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# Direct and indirect effects of shifting rainfall on soil microbial respiration and enzyme activity in a semi-arid system

**Ellen H. Esch · David Lipson · Elsa E. Cleland**

**Abstract**

*Background and aims* Higher interannual precipitation variability is predicted for Southern California's shrub-dominated systems, promoting soil moisture variation and changing community composition. We asked if soil microbial responses to rainfall regime will depend on litter inputs; showing direct effects of altered precipitation through soil moisture and indirect effects resulting from shifting litter inputs.

*Methods* Soils were collected from a 2-year field rainfall manipulation experiment. Under lab conditions soils were subjected to high or low moisture pulses with litter amendments from native and exotic species in all combinations.

*Results* Soil respiration was higher with larger water pulses, but rose over time in low pulse treatments (direct response). Litter additions from exotic species promoted greater respiration, and results were stronger under higher soil moisture (indirect response). Extracellular enzyme activities generally were higher with exotic litter and under high moisture pulses. Those involved in N-cycling had much larger increases activity for the exotic litter addition - high moisture pulse scenarios compared to other treatments.

*Conclusions* Our results indicate the potential for microbial acclimation to drought conditions over short timescales and that below-ground processes are sensitive to direct and indirect effects of shifting rainfall regimes, especially where invasion is promoted by future climate change.

**Keywords** Chaparral · Exotic species · Extracellular enzyme activity · Microbial acclimation · Microbial biomass · Microbial respiration

**Abbreviations**

| | |
|---|---|
| NAG | β-1,4-N-acetylglucosaminidase |
| BGLUC | β-Glucosidase |
| CBH | Cellobiohydrolase |
| EEA | Extracellular enzyme activity |
| LAP | L-leucine aminopeptidase |
| MBM | Microbial biomass |
| PHOS | Phosphatase |

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## Introduction

In the coming century, rainfall patterns are predicted to change in intensity and frequency as a result of climate change (Collins et al. 2013; IPCC 2012), with direct ramifications for ecosystem functioning, especially in water-limited ecosystems. Over time, shifts in plant species composition could lead to additional, indirect

effects of shifting rainfall regimes on ecosystem functioning and biogeochemical cycling (Smith et al. 2009). For instance, in the semi-arid Mediterranean-type ecosystems of Southern California, rainfall is expected to decline overall, but also become more variable interannually (Berg and Hall 2015; Cayan et al. 2009). These systems are dominated by native shrubs, but are increasingly invaded by exotic herbaceous species, which often increase in abundance in high rainfall years (Ashbacher and Cleland 2015; Hobbs and Mooney 1991; Rao and Allen 2010). Other climate change factors such as increasing temperatures (Sandel and Dangremond 2012) or fire (D'Antonio 2000), could interact with shifting rainfall regimes to further increase invasion in these systems. Exotic and native species often differ in litter quality and quantity (Ehrenfeld 2003), in turn influencing belowground processes. Hence, interacting effects of altered precipitation and shifting litter inputs will likely have implications on belowground activity and functioning.

In arid and semiarid systems, episodic rain events play fundamental roles in determining microbial activity and resulting effects on ecosystem level biogeochemical cycling (Austin et al. 2004; Blazewicz et al. 2014; Chen et al. 2009; Collins et al. 2008; Schwinning and Sala 2004). During dry conditions, overall soil microbial activity is generally low with rewetting episodes causing large spikes in microbial respiration and activity - the well-known Birch Effect (Birch 1958). Pulses in activity after rewetting episodes are attributed to increased substrate availability, both microbial (resulting from lysis and rapid reassimilation of microbial cells) and nonmicrobial (substrate becoming accessible via diffusive transport) (as reviewed by Borken and Matzner 2009). Frequency and intensity of the rewetting pulse are known to affect soil activity in the short term (Borken and Matzner 2009; Fierer and Schimel 2002; Mikha et al. 2005; Shi and Marschner 2014; Yu et al. 2014), but it is less well understood how legacy effects of prior precipitation regimes may affect the dynamics of post-rewetting activity spikes under new regimes (Hawkes and Keitt 2015). With predictions of increased interannual precipitation variability and the importance of belowground processes on ecosystem functioning, it is important to understand the effects of past precipitation regimes on current soil activity.

While drought conditions can reduce overall microbial activity, they can also have effects on the aboveground community, which can indirectly affect microbial activity, potentially across years. Drought can promote litter fall and accumulation (Brando et al. 2008), change belowground carbon (C) allocation patterns (Gorissen et al. 2004), and cause shifts in plant community composition (Ashbacher and Cleland 2015; Cleland et al. 2013), all of which combine to alter litter chemistry inputs. With increased litterfall combined with slowed decomposition, historic drought conditions may poise soils to have a greater response to a large moisture pulse as a result of access to accumulated substrate and nutrient pools (Meisner et al. 2015). Large moisture pulses associated with wet years are also linked to high production of fast-growing exotic annuals (Hobbs and Mooney 2005), that are often associated with higher nitrogen (N) litter content (Ehrenfeld 2003) and faster rates of decomposition (Pysek et al. 2012). As an indirect effect of altered precipitation, microbial activity may rapidly increase and result in faster nutrient cycling in high rainfall scenarios, resulting from shifting litter inputs (Ehrenfeld 2003; Esch et al. 2013; Wardle et al. 2004; Wolkovich et al. 2010).

The goal of this study was to test the hypothesis that ecosystem responses to shifting precipitation regimes would depend on the identity of litter inputs, specifically that labile litter inputs from exotic species would enable more positive soil microbial responses to large pulses of precipitation. To accomplish this goal we examined a) the direct effects of past and present precipitation change on microbial activity and b) the indirect effects precipitation change on microbial activity arising from precipitation's effect on plant community composition, specifically invasion scenarios, and associated shifting litter inputs in a chaparral system in southern California. We collected soils from a rainfall manipulation experiment in the field, and incubated the soils in the laboratory, adding plant litter of either native or exotic species, and imposing high versus low pulses of moisture. Measurements of microbial respiration rate and microbial biomass (MBM), provided estimates of overall microbial activity and community size. Extracellular enzyme activities (EEA) were measured to give an estimate of microbial demand for different elements (Allison and Vitousek 2005), recognizing that initial breakdown of organic material by EEAs is the rate limiting step of decomposition (Sinsabaugh 1994) and that N cycling is constrained by the degradation of proteins into oligomers (Sinsabaugh et al. 2009).

