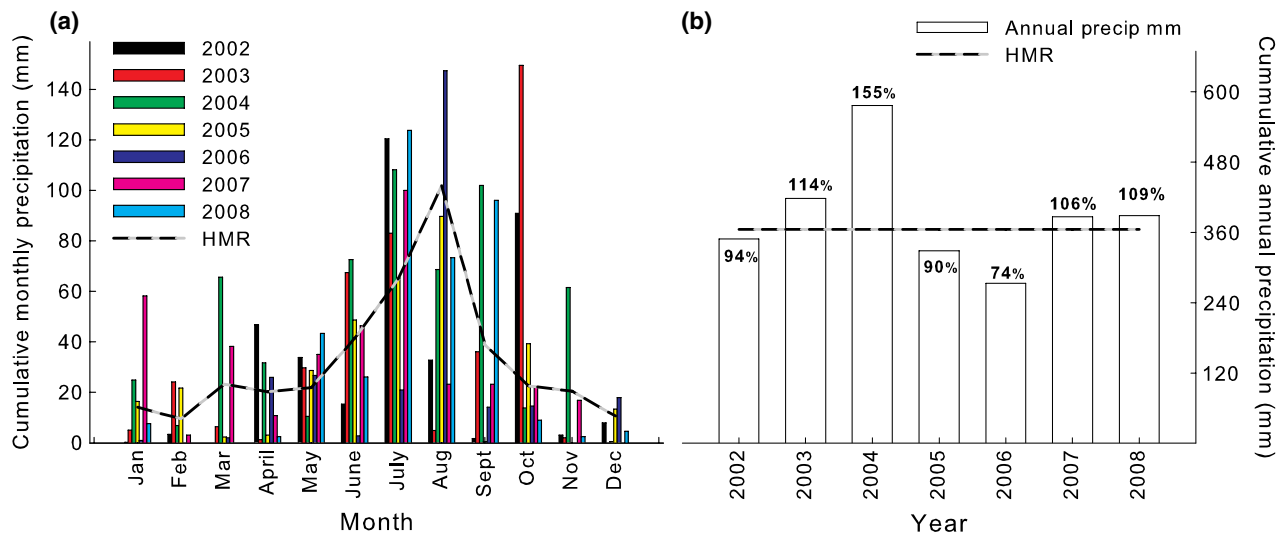
Fig. 2 (a) Monthly precipitation comparing historical mean rainfall (HMR) for 1976–2000 to observed rainfall during the study period (2002–2008). (b) Cumulative annual rainfall during 2002–2008 compared to HMR (1976–2000). Percentages indicate measured annual vs. average precipitation for each year.

the field and stored at 4 °C. All soils were analyzed within 2 weeks of collection.

Prior to the initial water application in 2002, we measured microbial biomass carbon (MBC), soil moisture, soil organic matter (SOM), soil pH, extractable soil NO_3–N, and NH_4–N in each plot to establish baseline values. Soil MBC was measured in years 1–5 (2002–2006). In years 3–5 (2004–2006), enzyme activity and microbial fatty acid methyl ester analysis (FAME) were measured for a more in-depth assessment of microbial community structure and functional dynamics. In years 3–7 (2004–2008), microbial C substrate utilization profiles were measured for a longer term assessment of microbial functional dynamics. The edaphic and soil nutrient parameters measured at the beginning of the experiment (e.g., soil moisture, SOM, soil pH, extractable soil NO_3–N and NH_4–N) were measured every year during the 7-year study. Available soil phosphorus (P) measurements were assessed during years 5–7 (2006–2008).

Microbial biomass carbon and FAME analysis

Microbial biomass carbon (MBC) was measured in 2002–2006 using chloroform fumigation and extraction techniques (Vance *et al.*, 1987). Two replicate, 10 g dry wt. equivalent, subsamples from each composite sample were fumigated with chloroform for 48 h and extracted using 50 ml of a 0.5 M K_2SO_4 solution. Soil extracts were filtered using Fisherbrand-P2 fine-porosity filter paper and measured spectrophotometrically at 280 nm (Nunan *et al.*, 1997).

Fatty acid methyl ester (FAME) analysis was conducted on field-moist soils (2004–2006) following procedures developed for pure culture isolates and soil applications (Acosta-Martinez *et al.*, 2003). Microbial community structure was assessed using methyl ester derivatives from the extracted lipids, in which known microbial markers were recorded as output

peaks via gas chromatography. MIDI Sherlock peak identification software was used to associate fatty acid markers with different microbial functional groups (Sasser, 2001). Microbial community structural groups were categorized by specific lipid markers into fungal and bacterial functional subgroups: saprophytic fungi (SAP) and arbuscular mycorrhizae (AM), gram-positive bacteria (G+), gram-negative bacteria (G−), and *Actinomycete* (Actino) bacteria (Table S1).

Soil microbial enzyme activities and bacterial and fungal carbon utilization profiles

Soil microbial biogeochemical cycling related to enzyme C, N, and P acquisition activities (β-Glucosidase, β-Glucosaminidase, and Phosphodiesterase, respectively), were assayed biannually during the middle 3 years of this study (2004–2006) as described elsewhere (Tabatabai, 1994; Parham & Deng, 2000) using 1 g of soil (<2 mm, air dried) and incubated for 1 h at 25 °C (at pH 6.4). Enzyme activities were assayed in duplicate with one control to which the substrate was added after stopping the reaction. The released product (p-nitrophenol) was determined colorimetrically at 400 nm.

Microbial carbon substrate utilization patterns were assessed in years 3–7 (2004–2008) as described by the Biolog method for bacteria (BSA) (Garland & Millis, 1991; Zak *et al.*, 1994) and the FungiLog method for fungi (FSA) (Sobek & Zak, 2003). In brief, bacterial responses were quantified using soil sample dilutions (10^{-4}), of which 150 μl of the soil dilution was inoculated into each well of the Biolog GN2 96-well microtiter plates. Fungal responses were assessed by inoculating Biolog SFN2 96-well microtiter plates with 100 μl of a 20 ml inoculation mixture containing 50 mg SOM particles, following the Soil FungiLog procedures (Sobek & Zak, 2003). The carbon substrates in the Biolog GN2 and SFN2 96-well microtiter plates (95 different substrates) can be categorized into

Table 1 Supplemental water added to treatment plots over the 7-year study in the Sotol Grasslands of Big Bend National Park in the Chihuahuan Desert. Water additions in summer were calculated as 25% of rainfall received the month prior to the watering event. Water additions in winter were calculated as 25% of total rainfall received in November, January, and February

Year	Month	Season	Volume of H_2O/3 × 3 m Plot (mm)
Year 1 (2002)	Aug 14	Summer	13.0
	Sept 15		13.0
Year 2 (2003)	Mar 1	Winter	6.8
	June 3	Summer	7.4
	July 22		16.9
	Aug 3		20.8
Year 3 (2004)	Feb 4	Winter	6.7
	June 11	Summer	2.6
	July 9		18.1
	Aug 12		27.1
Year 4 (2005)	Feb 19	Winter	19.6
	June 17	Summer	7.2
	July 8		12.2
	Aug 20		16.4
Year 5 (2006)	Feb 19	Winter	3.6
	June 9	Summer	6.7
	July 21		0.7
	Aug 20		5.4
Year 6 (2007)	Feb 24	Winter	19.0
	June 24	Summer	8.8
	July 20		11.6
	Aug 11		25.0
Year 7 (2008)	Feb 23	Winter	8.5
	June 3	Summer	10.8
	July 8		6.5
	Aug 12		16.5

seven different carbon guilds: simple carbohydrates, carboxylic acids, amino acids, complex carbohydrates, polymers, amines-amides, and nucleotides (Dobranic & Zak, 1999; Sobek & Zak, 2003). Functional responses for fungi and bacteria were quantified as the sum of all substrate activity (i.e., total combined activity among all seven different carbon guilds) after a 72-h incubation period at 25 °C (Zak et al., 1994).

Soil abiotic and nutrient properties

Soil moisture was measured by drying soils in an oven at 60 °C for 48 h (Jarrell et al., 1999). Air and soil temperatures (1 m above soil surface and 15 cm depth, respectively) were measured using Onset Computer Corporation HOBO-H8 Pro Series data loggers at 36-min intervals (Onset, 2004), and subsequently averaged categorically by month.

Soil environmental parameters were measured at each sampling period throughout the entire study. SOM was estimated via the loss-on-ignition method (Sollins et al., 1999). Exchangeable soil ammonium was determined via a colorimetric assay using a 50 ml 2 M KCl solution from 5 g field-moist (dry weight equivalent) soil samples (Miller & Keeney, 1982). Levels of extractable soil NO_3–N were determined by A & L Soil Laboratories (Lubbock, TX, USA) using ion-specific probes. Soil pH was measured using a 2 : 1 soil-DI H_2O paste extract with an ion-specific probe (Robertson et al., 1999). During the last 3 years of this study, available soil P measurements were analyzed using Mehlich III soil extraction methods (Mehlich, 1984) from soil subsamples sent to Waters Agricultural Laboratories, Inc. (Owensboro, KY, USA).

Statistical analyses

Multivariate analysis of variance (MANOVA) was used to identify shifts in microbial community structure (FAME), function (enzyme assays and carbon substrate utilization profiles), and soil properties with respect to water treatments. Microbial community and soil parameters were pooled within years to observe intraseasonal responses to watering treatments. Significant multivariate differences suggest that the parameters fit into the model were influenced by shifts in rainfall patterns among the watering treatment plots. Following MANOVA, analysis of variance (ANOVA) and Tukey *post hoc* multiple comparisons were performed to assess univariate differences among watering treatment plots within each sample period. Two-way repeated measures analysis using MANOVA was also performed to assess season, watering treatments, and interactions between these main effects. Repeated measures analysis using MANOVA was chosen over traditional repeated measures analysis because it is free of sphericity assumptions that markedly affect the true Type I error rates and power for the mixed model tests (O'Brien, 1985; Vasey & Thayer, 1987). MANOVA results using the Wilks' Lambda test statistic (Bray & Maxwell, 1982) and Tukey *post hoc* multiple comparisons were considered significant at $P \leq 0.05$.

Pearson correlation coefficients were used to relate seasonal soil properties (SOM, soil inorganic N, soil P, soil pH, and soil moisture) with microbial community function (enzyme activities and bacterial and fungal carbon utilization profiles), and microbial community structure (FAME and MBC). Linear regression analysis was used to model soil moisture and soil P as a function of microbial community function and structure among watering treatment plots. All statistical analyses described above were performed using SPSS v 20.0 (SPSS: IBM Corp., Armonk, NY, USA).

Distance-based redundancy analysis (dbRDA) was used to assess microbial community structure and functional relationships in years 3–5 (2004–2006). Distance-based RDA is a three-step ordination technique that facilitates utilization of optional distance measures (we chose to use Bray's distance) to examine the effects of defined treatments on ecological communities (Legendre & Anderson, 1999). Subsequently, a principal coordinate analysis (PCoA) was performed on the distance matrix, and eigenvalues (obtained in the PCoA) were used within a redundancy analysis (RDA). The Vegan package within R was used for the dbRDA (R.Core.Team, 2013; Oksanen et al., 2011).

Results

Microbial biomass carbon (years 1–5: 2002–2006)

Overall (2002–2006), soil microbial biomass (μg C g^{-1} soil) was significantly higher in the summer (mean $\pm$ SE: 326.03 $\pm$ 30.38) relative to winter (213.2 $\pm$ 12.03; $P < 0.001$). During the first 3 years of the study, MBC did not differ among the additional watering treatment plots. However, in summer 2005 and 2006, MBC significantly increased in S + W watering plots compared to the S plots in 2005, and S and W plots in 2006 ($P \leq 0.03$; Fig. 3). MBC was positively correlated with soil moisture availability among all watering plots ($r = 0.25$ (or higher); $P \leq 0.02$; Fig. 3). In August 2006, MBC increased ca. 2-fold from previous years ($P = 0.05$) following a large soil moisture pulse from a heavy natural rain event in early August. MBC was negatively correlated with SOM in summer; however, these observations were only significant in the C and S plots ($r = -0.56$ (or lower); Table 2a).

FAME analysis (years 3–5: 2004–2006)

There were no seasonal differences in saprophytic or AM fungi, as well as for G− or Actino bacterial abundances. However, gram-positive bacterial relative abundances were significantly higher in summer
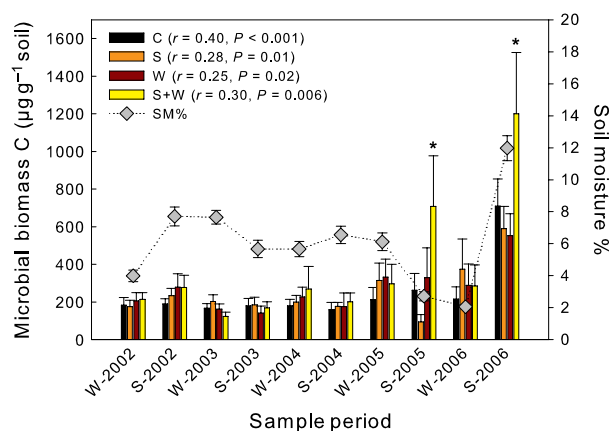

Fig. 3 Microbial biomass carbon (MBC) and soil moisture corresponding to winter and summer season water treatments (Years 1–5; 2002–2006). MBC values are mean $\pm$ SE; $N = 24$. Asterisk above error bars indicate significant differences among treatment plots within sample period at $P \leq 0.05$ using Tukey *post hoc* tests. Note: C, carbon; SM%, soil moisture, watering treatment plot codes: (C, natural rainfall only; S = natural + supplemental summer rainfall; W, natural + additional winter rainfall; S + W = natural + supplemental summer and winter rainfall); r = represents the Pearson correlation coefficient between MBC and soil moisture availability.

(2004–2006 mean $\pm$ SE = 9.49 $\pm$ 0.15) compared to winter (seasonal mean = 8.63 $\pm$ 0.29; as assessed by ANOVA, $P = 0.012$). Likewise, a seasonal trend began to emerge suggesting that bacterial abundances (overall) were negatively correlated with soil moisture availability in the winter, but positively correlated with soil moisture in the summer (2004–2006; Table S2a). Significant multivariate shifts in microbial abundances (overall) suggested increased microbial abundances in the S + W treatment plots during sampling events that correspond to periods experiencing relatively higher soil moisture (in summer 2004 and 2006, and winter 2005; $P \leq 0.05$; Table 3). However, the univariate microbial abundances were highly variable among treatment plots across the study (Table 3).

Across years 3–5, distinct patterns emerged with respect to AM fungi. Additional watering increased the relative abundances of AM fungi in the S + W watering plots in summer 2004, 2006, and in winter 2005 ($P \leq 0.05$; Table 3). F : B was higher in the S + W plots in winter and summer 2006 ($P \leq 0.02$; Table 3). AM fungal abundances were positively correlated with SOM levels in summer in the C and W plots ($r = 0.52$ (or higher); $P \leq 0.05$; Table 2a) and positively correlated with increased soil pH among all treatment plots in the summer ($P < 0.007$; Table 2d). These results suggest that AM fungal abundances may be relatively more responsive to increased seasonal precipitation variability in this desert grassland when compared to the other microbial groups.

Saprophytic fungi, G+, G−, and Actino bacteria mostly demonstrated negative correlations with SOM levels in summer and winter among all treatment plots (Table 2a). Furthermore, in the summer, Actino and G− bacteria predominantly demonstrated negative correlations with soil NO$_3$–N across most plots ($r = -0.42$ (or less); $P \leq 0.02$; Table 2b), but positively correlated with soil NH$_4$–N ($r = 0.6$ (or higher); $P \leq 0.05$; Table 2c). These results suggest that soil bacteria may have a strong influence on summer SOM dynamics with increased precipitation and inorganic N mineralization in the summer season regardless of increased precipitation variability.

Enzyme activities (years 3–5: 2004–2006)

In years 3–5 (2004–2006), microbial C and P acquisition activities (β-Glucosidase and Phosphodiesterase, respectively) trended higher in the S + W watering plots in 2004–2006 (Table 4). For example, β-Glucosidase activity was higher in the S + W treatment when compared to the C plots in summer 2004, 2006, and winter 2005 ($P \leq 0.05$; Table 4). Higher Phosphodiesterase activity was observed in the S + W treatment when

Table 2 Pearson correlations demonstrating seasonal microbial community functional and structural relationships with soil nutrient properties in response to winter and summer water treatment plots in Big Bend National Park in years 3–5 (2004–2006)

			(a) SOM (%)				(b) NO₃-N (ppm)				(c) NH₄-N (ppm)				(d) soil pH			
		Trt:	C	S	W	S + W	C	S	W	S + W	C	S	W	S + W	C	S	W	S + W
Microbial Function	Winter	β-Gluc	−0.06	0.25	**0.65**	−0.20	−0.25	−0.19	−0.27	0.26	0.13	−0.07	−0.23	**−0.53**	0.14	0.40	0.47	0.06
		β - Glsm	−0.36	0.17	0.35	**−0.50**	−0.44	0.34	−0.38	**−0.51**	0.29	**0.62**	0.10	−0.42	0.02	**0.69**	0.06	**−0.78**
		Phos	0.06	0.44	**0.68**	**0.60**	0.15	0.24	0.02	**0.73**	**0.61**	0.15	−0.36	0.05	**0.71**	**0.77**	**0.65**	**0.47**
		BSA	0.43	0.19	0.11	**0.56**	**0.77**	−0.08	0.21	**0.55**	−0.07	−0.07	0.24	−0.02	0.26	−0.02	−0.19	−0.16
		FSA	**0.57**	**0.49**	0.19	**0.65**	**0.58**	0.11	**0.47**	0.38	−0.02	−0.27	−0.25	0.12	0.47	−0.01	0.28	0.30
	Summer	β -Gluc	0.01	−0.37	0.17	−0.07	**−0.51**	−0.11	−0.30	−0.02	0.17	**0.55**	0.10	0.18	0.43	**−0.54**	0.37	−0.24
		β - Glsm	0.11	−0.27	0.09	0.11	−0.38	**0.62**	−0.39	−0.38	−0.06	0.46	0.29	0.33	0.45	−0.13	0.03	−0.32
		Phos	0.15	0.13	0.21	−0.31	−0.41	0.14	−0.33	**−0.78**	0.20	0.08	0.13	**0.66**	**0.85**	**0.49**	**0.56**	0.19
		BSA	−0.24	**−0.76**	−0.43	−0.36	**−0.47**	−0.28	**−0.58**	−0.16	−0.09	**0.79**	**0.68**	0.46	−0.17	−0.30	−0.14	−0.42
		FSA	**−0.67**	**−0.82**	**−0.60**	**−0.70**	−0.44	−0.07	−0.37	**−0.79**	**0.51**	**0.82**	**0.61**	**0.86**	−0.41	−0.24	−0.45	−0.06
Microbial Structure	Winter	Sap	**−0.60**	−0.24	−0.41	−0.06	−0.47	−0.21	−0.17	−0.01	**0.60**	−0.16	0.26	−0.04	−0.10	−0.01	−0.23	**−0.48**
		AM	−0.34	0.35	−0.26	−0.17	−0.04	−0.09	−0.16	−0.04	**0.60**	−0.29	0.24	−0.38	0.13	0.20	0.17	−0.34
		G +	−0.31	−0.38	**−0.54**	−0.06	−0.44	0.03	0.01	**−0.57**	**0.49**	−0.02	0.39	**0.62**	0.09	0.02	−0.36	−0.05
		G −	**−0.48**	**−0.51**	−0.37	0.00	−0.41	0.22	−0.13	0.19	**0.52**	0.08	0.31	−0.16	0.17	−0.01	−0.15	−0.14
		Actino	−0.31	−0.32	−0.45	−0.45	**−0.52**	0.35	−0.22	**−0.51**	0.30	**0.49**	**0.47**	0.26	0.07	0.34	−0.20	−0.14
		F : B	−0.31	0.36	0.13	−0.16	0.34	−0.25	0.02	0.17	0.11	−0.29	−0.17	−0.23	−0.13	0.04	0.29	−0.01
		MBC	0.16	0.35	0.28	−0.07	−0.13	**0.50**	−0.11	0.15	−0.10	**0.52**	−0.25	**−0.51**	0.26	**0.55**	0.35	−0.31
	Summer	Sap	0.16	−0.32	0.02	**−0.55**	0.18	−0.17	−0.20	−0.38	0.28	0.30	0.35	0.28	0.18	−0.42	−0.02	0.17
		AM	**0.66**	0.44	**0.52**	0.26	0.11	−0.21	−0.09	0.16	**−0.51**	**−0.51**	−0.45	**−0.50**	**0.66**	**0.73**	**0.67**	**0.62**
		G +	−0.09	**−0.79**	0.10	−0.04	−0.27	−0.33	0.00	−0.38	−0.28	**0.60**	0.16	0.35	−0.25	−0.35	−0.43	**−0.47**
		G −	−0.28	**−0.61**	−0.30	**−0.49**	**−0.54**	−0.37	**−0.55**	**−0.74**	0.17	**0.61**	**0.71**	**0.62**	0.01	0.06	0.11	0.22
		Actino	−0.32	**−0.78**	−0.24	**−0.51**	**−0.59**	−0.18	−0.42	**−0.78**	0.06	**0.79**	**0.60**	**0.84**	−0.07	−0.24	−0.08	−0.02
		F:B	0.41	**0.61**	**0.51**	0.03	**0.51**	−0.11	−0.01	0.27	0.13	**−0.64**	−0.41	**−0.47**	0.44	0.45	**0.68**	**0.54**
		MBC	**−0.56**	**−0.64**	−0.24	−0.40	**−0.73**	0.27	**−0.54**	**−0.50**	**0.56**	**0.77**	0.42	**0.58**	0.13	−0.25	0.27	**0.47**