## Materials and methods

### Study site and sample collection

Soil cores were collected from a rainfall manipulation at the Elliott Chaparral Reserve (San Diego County, CA, 32°53′32″N, 117°06′2″W) at the conclusion of the 2011–2012 growing season, following two years of experimental treatments. Rainout shelters were established prior to the 2010–11 growing season and created five rainfall treatments (0 %, 50 %, 100 %, 150 %, and 200 % of ambient rainfall with $n = 5$ for each treatment). These rainfall treatments were in operation during the 2010–2011 and 2011–2012 growing seasons (Ashbacher and Cleland 2015). The site has a Mediterranean climate with cool, wet winters and hot, dry summers. The growing season begins with winter rains, generally in November, and concludes with the onset of the dry summer period, usually ending in April. During the 2010–2011 growing season (Nov. 1 – Apr. 30), 362.2 mm of rain fell while 236.0 mm fell during the 2011–2012 season (www.wrcc.dri.edu). Average yearly rainfall is 260 mm at the site, and there is an average annual temperature of 17° Celsius. At the conclusion of the 2011–2012 growing season, five soil cores (0–10 cm depth, 1.5 cm diameter) were collected from a 0.5 m × 4 m strip on the south side of each plot, which had been planted with native shrub seedlings (*Adenostoma fasciculatum* Hook. and Arn., *Salvia mellifera* E. Greene, *Malosma laurina* (Nutt.) Abrams). Thus, all cores were collected from areas with similar plant species composition. All cores were subsequently composited, sieved through a 2 mm standard test sieve to remove rocks, and stored air-dry. The soil at the site has an organic carbon content of 1.812 % and nitrogen content of 0.154 % on average (elemental combustion, NCS 2500, CE Elantech, Lakewood, NJ) as determined by elemental analysis. Soil pH at the site was 5.38 on average, soils are classified as Alfisols with a sandy loam texture at 0–10 cm depth, and bulk density is 1.26 g cm$^{-3}$ on average.

Litter samples of recently senesced leaves shaken from the stem of two native focal shrub species (*A. fasciculatum* and *M. laurina*) were collected at the conclusion of the 2011–2012 growing season outside of experimental plots. Biomass from two exotic, annual species abundant at the site (*Erodium cicutarium* (L.) Aiton and *Festuca myuros* L.) was collected at peak ecosystem biomass in April 2012, at which point the exotic portion of the biomass had senesced. At this site, invasion is primarily by annual grasses and forbs, and perennial shrubs dominate the native community. The four species selected for the litter addition treatments represents the dominant aboveground community at the site. Litter from all species was ground to a uniform size in a Wiley mill (Thomas Scientific, Swedesboro NJ). Litter C and N were measured by combustion on a CE NCS 2500 elemental analyzer (CE Elantech, Inc., Lakewood NJ) and plant P was measured using sulfuric acid digestion followed by colorimetric analysis (American Public Health Association 1994).

### Laboratory experiment

Ten replicate 30 g of air-dried soil subsamples from each of the 25 experimental field rainfall manipulation plots were prepared in microcosms, and assigned to one of five litter addition treatments at each of two laboratory moisture pulse sizes ($n = 250$). Each microcosm received 0.50 g of litter, which was evenly mixed into the soil, except for the no-addition control treatment. Litter came from two species of native origin (*A. fasciculatum*, *M. laurina*) and from two species of exotic origin (*E. cicutarium*, *F. myuros*), and these species were chosen as collectively they represent over 80 % of the biomass at the site and allowed us to focus on community-level responses to invasion. Moisture pulse sizes mimicked the 150 % or 50 % field treatments (4.5 ml and 1.5 ml water per week, respectively) maintaining roughly 25 % and 15 % moisture at time of application, respectively. Moisture pulses were applied using de-ionized water once each week for eight weeks. Microcosms were contained in 100 ml plastic sample cups, left open to dry down between moisture pulses in an area with substantial air flow and ventilation, and incubated at 23 °C. After 24 h of dry-down time, the high moisture pulse maintained on average 11 % moisture while the low pulse treatment on average had 2 % moisture. After drying-down 72 and 144 h after the pulse, percent moisture of all treatments was on average 1 %. In the laboratory, soil dry-down time after moisture pulses was accelerated in comparison to measurements seen in the field (Ashbacher and Cleland 2015), possibly due to differences in air temperature. During the two experimental years in the field, an average of 13.7 °C seen during the November 1 – March 31 main growing seasons (www.wrcc.dri.edu) in

contrast to our laboratory incubation temperature of 23 °C. Hence, the laboratory measurement taken 24 h after each moisture pulse application may be more representative of growing season trends likely to occur in a natural system.

Soil respiration was measured at 24, 72, and 144 h following each pulse (Li-Cor 6400XT (Lincoln, NE) with soil chamber attachment). Each sample was placed into the chamber for three minutes, and an insert was used to occupy empty space in the chamber. There were no differences in microbial respiration associated with the prior rainfall treatment for the field collected soils (see Results), so ten random samples were selected from each treatment on which to perform subsequent analyses. Other measures of microbial activity were measured only once at the conclusion of the experiment (microbial biomass carbon (MBM C), microbial biomass nitrogen (MBM N), and extracellular enzyme activities (EEA)). Microbial biomass C and N were measured with chloroform fumigation methods (Beck et al. 1997; Brookes et al. 1985). Extracellular enzyme activities of five enzymes, including two involved in C- acquisition (β-glucosidase (BGLUC) and cellobiohydrolase (CBH)), two involved in N- acquisition (L-leucine aminopeptidase (LAP) and β-1,4-N-acetylglucosaminidase (NAG)), and one involved in P- acquisition (phosphatase (PHOS)) were measured using methods described in Saiya-Cork et al. (2002). Enzyme activity was converted to micromoles of substrate converted per hour per gram of dry weight soil sample. Biomass specific activity was calculated by dividing EEA production for each of the enzymes by both MBM C and MBM N.