Bold indicates significant correlations. Trt, watering treatments: (C, natural rainfall only; S, natural + supplemental summer rainfall; W, natural + supplemental winter rainfall; S + W, natural + supplemental summer and winter rainfall). Dependent soil variables include: SOM, soil organic matter; NO₃–N, extractable soil nitrate; NH₄–N, extractable soil ammonium; and Soil P, soil phosphate. Microbial Community Function Predictors entered into the regression analysis include enzyme activities for β-Gluc, β-Glucosidase; β-Glsm, β-Glucosaminidase; and Phos, Phosphodiesterase with units expressed as (mg p-nitrophenol (kg⁻¹ soil h⁻¹); and BSA, Bacterial C utilization using Biolog; and FSA, Fungal C utilization using FungiLog. Microbial Community Structural Predictors include: Sap, saprophytic fungi; AM, arbuscular mycorrhiza; G+, gram-positive bacteria; G−, gram-negative bacteria; Actino, actinomycete bacteria; FAME F:B ratios, (Sap + AM)/(G+) + (G−) + (Actino); and SM%, soil moisture%.

Table 3 Microbial community structural dynamics (using FAME analysis) in response to season to season water treatments in the Sotol Grasslands in Big Bend National Park in years 3–5 (2004–2006)

FAME (%)	Trt	Year 3 (2004) W (P = 0.24) Mean (SE)	Sig.	S (P = 0.03) Mean (SE)	Sig.	Year 4 (2005) W (P = 0.05) Mean (SE)	Sig.	S (P = 0.34) Mean (SE)	Sig.	Year 5 (2006) W (P = 0.18) Mean (SE)	Sig.	S (P = 0.02) Mean (SE)	Sig.
Sap	C	9.84 (1.02)	0.18	10.31 (0.85)	0.19	6.76 (1.56)^b	**0.01**	16.42 (1.05)	0.70	17.69 (1.04)	0.30	12.43 (0.33)^b	**0.04**
	S	11.19 (1.12)		9.64 (1.39)		14.90 (1.73)^a		15.92 (1.65)		14.82 (1.06)		14.84 (1.21)^{ab}	
	W	9.20 (0.47)		10.10 (0.74)		12.24 (0.87)^{ab}		16.61 (2.05)		15.62 (1.27)		16.37 (0.93)^a	
	S + W	12.91 (1.85)		13.37 (1.83)		16.73 (2.87)^a		14.37 (0.80)		16.92 (1.11)		15.82 (1.11)^{ab}	
AM	C	5.42 (0.83)^b	**0.02**	9.27 (2.01)	0.99	4.58 (0.62)^b	**<0.01**	6.94 (0.63)	0.25	7.48 (1.57)	0.06	4.65 (0.77)^b	**0.01**
	S	6.33 (0.62)^b		9.70 (1.10)		11.17 (1.16)^a		11.40 (3.09)		7.88 (0.85)		4.70 (0.84)^b	
	W	6.51 (1.04)^b		9.03 (1.43)		7.64 (1.41)^{ab}		8.19 (2.60)		8.90 (1.18)		5.18 (0.82)^b	
	S + W	9.63 (0.95)^a		9.31 (0.36)		11.32 (1.00)^a		12.42 (1.26)		11.77 (0.77)		8.38 (0.45)^a	
G+	C	8.41 (0.99)	0.66	9.37 (0.28)^{ab}	**0.03**	4.82 (1.53)^b	**0.05**	9.22 (0.91)	0.74	11.58 (0.87)^a	**0.03**	9.29 (0.30)	0.07
	S	9.43 (0.79)		8.98 (0.36)^{ab}		9.30 (0.57)^a		8.66 (0.47)		10.27 (0.42)^{ab}		10.76 (0.50)	
	W	8.39 (0.56)		9.99 (0.22)^a		6.42 (1.20)^{ab}		9.80 (0.82)		9.87 (0.39)^{ab}		10.10 (0.36)	
	S + W	9.20 (0.38)		8.75 (0.27)^b		6.82 (0.63)^{ab}		9.14 (0.61)		9.06 (0.31)^b		9.77 (0.31)	
G-	C	1.66 (0.10)	0.35	1.73 (0.08)	0.09	1.47 (0.31)	0.13	1.99 (0.47)	0.21	3.15 (0.29)	0.62	2.58 (0.32)^b	**<0.01**
	S	1.78 (0.07)		1.64 (0.05)		2.17 (0.51)		2.84 (0.32)		2.99 (0.33)		3.40 (0.25)^{ab}	
	W	1.77 (0.19)		1.82 (0.09)		1.82 (0.41)		2.64 (0.29)		2.77 (0.14)		3.78 (0.12)^a	
	S + W	3.61 (1.73)		1.91 (0.08)		3.51 (0.98)		3.06 (0.32)		2.78 (0.13)		3.56 (0.20)^a	
Actino	C	1.76 (0.16)	0.43	1.69 (0.26)	0.24	1.94 (0.56)	0.48	1.96 (0.41)	0.87	3.42 (0.32)	0.09	2.34 (0.04)^b	**0.02**
	S	1.94 (0.21)		1.46 (0.09)		2.39 (0.21)		2.27 (0.07)		3.00 (0.25)		2.96 (0.13)^{ab}	
	W	1.63 (0.08)		1.93 (0.13)		1.70 (0.15)		2.05 (0.43)		3.09 (0.16)		3.24 (0.34)^a	
	S + W	2.01 (0.29)		1.64 (0.09)		1.93 (0.13)		1.95 (0.14)		2.52 (0.17)		2.74 (0.10)^{ab}	
FAME F:B ratios	C	1.30 (0.09)	0.43	1.54 (0.19)	0.21	1.52 (0.24)	0.16	2.11 (0.55)	0.91	1.44 (0.16)^b	**0.02**	1.21 (0.07)^b	**<0.01**
	S	1.36 (0.12)		1.61 (0.13)		1.97 (0.31)		1.99 (0.15)		1.40 (0.09)^b		1.14 (0.05)^b	
	W	1.35 (0.12)		1.39 (0.10)		2.09 (0.25)		1.78 (0.23)		1.57 (0.11)^{ab}		1.25 (0.07)^{ab}	
	S + W	1.58 (0.16)		1.85 (0.16)		2.33 (0.14)		1.96 (0.23)		2.03 (0.18)^a		1.51 (0.09)^a	

Responses to water treatments were analyzed using MANOVA followed by univariate ANOVA for all parameters within each sample period. Significant multivariate differences are indicated in the table header under each year with *P* values (**in bold** if significant). Univariate pair-wise differences were calculated using Tukey *post hoc* analysis (also in **bold** if significant). The treatment sample size for all soil parameters (2004–2006) is $N = 24$. DV, dependent variables with (unit of measure); Trt, watering treatments, as in Table 2; Sap, saprophytic fungi; AM, arbuscular mycorrhiza; G+, gram-positive bacteria; G−, gram-negative bacteria; Actino, actinomycete bacteria; FAME F : B ratios, (Sap + AM)/(G+) + (G−) + Actino).

Table 4 Key soil enzymes in response to winter and summer season water treatments in the Sotol Grasslands in Big Bend National Park in years 3–5 (2004–2006)

Ea's (units in footnote)	Trt	Year 3 (2004) Winter (P = 0.24) Mean (SE)	Sig.	Summer (P = 0.03) Mean (SE)	Sig.	Year 4 (2005) Winter (P = 0.05) Mean (SE)	Sig.	Summer (P = 0.34) Mean (SE)	Sig.	Year 5 (2006) Winter (P = 0.18) Mean (SE)	Sig.	Summer (P = 0.02) Mean (SE)	Sig.
β-Gluc	C	284.63 (69.92)	0.70	305.86 (60.09)[b]	0.01	414.60 (79.04)[b]	0.05	443.50 (105.57)	0.38	555.83 (91.39)	0.57	483.19 (66.92)	0.20
	S	385.91 (109.07)		378.13 (63.61)[ab]		507.05 (96.43)[ab]		375.68 (55.19)		517.26 (59.48)		534.31 (77.50)	
	W	429.07 (118.15)		501.57 (63.99)[ab]		604.85 (69.41)[ab]		381.62 (76.54)		435.15 (78.88)		503.61 (22.91)	
	S + W	395.15 (34.44)		643.69 (83.88)[a]		751.42 (81.56)[a]		555.59 (75.60)		562.01 (41.94)		648.67 (42.95)	
β-Glsm	C	66.21 (8.31)	0.94	95.49 (12.10)	0.65	76.21 (4.70)	0.81	81.39 (7.72)	0.18	119.47 (11.87)	0.81	94.26 (6.03)	0.49
	S	61.99 (8.52)		77.67 (14.67)		84.31 (13.29)		67.49 (5.52)		109.39 (21.50)		118.03 (25.77)	
	W	70.28 (10.55)		98.60 (12.50)		93.45 (20.67)		102.64 (15.87)		107.27 (13.36)		123.33 (6.87)	
	S + W	65.00 (9.40)		91.47 (9.82)		88.12 (6.19)		82.10 (11.07)		99.51 (7.67)		104.21 (9.06)	
Phos	C	175.42 (35.26)	0.44	156.78 (30.36)	0.27	118.10 (20.48)[b]	0.05	154.16 (18.64)[b]	0.05	203.81 (41.23)	0.43	189.69 (45.14)[b]	< 0.01
	S	168.98 (24.29)		181.87 (25.64)		198.83 (18.46)[ab]		167.22 (15.77)[ab]		193.86 (28.31)		181.46 (13.21)[b]	
	W	178.53 (21.45)		230.76 (29.17)		235.52 (52.81)[ab]		179.39 (44.47)[ab]		149.99 (9.94)		250.50 (32.12)[b]	
	S + W	222.19 (14.75)		203.09 (19.64)		237.96 (21.05)[a]		267.67 (28.68)[a]		159.90 (14.15)		352.52 (15.84)[a]	