Statistical analysis

Statistical analyses were performed in R v. 3.02 (The R Development Core Team, 2016). Respiration was evaluated with a linear mixed effect model ('lme4' package in R) where the laboratory-added water pulse size (low or high), previous field treatment (0 %, 50 %, 100 % 150 %, 200 % of ambient rainfall, as continuous variable), litter addition treatment (native or exotic origin, or no litter addition control), and week of experiment (1–8) were included as fixed factors, and sample ID was included as a random-factor to account for repeated measures over time on each sample at one, three, or six days post-pulse. The litter addition treatments were grouped into an origin factor to address the a priori

hypothesis of invasion on community-level responses, yet mean species responses were also analyzed for each response as percent increase or decrease for each litter treatment as compared to the no litter addition treatment. Microbial biomass C and N, and EEA were analyzed with linear models where laboratory pulse size and litter addition treatment were factorially-crossed fixed factors. Tukey's post hoc tests were performed when there were significant effects of litter addition on microbial response. Significance of all factors was evaluated with Type II tests using the *Anova* function in the *car* package (Fox and Weisberg 2011). These data are published in the Knowledge Network for Biocomplexity database under the same name as this manuscript.

Results

Litter quality, as measured by C, N, and P content, varied with species origin. Average plant P content was higher for species of exotic origin (0.9 ± 0.05 mg P/g litter) than for those of native origin (0.3 ± 0.04 mg P/g litter; $p < 0.001$). The opposite trend was true for percent C, with litter from exotic origin having lower percent C than litter from native origin (391 ± 7.9 mg C/g litter and 454.7 ± 8.5 mg C/g litter for exotic and native species, respectively; p < 0.001). Average percent N content was not statistically significant between species origin ($p = 0.355$), though was higher for species of exotic origin (7.9 ± 0.7 mg N/g litter) compared to native origin (6.8 ± 0.8 mg N/g litter). Differences in mean response for the four species litter additions show variability in the degree to which species increase activity depending on the measurement, yet in all cases both exotic species had the highest increase in activity over the control treatment. *E. cicutarium* promoted the largest activity increases in 3 cases (soil respiration, MBM C and N), while *F. myuros* additions had the largest activity increases in 5 cases (BGLUC, CBH, LAP, NAP, and PHOS) (Appendix Table 3). Additions of *A. fasciculatum* had higher activity than *M. laurina* additions in 6 cases (BGLUC, CBH, LAP, NAG, MBM N, and PHOS) while *M. laurina* had higher activity than *A. fasciculatum* for soil respiration and MBM C responses (Appendix Table 3).

Soil respiration was not significantly affected by prior field rainfall treatment nor were there any

significant interactions with prior field treatment in any case. Overall, soils receiving high soil moisture pulses had greater respiration than low pulse soils, with day one following a pulse having the greatest respiration rate (Fig. 1); however soil respiration was influenced by higher order interactions between pulse size and the other variables (Table 1). On days one and three post-pulse, there was a significant laboratory pulse size × week of experiment × litter addition treatment interaction (Table 1). In the soils receiving a high moisture pulse, soil microbial respiration was influenced by the origin of litter addition (Fig. 1), whereby litter additions from exotic species stimulated soil microbial respiration more than amendments of litter from native plant species (Fig. 1a). The effect of the litter addition treatment in the high pulse soils became less pronounced throughout the course of the experiment. In the soils receiving the lower moisture pulse, respiration was less affected by litter addition treatment for the entirety of the experiment (Fig. 1b). Weekly average activity for high pulse soils decreased over time, but the opposite was true for the low pulse soils (Fig. 1). Day six post-pulse was not affected by the three-way interaction, but did have significant two-way interactions of week of experiment × litter addition treatment and pulse size × experiment week (Table 1). Similar trends were shown as in days one and three with high pulse soil respiration generally decreasing and low pulse soil respiration increasing as experiment week progressed (pulse × week), and litter substrate addition treatment having less of an effect of respiration over the course of the experiment (week × litter) (Fig. 1). Mean species responses showed that the *E. cicutarium* and *F. myuros* had 24.9 % and 34.4 % greater respiration, respectively, over the course of the experiment in comparison to the no litter addition control treatment. The *A. fasciculatum* and *M. laurina* treatments only showed 11.6 % and 7.0 % increases in respiration, respectively, when compared to the control treatment (Appendix Table 3).

Microbial biomass C measured at the end of the experiment was affected by litter origin (Table 2, Fig. 2a), with exotic litter addition soils having greater MBM C than native addition soils (Tukey HSD, $p = 0.023$), but no significant differences between no-litter addition soils and either native or exotic litter additions. Laboratory pulse size had a marginally significant effect on MBM C (Table 2), with trends of reduced biomass sizes in the low

pulse soils (Fig. 2a). Microbial biomass N showed a significant pulse size × litter treatment interaction, as well as individual pulse size and litter treatment effects, driven largely with the exotic litter addition treatment having greater activity in the high pulse treatment than the low pulse treatment (Table 2, Fig. 2b). Microbial biomass C:N ratios were not significantly affected by experimental treatments (Table 2). Mean species differences in MBM C and N followed our expectations, with the exception of lower MBM C found for the *A. fasciculatum* treatment (Appendix Table 3).

Both litter origin and pulse size significantly influenced the activity of enzymes involved in C- acquisition (BGLUC and CBH), with no significant interaction (Table 2, Fig. 3a and b). C-acquiring enzyme activity (C-EEA) was greater for soils receiving a high moisture pulse than for soils receiving a low moisture pulse. Overall litter additions stimulated C-EEA, though exotic litter stimulated C-EEA more than native litter additions. Mean species responses for C-EEA ranged from 277.8 % to 602.1 % increases over the control treatment for the *E. cicutarium* and *F. myuros* additions while the *A. fasciculatum*, *M. laurina* additions increased C-EEA from minimum of 64.9 % to a maximum 270.4 % increase over the control treatment (Appendix Table 3). Enzymes involved in N- acquisition (N-EEA, LAP and NAG) showed significant pulse size and litter origin interactions, as well as pulse size and litter origin effects (Table 2, Fig. 3c and d). Again, soils with exotic litter additions had much greater LAP and NAG activity under the high water pulse treatment than under the low pulse treatment, similar to the trend seen for MBM N and likewise driven by the disproportionately large response to water pulse in the exotic litter addition treatment. Additions of *M. laurina* litter stimulated N-EEA the least of any species additions, and in the case of LAP activity, both native species litter additions decreased activity relative to the control treatment (Appendix Table 3). PHOS activity was the only measure of enzyme activity not affected by pulse size, though was significantly affected by litter origin (Table 2, Fig. 3d), and showed similar trends to other the activities of other enzymes, with the no-litter addition treatment having the lowest activity, and soils with exotic litter additions having the highest activity. Species-level responses show that *F. myuros* increased activity the most over the
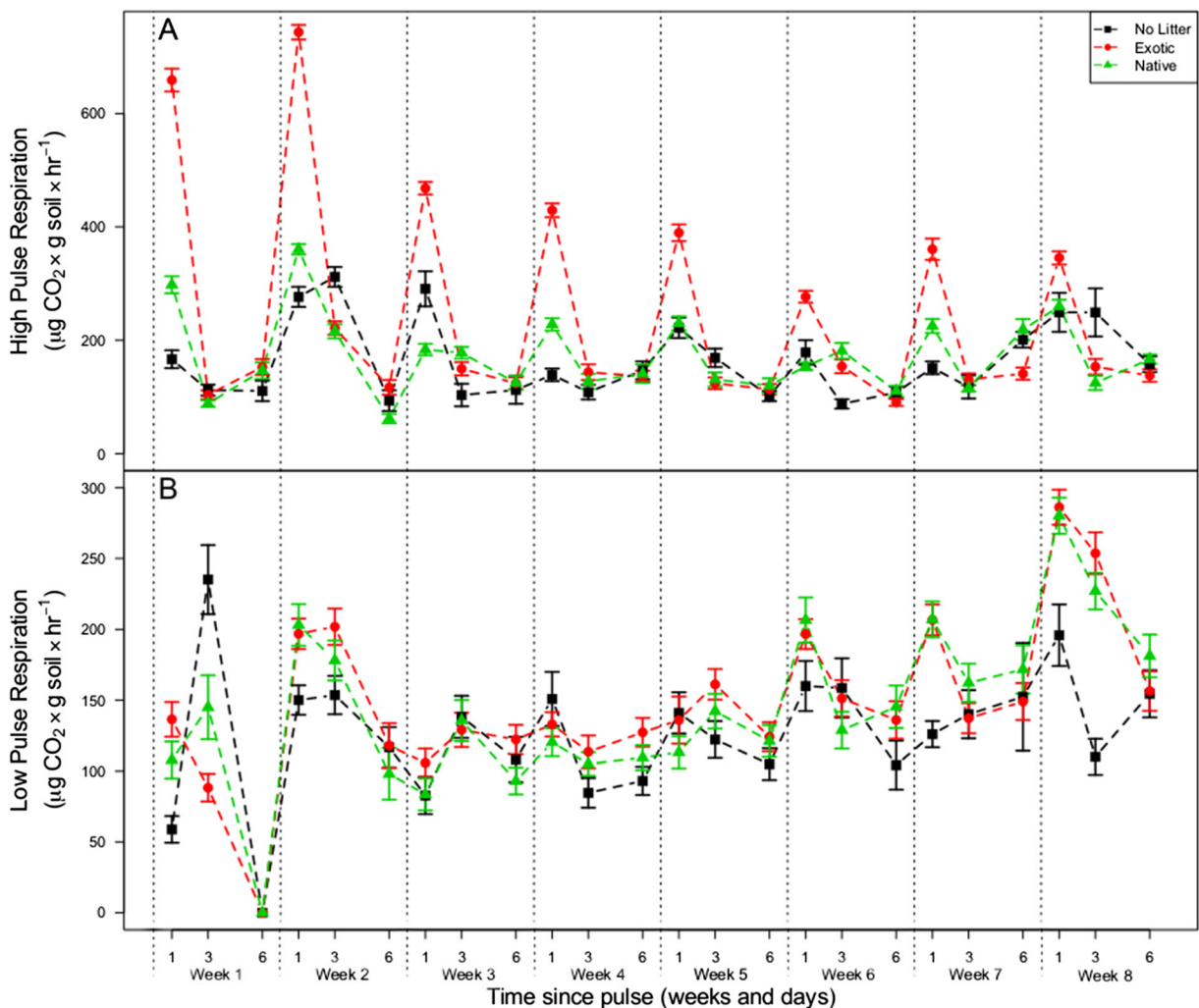
**Fig. 1** Soil respiration response ($\mu g$ $CO_2$ g soil $^{-1}$ h $^{-1}$) of soils receiving high moisture pulses (**a**) and low moisture pulses (**b**). Dashed vertical lines indicate time of moisture application. The no litter addition treatments are indicated by black squares, the exotic litter amendment by red circles, and the native treatment with green triangles. Error bars represent ±1 standard error

control treatment, while *M. laurina* increased activity the least over the control treatment (Appendix Table 3). Biomass specific activity (for both MBM C and MBM N) was not affected by treatment for any of the enzymes measured (Appendix Table 4).