Responses to water treatments were analyzed using MANOVA for all parameters within each year. Significant multivariate (within season) and pair-wise differences are indicated in the table header under each year with P-values (in bold if significant). Pair-wise differences were calculated using Tukey *post hoc* analysis. The annual treatment sample size for all soil parameters (2004–2006) is N = 24. Watering treatments are as described in Table 2. Enzyme Activity (Ea's) units are expressed as (mg p-nitrophenol (kg⁻¹ soil h⁻¹); β-Gluc, β-Glucosidase activity; β-Glsm, β-Glucosaminidase activity; and Phos, Phosphodiesterase activity.

compared to the C plots in winter 2004 and summer 2005, and 2006 ($P \leq 0.05$; Table 4). In the summer and winter, microbial P enzyme acquisition activities were positively correlated with temporal soil moisture availability (%) in the S + W plots (Table S2b). Phosphodiesterase activity was positively correlated with soil pH in both summer and winter among all treatment plots (Table 2d). There were no significant seasonal differences in soil microbial C-, N-, or P-degrading enzyme activities. However, multivariate differences in enzyme activities among treatment plots were observed in the periods experiencing relatively higher seasonal soil moisture (i.e., summer 2004, 2006, and winter 2005; $P \leq 0.05$; Table 4).

Annual shifts in microbial community structure and function (2004–2006)

In years 3–5, microbial community structure and function (i.e., MBC, FAMEs, and soil enzyme activity) in the S + W plots significantly differed from the C plots (as assessed using dbRDA; (a) 2004 and (b) 2005: $P = 0.005$; (c) 2006: $P \leq 0.03$; Fig. S1a–c). These findings demonstrated that an overall shift in microbial community structure and function was initiated after 2 years in 2004 and persisted through 2006 in response to supplemental water treatments, regardless of natural climate variability (i.e., rainfall or drought) over these 3 years.

Soil bacterial and fungal carbon utilization (2004–2008)

Soil bacterial and fungal carbon utilization did not exhibit significant seasonal differences. However, FSA was highly negatively correlated with SOM in summer among all plots ($r = -0.67$ (or less); $P \leq 0.009$; Table 2a) and positively correlated with SOM in winter ($r = 0.49$ (or higher), $P \leq 0.038$, Table 2a; note: winter correlations were only significant in the C, S, and S + W plots). BSA and FSA were positively correlated with temporal soil moisture availability among all treatment plots ($r = 0.33$ (or higher); $P \leq 0.01$) with the strongest correlations observed in the S + W plots (Fig. 4a and b). A pattern emerged in which FSA and BSA steadily declined with decreased soil moisture availability from winter 2004 through winter 2006. However, in late summer 2006, FSA and BSA increased in response to a large rain event that occurred in August (Fig 4a,b). Following this rain event, BSA levels declined with soil moisture (Fig. 4a), while higher FSA levels persisted for the remaining 2 years of the study, regardless of fluctuating soil moisture patterns (Fig 4b). These results suggest that soil bacterial C cycling is dependent on short-term temporal soil moisture pulses. Fungi maintain relatively higher C cycling functionality compared
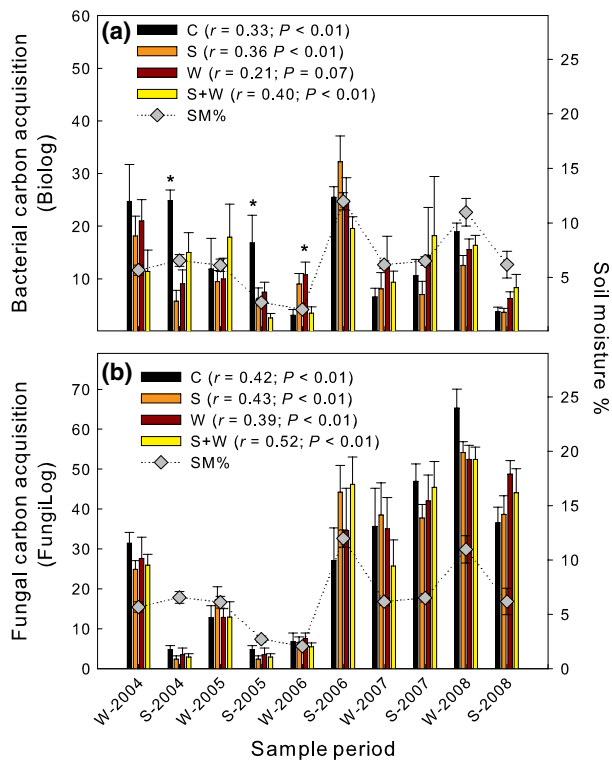
Fig. 4 Years 3–7 soil microbial community function and soil moisture levels corresponding to winter and summer season water treatments for 2004–2008. Soil microbial community function is represented as (a) bacterial and (b) fungal carbon substrate utilization using Biolog and FungiLog procedures, respectively, after 72-h incubations. Bacterial and fungal community functional values are mean ± SE; $N = 24$. Asterisk above error bars indicate significant differences among treatment plots within sample period at $P \leq 0.05$ using Tukey *post hoc* tests. Note: Biolog, bacterial C utilization; FungiLog, fungal C utilization; SM%, soil moisture, watering treatment plot codes: (C, natural rainfall only; S = natural + supplemental summer rainfall; W = natural + additional winter rainfall; S + W = natural + supplemental summer and winter rainfall), r = represents Pearson correlation coefficients between microbial C acquisition within each watering treatment and soil moisture availability.

to bacteria for much longer periods (potentially years) after an unusually large rainfall event regardless of successive rainfall patterns.

SOM, inorganic N, and soil pH (years 1–7: 2002–2008)

There was a 2.5 year lag in edaphic responses to supplemental watering (Table 5). Multivariate differences in SOM, soil inorganic N, and soil pH were observed among the treatments in summer 2004, and winter and summer 2005 ($P \leq 0.03$; Table 5). These differences persisted throughout winter and summer sample periods of 2007 and 2008 ($P \leq 0.03$; Table 5). Across the 7-year

study (2002–2008), soil pH was significantly higher in the winter (mean ± SE: 6.18 ± 0.04) compared to the summer (mean ± SE: 6.06 ± 0.04; $P = 0.021$). However, seasonal soil moisture was not significantly correlated with soil pH (Table S2). Furthermore, beginning in Year 4, soil pH trended higher in the S + W plots across the study ($P \leq 0.02$; Table 5), and this pattern persisted throughout 2008. Across years 3–5, AM fungi and Phosphatase enzyme activities were significantly correlated with soil pH (Table 2; Table S5).

Overall, SOM demonstrated inverse seasonal trends with soil moisture availability, and was positively correlated with temporal soil moisture availability in the winter ($r = 0.58$ (or higher); $P \leq 0.012$; Table S2). SOM was negatively correlated with increased soil moisture availability in the summer ($r = -0.52$ (or less); $P \leq 0.028$; Table S2). Inorganic soil N was highly variable among the supplemental watering plots (Table 5). However, a trend emerged in which soil NO_3–N was negatively correlated with soil moisture availability across most watering plots in the summer ($r = -0.56$ (or less); $P \leq 0.015$; Table S2), while NH_4–N demonstrated the inverse pattern ($r = 0.50$ (or higher); $P \leq 0.034$; Table S2), suggesting that soil moisture availability has a strong influence on soil inorganic N dynamics.

Soil P (Years 5–7: 2006–2008)

Soil P was not significantly correlated with seasonal soil moisture availability (Fig. 5). However, soil P levels were significantly lower in the S + W additional watering plots in winter 2006, summer 2007, and in winter and summer 2008 (Fig. 5). In winter, MBC and β-Glucosaminidase activity demonstrated strong positive relationships with soil P ($r = 0.76$ (or higher); $P \leq 0.02$; Table S3a). In summer, β-Glucosidase along with fungal abundances demonstrated the strongest relationships with soil P among most treatment plots ($r^2 = 0.67$ (or higher); $P \leq 0.047$; Table S3b; Table S4). Overall, declines in soil P levels observed in the additional watering plots strongly correspond to specific microbial activities predominantly occurring in the summer season.