**Discussion**

This experiment revealed complex interactions between direct responses to variation in rainfall quantity, and indirect responses to litter inputs from native versus exotic species in this semi-arid ecosystem. Current soil moisture was the most important

factor predicting microbial activity. Greater overall activity of soils receiving a high moisture pulse compared to a low moisture pulse was expected, and is likely a cause of low moisture environments restricting MBM and access to nutrients (Austin et al. 2004; Borken and Matzner 2009; Cook and Orchard 2008). However there were strongly divergent temporal trends in soil microbial respiration between the soils receiving high and low soil moisture pulses, identified even with just three snapshot measurements each week. Respiration rates declined over time in the soils receiving high soil moisture pulses while the soils receiving low moisture pulses in the laboratory showed increases in average

**Table 1** Summary of F- and p- values for the linear mixed effect model on soil respiration with laboratory pulse size (P), week of experiment (W), litter addition treatment (L), prior field precipitation treatment (F), and all possible interactions at 1, 3, and 6 days post-pulse. Degrees of freedom (d.f.) are given as numerator, denominator

| | | Day 1 | | Day 3 | | Day 6 | |
|---|---|---|---|---|---|---|---|
| Factor | d.f. | F-value | *p*-value | F-value | p-value | F-value | p-value |
| P | 1, 238 | **1285.92** | **<0.001** | 0.55 | 0.460 | **19.97** | **<0.001** |
| W | 7, 1666 | **78.00** | **<0.001** | **33.35** | **<0.001** | **33.76** | **<0.001** |
| F | 1, 238 | 0.01 | 0.936 | 0.16 | 0.686 | 0.17 | 0.682 |
| L | 2, 238 | **385.52** | **<0.001** | 0.09 | 0.910 | 1.50 | 0.226 |
| P:W | 7, 1666 | **105.98** | **<0.001** | **10.47** | **<0.001** | **24.81** | **<0.001** |
| P:F | 1, 238 | 1.08 | 0.300 | 0.00 | 0.949 | 0.30 | 0.587 |
| W:F | 7, 1666 | 0.91 | 0.496 | 0.89 | 0.511 | 0.60 | 0.760 |
| P:L | 2, 238 | **260.34** | **<0.001** | 2.34 | 0.099 | 1.28 | 0.280 |
| W:L | 14, 1666 | **20.23** | **<0.001** | **3.71** | **<0.001** | **2.64** | **<0.001** |
| F:L | 2, 238 | 0.17 | 0.841 | 0.26 | 0.774 | 0.23 | 0.791 |
| P:W:F | 7, 1666 | 0.62 | 0.740 | 1.25 | 0.273 | 0.64 | 0.726 |
| P:W:L | 14, 1666 | **17.36** | **<0.001** | **10.31** | **<0.001** | 1.10 | 0.357 |
| P:F:L | 2, 238 | 0.31 | 0.736 | 0.06 | 0.943 | 2.38 | 0.095 |
| W:F:L | 14, 1666 | 0.64 | 0.830 | 1.03 | 0.423 | 1.07 | 0.376 |
| P:W:F:L | 14, 1666 | 1.12 | 0.330 | 0.53 | 0.918 | 0.80 | 0.671 |

weekly activity over the course of the experiment. Decreasing activity over time in the high pulse soils could have been caused by microbial depletion of organic substrates (Borken and Matzner 2009; Fierer and Schimel 2002; Mikha et al. 2005; Xiang et al. 2008; Yu et al. 2014). Alternatively, the decline in respiration over time could reflect physiological adaptation in the microbial community to the osmotic shock associated with rewetting (Evans and Wallenstein 2014; Fierer and Schimel 2002; Schimel et al. 2007; Vangestel et al. 1993). In contrast, the increase in activity of soils receiving low moisture pulses potentially reflects microbial responses to low soil moisture, which may have

**Table 2** Summary of F and *p*-values for the linear model for effects of laboratory pulse size (d.f. = 1), litter origin (d.f. = 2), and the interaction (d.f. = 2) on activity of microbial biomass carbon (MBM C), nitrogen (MBM N), and the ratio of MBM C to MBM N (MBM C:N), along with extracellular enzymes β-glucosidase (BGLUC), cellobiohydrolase (CBH), L-leucine aminopeptidase (LAP), β-1,4-N-acetylglucosaminidase (NAG), and phosphatase (PHOS)

| | Pulse Size | | Litter Treatment | | Pulse Size * Litter Treatment | |
|---|---|---|---|---|---|---|
| Microbial Measure | F-value | P-value | F-value | P-value | F-value | P-value |
| MBM C | 3.68 | 0.058 | **4.17** | **0.018** | 0.18 | 0.832 |
| MBM N | **9.88** | **0.002** | **5.16** | **0.008** | **4.66** | **0.012** |
| MBM C:N | 0.09 | 0.765 | 0.16 | 0.857 | 0.44 | 0.646 |
| BGLUC | **7.33** | **0.008** | **110.92** | **<0.001** | 0.86 | 0.426 |
| CBH | **10.31** | **0.002** | **78.91** | **<0.001** | 1.65 | 0.197 |
| LAP | **51.68** | **<0.001** | **10.90** | **<0.001** | **4.14** | **0.019** |
| NAG | **67.08** | **<0.001** | **108.18** | **<0.001** | **25.19** | **<0.001** |
| PHOS | 1.01 | 0.317 | **59.22** | **<0.001** | 2.00 | 0.141 |

**Fig. 2** Microbial biomass (mg g soil $^{-1}$) carbon (**a**) and nitrogen (**a**) in response to litter addition treatments and laboratory high (*black bars*) and low (*grey bars*) pulse sizes. Error bars represent ±1 standard error
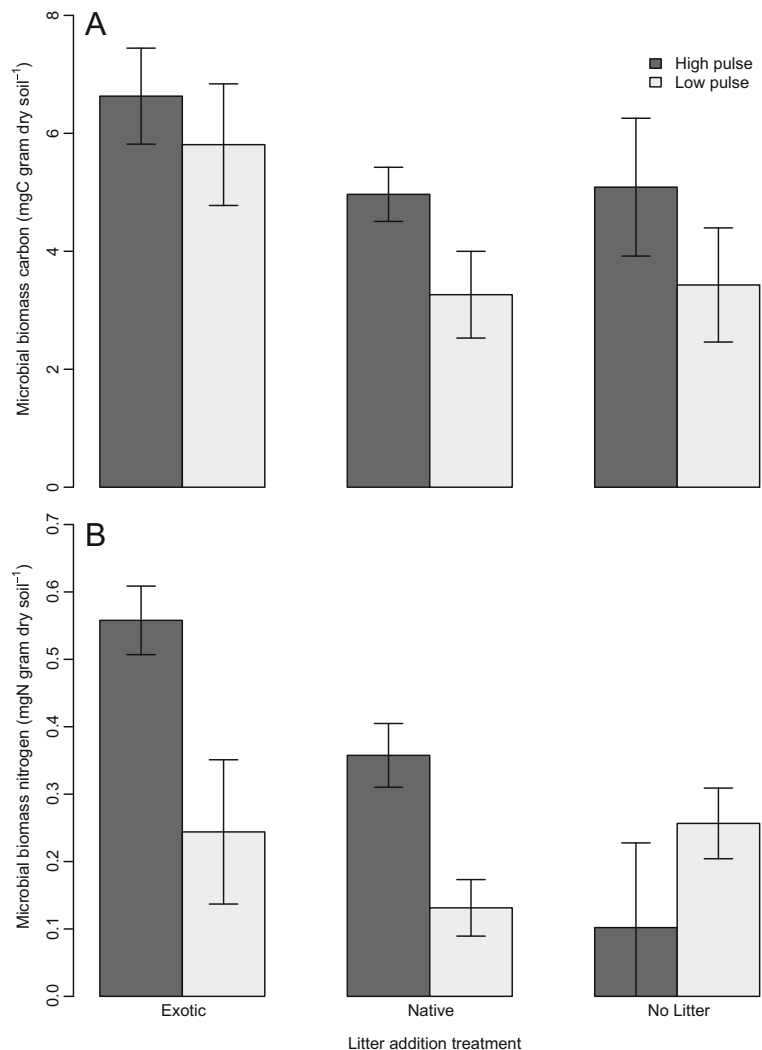


included physiological responses, shifts of community composition, or evolutionary responses community composition (Hawkes and Keitt 2015). Changes in community composition could have included shifts towards greater abundances of microbes with more drought resistant (Evans and Wallenstein 2014), generalist (Lennon et al. 2012) or oligotrophic strategies (de Vries and Shade 2013), greater ratios of taxa with physiological traits allowing for substrate decomposition under low soil moisture environments (Schimel et al. 2007), or shifts in active and dormant communities over time (Placella et al. 2012). Respiration measurements at the beginning of the experiment were also comparable to respiration of California soils at a similar depth (Xiang et al. 2008).

These temporal trends demonstrate that microbial communities in soils of semi-arid ecosystems can functionally adapt to shifts in soil moisture over short time scales, a finding further supported by our finding that there were no legacy effects associated with the prior two years of rainfall treatments applied to these field collected soils. Semi-arid ecosystems of southern California experience large variation in environmental variability both seasonally and inter-annually, especially with respect to precipitation (Cayan et al. 2009), which may select for microbial communities able to quickly adapt to changing soil moisture regimes (Hawkes and Keitt 2015; Tiemann and Billings 2011). A previous study in a tallgrass prairie ecosystem (Evans and
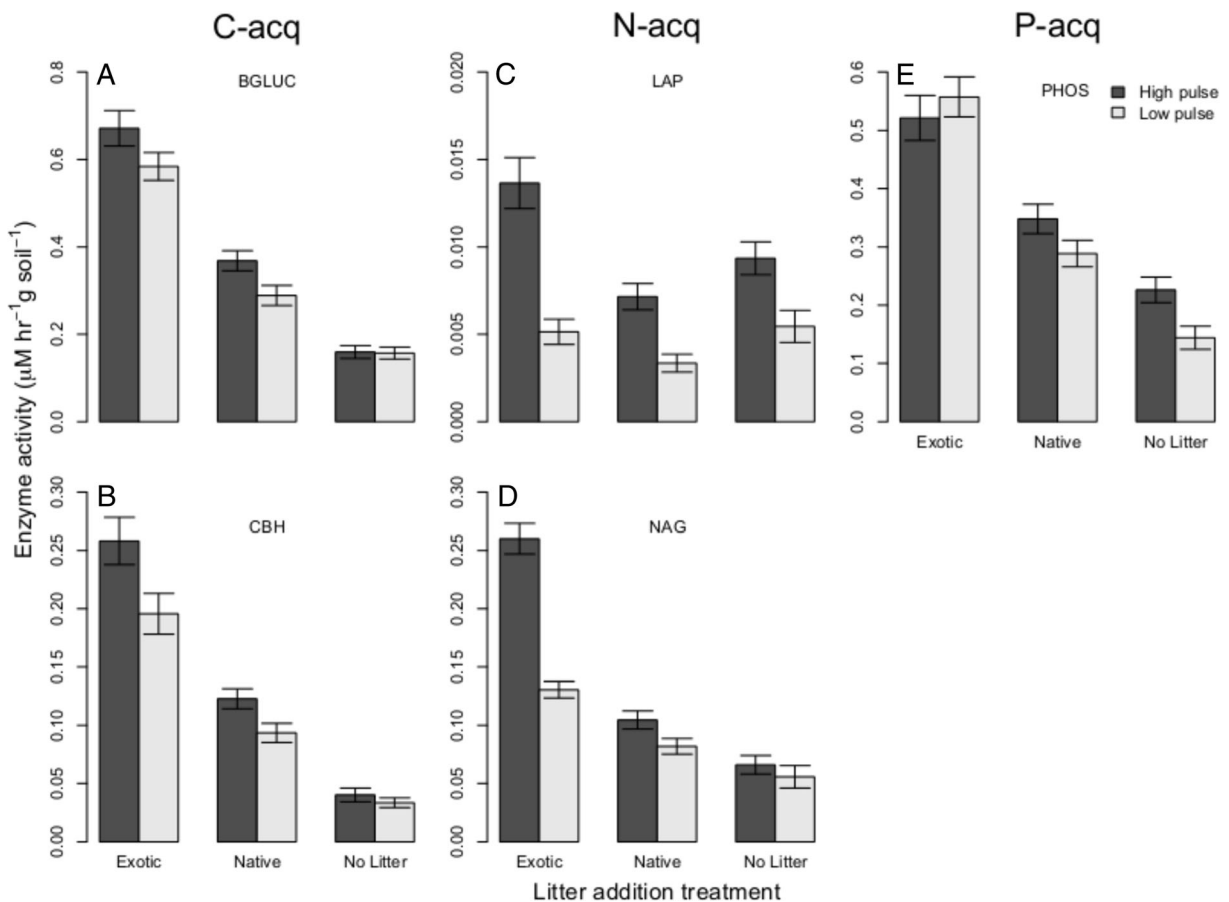
**Fig. 3** Activity (uM hr.$^{-1}$ g soil$^{-1}$) of five extracellular enzymes ((**a**) BGLUC = β-glucosidase, (**b**) CBH = cellobiohydrolase, (**c**) LAP = L-leucine aminopeptidase, (**d**) NAG = β-1,4-N-acetylglucosaminidase, and (**e**) PHOS = phosphatase) in response to laboratory litter additions and weekly moisture pulse size. *Black bars* correspond to soils receiving a high moisture pulse and *grey bars* for those receiving a low moisture pulse. Error bars represent ±1 standard error