Discussion

Soil microbial communities in the sotol grassland of Pine Canyon at Big Bend National Park experienced highly variable rainfall patterns across this 7-year study. Regardless of the naturally high rainfall variability that occurs at this grassland site (Wondzell & Ludwig, 1995; Bell *et al.*, 2009), we hypothesized that a persistent shift in the magnitude of seasonal

Table 5 Key soil responses to winter and summer season water treatments in the Sotol Grasslands in Big Bend National Park in years (2002–2008)

Soil Parameter (see units below)	Trt	Year 1 (2002) Winter (P = 0.61) Mean (SE)	Sig.	Year 1 (2002) Summer (P = 0.08) Mean (SE)	Sig.	Year 2 (2003) Winter (P = 0.21) Mean (SE)	Sig.	Year 2 (2003) Summer (P = 0.06) Mean (SE)	Sig.	Year 3 (2004) Winter (P = 0.56) Mean (SE)	Sig.	Year 3 (2004) Summer (P = 0.01) Mean (SE)	Sig.	Year 4 (2005) Winter (P = 0.03) Mean (SE)	Sig.	Year 4 (2005) Summer (P = 0.01) Mean (SE)	Sig.
SM (%)	C	3.84 (0.23)	0.82	7.68 (0.65)	0.93	8.10 (0.32)	0.15	5.91 (0.54)	0.46	6.77 (0.58)	0.1	7.08 (0.74)	0.09	6.01 (0.18)	0.43	3.04 (0.29)	0.35
	S	3.89 (0.43)		7.25 (0.38)		6.76 (0.30)		4.99 (0.47)		5.20 (0.36)		6.43 (0.35)		5.26 (0.33)		2.54 (0.28)	
	W	4.07 (0.42)		7.88 (0.77)		7.72 (0.59)		6.20 (0.64)		5.29 (0.43)		5.32 (0.35)		6.64 (0.67)		2.36 (0.27)	
	S + W	4.16 (0.40)		8.02 (0.57)		7.99 (0.51)		5.57 (0.54)		5.41 (0.53)		7.37 (0.72)		6.55 (1.03)		2.90 (0.33)	
SOM (%)	C	6.52 (0.40)	0.81	6.83 (0.35)	0.67	5.85 (0.27)	0.44	7.90 (0.39)	0.35	8.27 (0.39)	0.29	7.37 (0.47)	0.08	7.70 (0.59)	0.63	7.88 (0.44)	0.13
	S	6.04 (0.23)		6.89 (0.23)		6.03 (0.39)		7.44 (0.30)		6.74 (0.31)		5.89 (0.28)		7.35 (0.41)		5.96 (0.21)	
	W	6.67 (0.59)		6.84 (0.71)		6.48 (0.55)		7.35 (0.69)		7.45 (0.86)		5.79 (0.44)		7.59 (0.74)		6.79 (0.70)	
	S + W	6.73 (0.43)		6.21 (0.79)		6.67 (0.32)		6.71 (0.31)		8.15 (0.71)		7.25 (0.52)		6.74 (0.45)		7.17 (0.69)	
NO$_3$–N (ppm)	C	26.14 (0.82)	0.16	6.45 (0.99)	0.17	21.43 (1.15)	0.11	4.03 (0.35)	0.31	2.87 (0.06)	0.98	3.63 (0.24)b	0.04	2.41 (0.26)	0.12	3.87 (0.35)b	< 0.01
	S	31.63 (0.76)		4.80 (0.97)		15.77 (2.82)		3.11 (0.61)		2.81 (0.46)		3.94 (0.22)ab		2.33 (0.15)		3.92 (0.41)b	
	W	30.84 (0.43)		8.47 (1.59)		19.09 (1.98)		3.26 (0.31)		2.72 (0.45)		8.04 (2.17)a		2.73 (0.16)		7.77 (0.94)a	
	S + W	29.25 (0.28)		6.24 (0.76)		15.77 (1.16)		4.46 (0.82)		2.68 (0.21)		5.84 (0.52)ab		3.12 (0.34)		4.20 (0.26)b	
NH$_4$–N (ppm)	C	4.19 (0.82)	0.36	4.19 (0.82)	0.12	6.86 (2.23)	0.49	7.67 (2.94)	0.74	10.38 (3.28)	0.89	1.66 (0.68)	0.61	2.67 (0.77)	0.07	8.99 (2.67)	0.13
	S	5.56 (0.99)		5.56 (0.99)		5.75 (0.73)		5.07 (1.30)		9.19 (3.66)		1.62 (0.67)		1.99 (0.44)		4.19 (1.03)	
	W	6.37 (0.90)		6.37 (0.90)		9.01 (3.56)		6.30 (0.78)		12.11 (0.97)		3.74 (2.27)		4.48 (1.51)		5.67 (1.33)	
	S + W	5.56 (0.73)		5.56 (0.73)		4.41 (0.78)		5.84 (0.61)		10.32 (1.51)		2.76 (0.73)		6.01 (1.29)		3.82 (0.80)	
pH (H+ ion)	C	5.80 (0.16)	0.35	5.90 (0.25)	0.12	5.82 (0.24)	0.31	5.84 (0.46)	0.17	6.20 (0.40)	0.29	5.69 (0.23)	0.12	5.78 (0.05)b	0.01	5.90 (0.10)b	0.02
	S	5.89 (0.11)		5.80 (0.09)		5.93 (0.12)		6.54 (0.28)		6.20 (0.13)		6.23 (0.12)		6.34 (0.06)ab		6.36 (0.23)ab	
	W	6.25 (0.23)		6.05 (0.25)		6.20 (0.21)		5.71 (0.27)		6.21 (0.26)		5.88 (0.08)		6.27 (0.22)ab		5.95 (0.19)b	
	S + W	6.33 (0.20)		6.30 (0.19)		6.27 (0.18)		6.75 (0.45)		6.92 (0.38)		6.22 (0.23)		6.56 (0.21)a		6.78 (0.22)a	

Table 5 (Continued)

Soil Parameter (see units below)	Trt	Year 5 (2006) Winter (P = 0.11) Mean (SE)	Sig.	Summer (P = 0.14) Mean (SE)	Sig.	Year 6 (2007) Winter (P < 0.001) Mean (SE)	Sig.	Summer (P = 0.008) Mean (SE)	Sig.	Year 7 (2008) Winter (P = 0.03) Mean (SE)	Sig.	Summer (P < 0.001) Mean (SE)	Sig.
SM (%)	C	2.29 (0.14)	0.07	11.79 (0.92)	0.47	6.64 (0.39)[ab]	<0.01	6.51 (0.25)	0.5	10.89 (1.29)	0.96	6.76 (1.26)	0.81
	S	1.81 (0.09)		11.37 (0.85)		5.36 (0.21)[b]		5.99 (0.32)		10.44 (1.36)		5.54 (1.11)	
	W	1.98 (0.15)		11.68 (0.55)		5.37 (0.24)[b]		6.68 (0.50)		11.37 (1.19)		5.61 (1.18)	
	S + W	2.15 (0.12)		13.06 (0.82)		7.29 (0.54)[a]		6.84 (0.49)		11.18 (1.20)		6.79 (1.31)	
SOM (%)	C	4.67 (0.48)	0.97	3.68 (0.31)	0.5	3.65 (0.15)[a]	0.01	4.53 (0.36)	0.09	4.08 (0.16)	0.51	4.69 (0.21)[a]	**0.01**
	S	4.40 (0.66)		3.38 (0.20)		2.75 (0.15)[b]		3.47 (0.20)		3.58 (0.38)		3.98 (0.09)[b]	
	W	4.48 (0.68)		3.85 (0.20)		2.97 (0.29)[ab]		4.10 (0.39)		3.57 (0.28)		4.28 (0.15)[ab]	
	S + W	4.27 (0.49)		4.08 (0.50)		3.48 (0.13)[ab]		4.68 (0.40)		3.80 (0.20)		4.64 (0.19)[ab]	
NO$_3$-N (ppm)	C	1.80 (0.18)	0.17	2.29 (0.26)	0.16	6.79 (1.41)[b]	<0.01	6.31 (0.56)[b]	<0.01	22.25 (2.84)	0.6	54.17 (5.55)	0.3
	S	2.54 (0.70)		3.59 (1.04)		7.30 (0.64)[b]		7.51 (1.71)[b]		19.79 (2.39)		41.15 (5.09)	
	W	2.51 (0.20)		2.70 (0.39)		9.20 (0.54)[b]		12.52 (1.38)[ab]		24.83 (3.34)		53.47 (6.52)	
	S + W	1.48 (0.14)		1.69 (0.18)		12.05 (0.79)[a]		17.68 (3.74)[a]		20.79 (2.31)		43.66 (6.38)	
NH$_4$-N (ppm)	C	15.45 (2.26)	0.26	19.83 (0.84)	0.28	1.99 (0.24)	0.82	0.69 (0.29)	0.58	1.84 (0.28)	0.85	8.45 (2.28)	0.22
	S	20.80 (8.20)		22.05 (1.53)		2.80 (0.39)		0.88 (0.23)		1.89 (0.32)		5.89 (1.35)	
	W	20.33 (3.26)		19.60 (1.16)		2.64 (0.39)		0.87 (0.27)		2.15 (0.42)		11.57 (2.53)	
	S + W	8.96 (1.13)		18.90 (1.01)		2.69 (1.18)		1.18 (0.19)		2.56 (1.14)		11.92 (2.81)	
pH (H+ ion)	C	5.82 (0.21)	0.28	5.65 (0.26)[b]	**0.01**	6.20 (0.22)[ab]	0.01	5.95 (0.18)[b]	<0.01	5.73 (0.19)[c]	<0.01	5.56 (0.13)[c]	<0.01
	S	6.37 (0.27)		6.05 (0.06)[ab]		6.32 (0.09)[ab]		6.18 (0.05)[ab]		6.18 (0.12)[bc]		6.08 (0.04)[ab]	
	W	5.88 (0.25)		5.88 (0.14)[b]		5.98 (0.12)[b]		6.02 (0.15)[b]		6.39 (0.12)[b]		5.73 (0.13)[bc]	
	S + W	6.27 (0.19)		6.48 (0.12)[a]		6.77 (0.13)[a]		6.60 (0.07)[a]		7.07 (0.21)[a]		6.30 (0.12)[a]	

Responses to water treatments were analyzed using MANOVA for all parameters within each year. Significant multivariate and pair-wise differences are indicated in the table header under each year with *P*-values (in **bold** if significant). Pair-wise differences were calculated using Tukey *post hoc* analysis. The annual treatment sample size for all soil parameters (2002–2008) is *N* = 24. Note: Watering treatments are as described in Table 2. Soil Parameter, dependent variables; SM, gravimetric soil moisture; SOM, soil organic matter; NO$_3$–N, extractable soil nitrate; NH$_4$–N, extractable soil ammonium; and pH, soil hydrogen ion concentrations (pH).
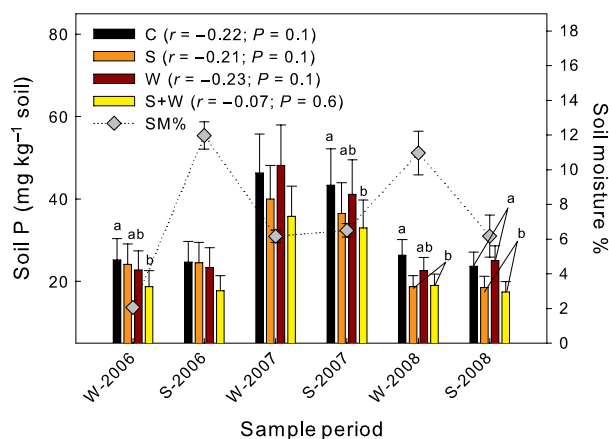
Fig. 5 Soil phosphorus levels corresponding to winter and summer season water treatments for 2006–2008. Responses to water treatments were analyzed using MANOVA using treatment groups as the fixed factor and each sample period as a dependent variable. Letters (in bold) above error bars indicate significant differences among treatment plots within sample period at $P \leq 0.05$ using Tukey *post hoc* tests. The annual treatment sample size for all soil parameters (2002–2008) is $N = 24$. Note: P, phosphorus, SM%, soil moisture, watering treatment plot codes: (C, natural rainfall only; S = natural + supplemental summer rainfall; W = natural + additional winter rainfall; S + W = natural + supplemental summer and winter rainfall).