Wallenstein 2012) likewise showed that a decade of altered precipitation regimes did not produce any detectable differences in soil respiration at the end of a 16-week, 4-moisture pulse, laboratory experiment, further supporting the idea that microbial communities acclimate on seasonal time frames to current environmental conditions. However, in the same experiment, Evans and Wallenstein (2012) did find initial differences in respiration rates from field collected soils that experienced experimental manipulations of pulse size and frequency, but the same total rainfall. Comparatively lower soil fertility, annual ecosystem productivity, and precipitation reliability in chaparral compared to tallgrass prairie ecosystems may predispose microbes found in chaparral soils to be poised to take advantage of sporadic periods of high resource availability with

more generalist, rather than specialist, niche strategies – and therefore resulting in microbial communities adapted to faster acclimation (Hawkes and Keitt 2015). This in turn may mean highly variable environments favoring fast acclimating microbial communities will be less likely to show legacy effects across years than soils from more mesic sites whose soil microbial communities are more suited towards relative stability in resource availability.

In addition to these direct effects, soil microbial activity was also sensitive to the identity of litter inputs, and this sensitivity was dependent on soil moisture. Differences in respiration rates associated with litter additions were more prominent in the soils in the high pulse treatment versus in the low pulse treatment, suggesting that substrate limitation

is secondary to soil moisture limitations for microbes in drought conditions (Ng et al. 2015). In soils with high moisture, our results finding that litter from the exotic species was associated with increased soil activity was likely due to associated faster decomposition rates of exotic species (Liao et al. 2008). Considering the duration of this experiment, it is likely that initial litter chemistry differences are contributing to the observed changes in microbial community activities, rather than changes in the litter substrate as it breaks down (Bray et al. 2012). Community composition changes in this system likely only affect belowground processes through immediate litter inputs, since there was no legacy effect of rainfall treatments applied in the field on microbial activity. At our experimental site, exotics have shown greater proportional growth when compared to native shrubs, especially under high rainfall scenarios (Ashbacher and Cleland 2015). These shifts in aboveground production and hence litter quantities, in addition to shifts in litter identities resulting from differential response between exotics and natives to rainfall, were expected to influence microbial activity and associated C and N cycling in this system. However, these changes in aboveground growth and associated annual litter inputs may have a small contribution to the soil nutrient pool in comparison to the existing pool. Shifts in litter inputs as a legacy of drought may need more than two years of altered precipitation in this system to influence soil nutrient pools and influencing soil respiration. It should be noted that this experiment only added litter to soil mesocosms and no growing plants were included, therefore, we were not able to identify the potential role of altered precipitation on root exudates and subsequent effects on microbial activity. Since the effect of litter chemistry on respiration was more prominent in the treatment with high soil moisture, this may mean that ecosystem responses to shifting community composition may only be evident in high rainfall years in this system. Differences in mean species responses indicate that individual species do have slightly different effects on belowground processes, yet overall trends support that the differences between exotics and natives are more substantial than the differences between the representative species we chose of each origin.

Enzyme activities and MBM further emphasized that soil moisture is very important to microbial activity, though enzymes involved in N- cycling and MBM N were more sensitive to pulse size depending on the litter addition treatment than were enzymes involved in C- cycling and MBM C. Differential sensitivities of EEAs and MBM may foreshadow a decoupling of C and N cycling with increased pressure from the interactive effects of altered precipitation and invasion. Previous findings support that altered precipitation can decouple C and N cycles in semi-arid systems (Evans and Burke 2013), though this research suggests that changes in soil microbial activity, and not just changes in plant resource use and production, can play a role in the decoupling of biogeochemical cycles. Enzymes involved in N- cycling may have shown greater sensitivity as cellular N- rich osmolytes can reduce moisture stress on microbes through decreasing internal water potential (Kempf and Bremer 1998; Schimel et al. 2007). The high pulse – exotic litter amended soils experienced the greatest spikes in respiration post-pulse, and may have invested more resources in acquiring N in order to protect themselves from intense and repeated osmotic shock (Tiemann and Billings 2011). Changing litter chemistry inputs resulting from invasion by exotic species can accelerate N cycling (as reviewed by Castro-Diez et al. 2014), and this may be further accentuated in Mediterranean climates with soil undergoing frequent rewetting episodes, due to the interactive effects of litter chemistry and rewetting on soil microbes. Overall reduced EEA in drought treatments can lead to slower nutrient turnover (Li and Sarah 2003; Sardans and Penuelas 2005) and subsequently reduce future nutrient availability to plants. Our finding that biomass specific EEA was not significant in any case suggests that microbial biomass may drive EEA fluctuations. On average, soil activities of found in this experiment were similar to enzyme activities found in other California soils (higher than Esch et al. (2013) and Henry et al. (2005), but lower than Alster et al. (2013), though soil pH has been shown to be a better comparative measure of cross-site EEAs (Sinsabaugh et al. 2008).