precipitation, provided as one large winter event and three large summer events, would (over time) shift microbial community structure toward higher F : B and increased fungal abundances, which would subsequently influence soil biogeochemical cycling and soil nutrient availability (Schwinning & Sala, 2004; Owens *et al.*, 2012; Placella *et al.*, 2012). We found that increased supplemental seasonal precipitation, particularly during naturally wet periods, initiated higher microbial biomass carbon, higher F : B (as a result of increased fungal abundances), higher P-degrading enzyme activities and (to a lesser degree) higher C-degrading enzyme activities. Furthermore, higher fungal abundances and soil enzyme activities in the additional watering plots were significantly correlated with soil pH and lower soil P levels (Table S3–S5). These findings indicate that a modest (but sustained) 25% increase in seasonal precipitation can substantially modify soil microbial functional and structural properties as well as soil nutrient availability (Allison *et al.*, 2007; Borken & Matzner, 2009b; Henry, 2013). In turn, these factors can influence plant species success, and ultimately alter ecosystem function in this desert grassland (Kardol *et al.*, 2010; Wu *et al.*, 2011; Wallenstein & Hall, 2012).

Bacterial functional and structural characteristics (as a whole) did not strongly respond to supplemental seasonal watering treatments, but rather, mimicked

natural precipitation patterns by exhibiting higher activity during relatively wetter seasonal periods. These findings suggest that soil bacterial communities are more strongly influenced by short-term temporal rainfall variability relative to persistent, longer term shifts in seasonal rainfall patterns. On the contrary, although soil fungi appeared less responsive to short-term soil moisture pulses, a 25% increase to historic seasonal precipitation was sufficient to significantly increase fungal abundances (along with soil P and C cycling characteristics) between years 3 and 5 of this 7-year study.

Shifts in AM and saprophytic fungal abundances, microbial functional dynamics, and soil nutrient and edaphic properties were triggered (and persisted) by a modest (25%) increase in seasonal precipitation regardless of the high interannual and intraannual rainfall variability that occurred during this 7-year study. These findings strongly suggest that ecosystem functioning will shift in this desert grassland in response to future climate change. For example, AM fungi, as plant symbionts, are particularly important components of belowground biogeochemical cycling in arid ecosystems (Collins *et al.*, 2008; Crenshaw, 2008; Wu, 2010) because they facilitate more effective water and nutrient uptake for their plant hosts (Allen, 2009; Manzoni *et al.*, 2012). Likewise, shifts in AM fungal abundances have been suggested to promote the fitness of some C_4 grass species (Püschel *et al.*, 2007; Wu, 2010; Klironomos *et al.*, 2011) by influencing nutrient availability (Schlesinger, 1996; Wardle *et al.*, 1999; Mcguire *et al.*, 2010) and water uptake (Collins *et al.*, 2008; Allen, 2009; Owens *et al.*, 2012), corresponding to increased grass densities (Hetrick *et al.*, 1990; Pezzani, 2006; Owens *et al.*, 2012). Therefore, future changes in seasonal precipitation could influence specific plant species successes (Reynolds *et al.*, 2004; Schwinning & Sala, 2004; Miller *et al.*, 2012) by promoting plant–microbe (AM fungal) symbiotic relationships.

Past studies at this research site in Big Bend National Park reported increased *Bouteloua curtipendula* (C_4 grass) densities as well as higher soil pH levels in response to increased summer and winter precipitation (Robertson *et al.*, 2009, 2010). Although soil microbial communities typically have the capacity to self-regulate in response to environmental change (Walker, 1992; Naeem, 1997; Reynolds *et al.*, 2004; Sistla & Schimel, 2012), shifts in climate, edaphic properties, and specific plant species interactions can nonetheless generate soil feedbacks by influencing microbial community structural and/or functional characteristics (Schwinning & Sala, 2004; De Graaff *et al.*, 2010; Placella *et al.*, 2012; Bell *et al.*, 2013; Fanin *et al.*, 2013). For example, plant species can influence microbial communities and

extracellular enzyme activities via root exudates as well as by shifting soil pH within the rhizosphere zone (Dinkelaker et al., 1989; Richardson et al., 2009). Likewise, changes in microbial community structure, function, and soil chemistry can influence soil nutrient dynamics, thus promoting plant nutrient uptake (Dinkelaker & Marschner, 1992; Treseder & Vitousek, 2001) and root growth (Bever et al., 2010). Our research findings indicate that increased seasonal rainfall for ≥3 year is sufficient to increase AM fungal abundances and subsequently lower soil P levels in the S + W plots. Increased plant densities in response to additional seasonal watering (i.e., observed by Robertson et al. (2010)) is likely due (at least in part) to increased water and soil P availability as facilitated by AM fungal associations. We suggest that the lower soil P levels observed in the S + W watering plots may be due (at least in part) to increased plant and microbial P uptake.

Plants are well known to facilitate the mobilization of soil P and Fe primarily through rhizosphere exudation (Dinkelaker et al., 1989; Richardson et al., 2009). Although we did not directly examine plant community rhizosphere exudate characteristics, the higher soil pH levels observed in the S + W watering plots were well within the range considered to be effective for mobilizing P bound in Fe and Ca organometallic complexes (Gerke, 1993; Gustafsson et al., 2012). Therefore, our research findings suggest that alterations in seasonal precipitation influence soil P mineralization as well as plant and microbial P assimilation.

The increased grass densities reported by Robertson et al. (2010) in 2005 and 2006 could indicate positive soil P feedbacks (Bever, 2002) with increased seasonal watering. In this scenario, the lower soil P levels observed in this study (in S + W plots) could be sufficient to escalate plant interspecific competition for soil P, and ultimately result in decreased plant diversity (Bever, 2003; Levine et al., 2006). Alternatively, soil P may be taken up equally (generally speaking) among plant species in the S + W plots (and/or potentially lost via soil leaching), suggesting negative soil feedbacks (Bever, 2002) which would promote plant species coexistence (Diez et al., 2010; Harrison & Bardgett, 2010). In the latter scenario, increased competition for water would be the likely mechanism for increased plant success. However, we did not directly test potential competitive relationships among plant species by conducting nutrient fertilization experiments or assessing plant tissue P. Furthermore, assessing soil organic P pools (extracted by NaOH and NaHCO₃ using fractionation methods) would likely provide a higher resolution of potential P cycling dynamics (Lajtha & Schlesinger, 1988; Condron et al., 2005) to better assess potential positive vs. negative feedbacks related to

shifts in seasonal precipitation. Nevertheless, our findings suggest that plant–microbe feedbacks will be highly deterministic for ecosystem functioning in response to climate change in this desert grassland (Bever, 2002; Diez et al., 2010).

In conclusion, our results suggest that a 25% increase in seasonal rainfall in this desert grassland will alter microbial community structure (wider F : B via increased fungal abundances), microbial functional dynamics (soil C and P cycling activities), and soil nutrient and edaphic properties. Likewise, prolonged shifts in seasonal precipitation may decrease soil microbial community diversity toward a more fungal-dominated system (Carson et al., 2010; Hawkes et al., 2011). AM fungal symbionts that facilitate plant nutrient and water uptake are highly responsive to alterations in seasonal precipitation (Collins et al., 2008; Schaeffer et al., 2011; Miller et al., 2012). Therefore, plant–microbe interactions may be substantially influenced by climate variability in this region (Knapp et al., 2006; Bernstein et al., 2007), which could alter existing plant–soil feedbacks and potentially stimulate interspecific plant competition for soil nutrients (Klironomos, 2002; Johnson et al., 2004; Klironomos et al., 2011). It is yet to be determined whether the shifts in nutrient or microbial characteristics will introduce soil feedbacks promoting above- and belowground competitive exclusions in this desert grassland. Finally, we acknowledge that intermittent breaks in data continuity may somewhat lessen the power of inference in this study. However, we feel that the ≥3 year of seasonal data presented here (for any given parameter) provides substantial insight into potential soil microbial and nutrient responses to shifts in seasonal precipitation.

References

Acosta-Martinez V, Zobek TM, Gill TE (2003) Enzyme activities and microbial community structure in semiarid agricultural soils. Biology and Fertility of Soils, 38, 216–227.

Aide M, Aide C, Dolde J, Guffey C (2003) Geochemical indicators of external additions to soils in Big Bend National Park, Texas. Soil Science, 168, 200–208.

Allen MF (2009) Bidirectional water flows through the soil–fungal–plant mycorrhizal continuum. New Phytologist, 182, 290–293.

Allison VJ, Condron LM, Peltzer DA, Richardson SJ, Turner BL (2007) Changes in enzyme activities and soil microbial community composition along carbon and nutrient gradients at the Franz Josef chronosequence, New Zealand. *Soil Biology and Biochemistry*, **39**, 1770–1781.

Austin AT, Yahdjian ML, Stark JM *et al.* (2004) Water pulses and biogeochemical cycles in arid and semiarid ecosystems. *Oecologia*, **141**, 221–235.

Behan-Pelleteir VM, Newton G (1999) Linking soil biodiversity and ecosystem function-The taxonomic dilemma. *BioScience*, **49**, 149–153.

Bell CW, Acosta-Martinez V, Mcintyre N, Cox S, Tissue DT, Zak JC (2009) Linking microbial community structure and function to seasonal differences in soil moisture and temperature in a Chihuahuan Desert grassland. *Microbial Ecology*, **58**, 827–842.

Bell C, Carrillo Y, Boot CM, Rocca JD, Pendall E, Wallenstein MD (2013) Rhizosphere stoichiometry: are C:N:P ratios of plants, soils, and enzymes conserved at the plant species-level? *New Phytologist*, in press.

Bernstein L, Bosch P, Canziani O *et al.* (2008) *Climate Change 2007: Synthesis Report: An Assessment of the Intergovernmental Panel on Climate Change* (eds Allali A, Bojariu R, Diaz S, Elgizouli I, Griggs D, Hawkins D, Hohmeyer O, Jallow BP, Kajfez-Bogataj L, Leary N, Lee H, Wratt D), IPCC, Valencia, Spain.

Bever JD (2002) Negative feedback within a mutualism: host–specific growth of mycorrhizal fungi reduces plant benefit. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **269**, 2595–2601.

Bever JD (2003) Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. *New Phytologist*, **157**, 465–473.

Bever JD, Dickie IA, Facelli E *et al.* (2010) Rooting theories of plant community ecology in microbial interactions. *Trends in Ecology & Evolution*, **25**, 468–478.

Borken W, Matzner E (2009a) Introduction: impact of extreme meteorological events on soils and plants. *Global Change Biology*, **15**, 781.

Borken W, Matzner E (2009b) Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. *Global Change Biology*, **15**, 808–824.

Bray JH, Maxwell SE (1982) Analyzing and interpreting significant MANOVAs. *Review of Educational Research*, **52**, 340–367.

Breshears DD, Huxman TE, Adams HD, Zou CB, Davidson JE (2008) Vegetation synchronously leans upslope as climate warms. *PNAS*, **105**, 11591–11592.

Carson JK, Gonzalez-Quiñones V, Murphy DV, Hinz C, Shaw JA, Gleeson DB (2010) Low pore connectivity increases bacterial diversity in soil. *Applied and Environmental Microbiology*, **76**, 3936.

Chapin FS, Walker BH, Hobbs RJ, Hooper DU, Lawton JH, Sala OE, Tilman D (1997) Biotic control over the functioning of ecosystems. *Science*, **277**, 500–504.

Coe KK, Belnap J, Sparks JP (2012) Precipitation-driven carbon balance controls survivorship of desert biocrust mosses. *Ecology*, **93**, 1626–1636.

Collins SL, Sinsabaugh R, Crenshaw C, Green L, Porras-Alfaro A, Stursova M, Zeglin LH (2008) Pulse dynamics and microbial processes in aridland ecosystems. *Journal of Ecology*, **96**, 413–420.

Condron LM, Turner BL, Cade-Menun BJ (2005) Chemistry and dynamics of soil organic phosphorus. In: *Phosphorus: Agriculture and the Environment* (eds Sims JT, Sharpley AN), pp. 87–121. American Society of Agronomy, Madison, WI.

Cregger MA, Schadt CW, Mcdowell NG, Pockman WT, Classen AT (2012) Response of the soil microbial community to changes in precipitation in a semiarid ecosystem. *Applied and Environmental Microbiology*, **78**, 8587–8594.

Crenshaw CL (2008) Fungal control of nitrous oxide production in semiarid grassland. *Biogeochemistry*, **87**, 17.

Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, **440**, 165–173.

De Boer W, Folman LB, Summerbell RC, Boddy L (2005) Living in a fungal world: impact of fungi on soil bacterial niche development★. *FEMS Microbiology Reviews*, **29**, 795–811.

De Graaff M-A, Classen AT, Castro HF, Schadt CW (2010) Labile soil carbon inputs mediate the soil microbial community composition and plant residue decomposition rates. *New Phytologist*, **188**, 1055–1064.

Dechesne A, Owsianiak M, Bazire A, Grundmann GL, Binning PJ, Smets BF (2010) Biodegradation in a Partially Saturated Sand Matrix: compounding Effects of Water Content, Bacterial Spatial Distribution, and Motility. *Environmental Science & Technology*, **44**, 2386–2392.

Diez JM, Dickie I, Edwards G, Hulme PE, Sullivan JJ, Duncan RP (2010) Negative soil feedbacks accumulate over time for non-native plant species. *Ecology Letters*, **13**, 803–809.

Dinkelaker B, Marschner H (1992) In vivo demonstration of acid phosphatase activity in the rhizosphere of soil-grown plants. *Plant and Soil*, **144**, 199–205.

Dinkelaker B, Römheld V, Marschner H (1989) Citric acid excretion and precipitation of calcium citrate in the rhizosphere of white lupin (Lupinus albus L.). *Plant, Cell & Environment*, **12**, 285–292.

Dobranic JK, Zak JC (1999) A microtiter plate procedure for evaluating fungal functional diversity. *Mycologia*, **91**, 756–765.

Easterling DR, Meehl GA, Parmesan C, Changnon SA, Karl TR, Mearns LO (2000) Climate extremes: observations, modeling, and impacts. *Science*, **289**, 2068–2074.

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

Fanin N, Fromin N, Buatois B, Hättenschwiler S (2013) An experimental test of the hypothesis of non-homeostatic consumer stoichiometry in a plant litter–microbe system. *Ecology Letters*, **16**, 764–772.

Garland JL, Millis AL (1991) Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. *Applied and Environmental Microbiology*, **57**, 2351–2359.

Gerke J (1993) Solubilization of Fe (III) from humic-Fe complexes, humic/Fe-oxide mixtures and from poorly ordered Fe-oxide by organic acids - consequences for P adsorption. *Zeitschrift für Pflanzenernährung und Bodenkunde*, **156**, 253–257.

Gustafsson JP, Mwamila LB, Kergoat K (2012) The pH dependence of phosphate sorption and desorption in Swedish agricultural soils. *Geoderma*, **189–190**, 304–311.

Harrison KA, Bardgett RD (2010) Influence of plant species and soil conditions on plant–soil feedback in mixed grassland communities. *Journal of Ecology*, **98**, 384–395.

Hawkes CV, Kivlin SN, Rocca JD, Huguet V, Thomsen MA, Suttle KB (2011) Fungal community responses to precipitation. *Global Change Biology*, **17**, 1637–1645.

Henry HAL (2013) Reprint of "Soil extracellular enzyme dynamics in a changing climate". *Soil Biology and Biochemistry*, **56**, 53–59.

Hetrick BAD, Wilson GWT, Todd TC (1990) Differential responses of C3 and C4 grasses to mycorrhizal symbiosis, phosphorus fertilization, and soil microorganisms. *Canadian Journal of Botany*, **68**, 461–467.

IPCC (2011) *Special Report on Renewable Energy Sources and Climate Change Mitigation*. Cambridge University Press, United Kingdom and New York, NY, USA.

Jacobson KM, Jacobson PJ (1998) Rainfall regulates decomposition of buried cellulose in the Namib desert. *Journal of Arid Environments*, **38**, 571–583.

Jarrell WM, Armstrong DE, Grigal DF, Kelly EF, Monger HC, Wedin D (1999) Soil water and temperature status. In: *Standard Soil Methods for Long-Term Ecological Research* (eds Robertson GP, Coleman DC, Bledsoe CS, Sollins P), pp. 55–73. Oxford University Press, New York, NY.

Johns TC, Carnell RE, Crossley JF *et al.* (1997) The second hadley centre coupled ocean-atmosphere GCM: model description, spinup and validation. *Climate Dynamics*, **13**, 103–134.

Johnson D, Vandenkoornhuyse PJ, Leake JR *et al.* (2004) Plant communities affect arbuscular mycorrhizal fungal diversity and community composition in grassland microcosms. *New Phytologist*, **161**, 503–515.

Johnson SL, Kuske CR, Carney TD, Housman DC, Gallegos-Graves LV, Belnap J (2012) Increased temperature and altered summer precipitation have differential effects on biological soil crusts in a dryland ecosystem. *Global Change Biology*, **18**, 2583–2593.

Kakumanu ML, Cantrell CL, Williams MA (2013) Microbial community response to varying magnitudes of desiccation in soil: a test of the osmolyte accumulation hypothesis. *Soil Biology and Biochemistry*, **57**, 644–653.

Kardol P, Cregger MA, Campany CE, Classen AT (2010) Soil ecosystem functioning under climate change: plant species and community effects. *Ecology*, **91**, 767–781.

Kelly AE, Goulden ML (2008) Rapid shifts in plant distribution with recent climate change. *PNAS*, **105**, 11823–11826.

Klironomos JN (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature*, **417**, 67–70.

Klironomos J, Zobel M, Tibbett M *et al.* (2011) Forces that structure plant communities: quantifying the importance of the mycorrhizal symbiosis. *New Phytologist*, **189**, 366–370.

Knapp A, Burns C, Fynn R, Kirkman K, Morris C, Smith M (2006) Convergence and contingency in production–precipitation relationships in North American and South African C4 grasslands. *Oecologia*, **149**, 456–464.

Lajtha K, Schlesinger WH (1988) The biogeochemistry of phosphorus cycling and phosphorus availablilty along a desert soil chronosequence. *Ecology*, **69**, 24–39.

Legendre P, Anderson MJ (1999) Distance based redundancy analysis: testing multi-species responses in multifactoral ecological experiments. *Ecological Monographs*, **69**, 1–24.

Levine JM, Pachepsky E, Kendall BE, Yelenik SG, Lambers JHR (2006) Plant–soil feedbacks and invasive spread. *Ecology Letters*, **9**, 1005–1014.

Loarie SR (2009) The velocity of climate change. *Nature*, **462**, 1052.

Manzoni S, Trofymow JA, Jackson RB, Porporato A (2012) Responses of soil microbial communities to water stress: results from a meta-analysis. *Ecology*, **93**, 930.

Mcguire K, Zak D, Edwards I, Blackwood C, Upchurch R (2010) Slowed decomposition is biotically mediated in an ectomycorrhizal, tropical rain forest. *Oecologia*, **164**, 785–795.

Mehlich A (1984) Mehlich 3 soil test extractant: a modification of Mehlich 2 extractant. *Communications in Soil Science and Plant Analysis*, **15**, 1409–1416.

Miller RH, Keeney DR (1982) *Methods of Soil Analysis*, 2nd edn. Academic Press, Madison, WI.

Miller RM, Wilson GWT, Johnson NC (2012) Arbuscular mycorrhizae and grassland ecosystems. In: *Biocomplexity of Plant–Fungal Interactions* (eds John Wiley & Sons Inc.), pp. 59–84. Wiley-Blackwell, Chichester.

Min SK (2011) Human contribution to more-intense precipitation extremes. *Nature*, **470**, 378.

Naeem S (1997) Species redundancy and ecosystem reliability. *Conservation Biology*, **12**, 39–45.

National Assessment Synthesis Team UGCRP (2001) *Climate Change Impacts on the United States: The Potential Consequences of Climate Variability and Change, Report for the US Global Change Research Program*. Cambridge University Press, New York, NY.

Noy-Meir I (1974) Desert ecosystems: higher trophic levels. *Annual Review of Ecology and Systematics*, **5**, 195–214.

Nunan N, Morgan MA, Herlihy M (1997) Ultraviolet absorbance (280 nm) of compounds released from soil during chloroform fumigation as an estimate of the microbial biomass. *Soil Biology and Biochemistry*, **30**, 1599–1603.

O'Brien RG (1985) MANOVA method for analyzing repeated measures designs: an extensive primer. *Psychological bulletin*, **97**, 316.

Oksanen J, Blanchet FG, Kindt R et al. (2011) Vegan: Community Ecology Package. R package version 1.17-9.

Onset CC (2004) *Hobo-H8 Pro-series Users Manual*. Onset Computer Corporation, Bourne, MA.

Oren A, Steinberger Y (2008) Catabolic profiles of soil fungal communities along a geographic climate gradient in Israel. *Soil Biology and Biochemistry*, **40**, 2578–2587.

Owens H, Lafantasie J, Adler PB (2012) Mycorrhization rates of two grasses following alterations in moisture inputs in a southern mixed grass prairie. *Applied Soil Ecology*, **60**, 56–60.

Parham JA, Deng SP (2000) Detection, quantification and characterization of B-glucosaminidase activity in soil. *Soil Biology and Biochemistry*, **32**, 1183–1190.

Patrick L, Ogle K, Bell CW, Zak JC, Tissue DT (2009) Physiological responses of two contrasting desert plant species to precipitation variability are differentially regulated by soil moisture and nitrogen dynamics. *Global Change Biology*, **15**, 1214–1229.

Pezzani F (2006) Associations between arbuscular mycorrhizal fungi and grasses in the successional context of a two-phase mosaic in the Chihuahuan Desert. *Mycorrhiza*, **16**, 285.

Placella SA, Brodie EL, Firestone MK (2012) Rainfall-induced carbon dioxide pulses result from sequential resuscitation of phylogenetically clustered microbial groups. *Proceedings of the National Academy of Sciences*, **109**, 10931–10936.

Püschel D, Rydlová J, Vosátka M (2007) Mycorrhiza influences plant community structure in succession on spoil banks. *Basic and Applied Ecology*, **8**, 510–520.

R.Core.Team (2013) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.

Reynolds JF, Kemp PR, Ogle K, Fernandez RJ (2004) Modifying the 'pulse-reserve' paradigm for deserts of North America: precipitation pulses, soil water, and plant responses. *Oecologia*, **141**, 194–210.

Richardson AE, Hocking PJ, Simpson RJ, George TS (2009) Plant mechanisms to optimise access to soil phosphorus. *Crop and Pasture Science*, **60**, 124–143.

Robertson GP, Wedin D, Groffman PM, Blair JM, Holland EA, Nadelhoffer KJ, Harris D (1999) Soil carbon and nitrogen availability: Nitrogen mineralization, nitrification, and soil respiration potentials. In: *Standard Soil Methods for Long-Term Ecological Research*. (eds Robertson GP, Coleman DC, Bledsoe CS, Sollins P), pp. 258–271. Oxford University Press, New York, NY.

Robertson TR, Bell CW, Zak JC, Tissue DT (2009) Precipitation timing and magnitude differentially affect aboveground annual net primary productivity in three perennial species in a Chihuahuan Desert grassland. *New Phytologist*, **181**, 230–242.

Robertson T, Zak J, Tissue D (2010) Precipitation magnitude and timing differentially affect species richness and plant density in the sotol grassland of the Chihuahuan Desert. *Oecologia*, **162**, 185–197.

Saiz G, Bird MI, Domingues T et al. (2012) Variation in soil carbon stocks and their determinants across a precipitation gradient in West Africa. *Global Change Biology*, **18**, 1670–1683.

Sasser M (2001) *Identification of bacteria by gas chromatography of cellular fatty acids*. MIDI, Inc. Automated Microbial Identification Solutions. Newark, DE.

Schaeffer S, Ziegler S, Belnap J, Evans R (2011) Effects of Bromus tectorum invasion on microbial carbon and nitrogen cycling in two adjacent undisturbed arid grassland communities. *Biogeochemistry*, **111**, 427–441.

Schimel JP, Gulledge JM, Clein-Curley JS, Lindstrom JE, Braddock JF (1999) Moisture effects on microbial activity and community structure in decomposing birch litter in the Alaskan taiga. *Soil Biology and Biochemistry*, **31**, 831–838.

Schimel JP, Balser TC, Wallenstein M (2007) Microbial stress-response physiology and it's implications for ecosystem function. *Ecology*, **88**, 1386–1394.

Schlesinger WH (1996) On the spatial pattern of soil nutrients in desert ecosystems. *Ecology*, **77**, 364–374.

Schwinning S, Sala OE (2004) Hierarchy of responses to resource pulses in arid and semi-arid ecosystems. *Oecologia*, **141**, 211–220.

Seager R, Ting M, Held I et al. (2007) Model projections of an imminent transition to a more arid climate in southwestern North America. *Science*, **316**, 1181–1184.

Sistla SA, Schimel JP (2012) Stoichiometric flexibility as a regulator of carbon and nutrient cycling in terrestrial ecosystems under change. *New Phytologist*, **196**, 68–78.

Sleator RD, Hill C (2002) Bacterial osmoadaptation: the role of osmolytes in bacterial stress and virulence. *FEMS Microbiology Reviews*, **26**, 49–71.

Sobek E, Zak JC (2003) The soil FungiLog procedure: method and analytical approaches toward understanding fungal functional diversity. *Mycologia*, **95**, 590–602.

Soini SM, Koskinen KT, Vilenius MJ, Puhakka JA (2002) Effects of high and fluctuating pressure on microbial abundance and activity in a water hydraulic system. *Applied Microbiology and Biotechnology*, **58**, 669–674.

Sollins P, Glassman C, Paul EA, Swanston C, Lajtha K, Heil JW, Elliot TE (1999) Soil carbon and nitrogen. In: *Standard Soil Methods for Long-Term Ecological Research* (eds Robertson GP, Coleman DC, Bledsoe CS, Sollins P), pp. 89–105. Oxford University Press, New York.

Solomon S (2009) Irreversible climate change due to carbon dioxide emissions. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 1704.

Solomon S, Qin D, Manning M et al. (2007) *IPCC Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, United Kingdom and New York, NY, USA.

Sponseller RA (2007) Precipitation pulses and soil CO_2 flux in a Sonoran Desert ecosystem. *Global Change Biology*, **13**, 426–436.

Tabatabai MA (1994) Soil Enzymes. In: *Methods of Soil Analysis* (eds Page AL, Miller RH, Keeney DR), pp. 903–947. American Society of Agronomy, Inc, Madison, WI.

Treseder KK, Vitousek PM (2001) Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiin rain forests. *Ecology*, **82**, 946–954.

Turner AC (1997) *Soil Survey of Jeff Davis County, Texas*, pp. 3–12. Department of Agriculture, Soil Conservation Service, United States.

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*, **19**, 703–707.

Van Gestel M, Merckx R, Vlassak K (1993a) Microbial biomass and activity in soils with fluctuating water contents. *Geoderma*, **56**, 617–626.

Van Gestel M, Merckx R, Vlassak K (1993b) Microbial biomass responses to soil drying and rewetting: the fate of fast- and slow-growing microorganisms in soils from different climates. *Soil Biology and Biochemistry*, **25**, 109–123.

Vasey MW, Thayer JF (1987) The Continuing Problem of False Positives in Repeated Measures ANOVA in Psychophysiology: a Multivariate Solution. *Psychophysiology*, **24**, 479–486.

Walker BH (1992) Biodiversity and ecological redundancy. *Conservation Biology*, **6**, 18–23.

Wallenstein M, Hall E (2012) A trait-based framework for predicting when and where microbial adaptation to climate change will affect ecosystem functioning. *Biogeochemistry*, **109**, 35–47.

Wardle DA, Bonner KI, Barker GM et al. (1999) Plant removals in perennial grassland: vegetation dynamics, decomposers, soil biodiversity, and ecosystem properties. *Ecological Monographs*, **69**, 535–568.

Williams MA, Rice CW (2007) Seven years of enhanced water availability influences the physiological, structural, and functional attributes of a soil microbial community. *Applied Soil Ecology*, **35**, 535–545.

Wondzell S, Ludwig JA (1995) Community dynamics of desert grasslands: influences of climate, landforms, and soils. *Journal of Vegetation Science*, **6**, 377–390.

Wood JM, Bremer E, Csonka LN, Kraemer R, Poolman B, Van Der Heide T, Smith LT (2001) Osmosensing and osmoregulatory compatible solute accumulation by bacteria. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **130**, 437–460.

Wu Y (2010) Arbuscular mycorrhiza fungi as an ecology indicator for evaluating desert soil conditions. *Frontiers of Agriculture in China*, **4**, 24.

Wu Z, Dijkstra P, Koch GW, Peñuelas J, Hungate BA (2011) Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. *Global Change Biology*, **17**, 927–942.

Zak JC, Willig MR, Moorhead DL, Wildman HG (1994) Functional diversity of microbial communities: a quantitative approach. *Soil Biology and Biochemistry*, **26**, 1101–1108.

Zak JC, Sinsabaugh R, Mackay W (1995) Windows of opportunity in desert ecosystems: their implications to fungal community development. *Canadian Journal of Botany*, **73**, S1407–S1414.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Microbial community structure - function relationships in Years 3–5 (2004–2006) of the study in the Sotol Grasslands in Big Bend National Park using distance-based redundancy analysis (dbRDA) and ordination plots.

Table S1. Specific lipid markers (FAMEs) used to assess microbial group abundances.

Table S2. Pearson correlations demonstrating seasonal soil moisture availability relationships.

Table S3. Stepwise linear regression output demonstrating seasonal microbial community functional.

Table S4. Pearson correlations demonstrating how available soil P seasonally correlates with microbial community functional and structural characteristics.

Table S5. Stepwise linear regression output demonstrating significant seasonal microbial community functional and structural relationships.