While laboratory pulse size played an important role in the activities of most microbial measures, for certain responses in aridland biogeochemistry,

factors other than soil moisture and water availability may be more important (Austin 2011). The lack of PHOS response to pulse size may indicate that phosphatase producing microbes in this experiment are less sensitive to moisture than previously found (Kramer and Green 2000; Sardans and Penuelas 2005), or that litter chemistry with regards to P content exerts greater control on PHOS production. Tight cycling of P in ecosystems resulting from low solubility (Stevenson and Cole 1999) could make PHOS activity less sensitive to moisture, but large differences in plant P content between litter origins, with exotic litter having three times the percent P of native litter used in this study, likely drives the PHOS response to litter origin. Lack of MBM C response to pulse size was surprising, since the activities of enzymes involved in C- acquisition and soil respiration were affected by pulse size, though this may further support evidence for acclimation in microbial communities to drought.

Overall, these results suggest that below-ground ecosystem processes are sensitive to the combination of direct effects of shifting rainfall regimes and indirect effects associated with shifting species composition, especially where invasion is promoted by future climate change. The divergent temporal trends in soil microbial activity between the soils receiving high and low moisture pulses give evidence of the potential for microbial communities to quickly adapt to changing rainfall regimes, which may be responsible for the lack of legacy effects from field rainfall manipulations. Legacy effects may indirectly manifest themselves, however, since in this system, precipitation influences plant community composition changes. Our findings that litter additions from exotic species promoted greater activity than those from native species, specifically under higher soil moisture scenarios, indicate that the strongest ecosystem responses to shifting community composition are likely to arise in high rainfall years. Finally, this experiment indicates that N cycling may be more sensitive to factors associated with climate change than is C or P cycling, and may lead to uncoupling of soil biogeochemical cycles due to the interaction between interannual rainfall variation and invasion predicted for this region in the coming decades. In sum, the strong responses of soil microbial activity to altered precipitation and invasion under high rainfall scenarios suggests that global change is likely to have important ramifications on belowground processes in this system.

# Appendix 1

Table 3 Summary of mean species responses to microbial measurements. Values are percent increases or decreases observed for each species as compared to the no litter addition control treatment. Microbial responses measured include soil respiration (Respiration), microbial biomass carbon and nitrogen (MBM C and MBM N), and activity of extracellular enzymes (β-glucosidase (BGLUC), cellobiohydrolase (CBH), L-leucine aminopeptidase (LAP), β-1,4-N-acetylglucosaminidase (NAG), and phosphatase (PHOS))

| | Species | Respiration | MBM C | MBM N | BGLUC | CBH | LAP | NAG | PHOS |
|---|---|---|---|---|---|---|---|---|---|
| Exotic | E. cicutarium | 34.9 | 64.4 | 187.4 | 277.8 | 432.6 | 11.3 | 211.5 | 149.5 |
| | F. myuros | 34.4 | 27.7 | 64.0 | 317.2 | 602.1 | 42.5 | 230.5 | 233.4 |
| Native | A. fasciculatum | 7.0 | -8.7 | 50.8 | 151.4 | 270.4 | -18.7 | 78.4 | 111.1 |
| | M. laurina | 11.6 | 1.9 | 21.8 | 64.9 | 118.1 | -39.4 | 28.0 | 32.8 |

## Appendix 2

**Table 4** Summary of F and *p*-values for the linear model for effects of laboratory pulse size (d.f. = 1), litter origin (d.f. = 2), and the interaction (d.f. = 2) on biomass specific activity of each extracellular enzyme (β-glucosidase (BGLUC), cellobiohydrolase (CBH), L-leucine aminopeptidase (LAP), β-1,4-N-acetylglucosaminidase (NAG), and phosphatase (PHOS)). Biomass specific activity is for both microbial biomass carbon and microbial biomass nitrogen

|  |  | Pulse Size | | Litter Treatment | | Pulse Size * Litter Treatment | |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | $F$ - value | $P$ - value | $F$ - value | $P$ - value | $F$ - value | $P$ - value |
| Microbial biomass carbon | BGLUC | 1.46 | 0.230 | 0.79 | 0.456 | 0.74 | 0.479 |
|  | CBH | 1.50 | 0.224 | 0.77 | 0.465 | 0.70 | 0.498 |
|  | LAP | 1.71 | 0.194 | 0.87 | 0.421 | 0.64 | 0.527 |
|  | NAG | 1.54 | 0.218 | 0.82 | 0.442 | 0.71 | 0.494 |
|  | PHOS | 1.61 | 0.208 | 0.72 | 0.488 | 0.70 | 0.502 |
| Microbial biomass nitrogen | BGLUC | 0.13 | 0.718 | 2.09 | 0.130 | 0.80 | 0.452 |
|  | CBH | 0.05 | 0.823 | 2.48 | 0.089 | 0.69 | 0.504 |
|  | LAP | 1.07 | 0.303 | 1.59 | 0.209 | 0.23 | 0.799 |
|  | NAG | 0.12 | 0.729 | 2.60 | 0.079 | 0.49 | 0.615 |
|  | PHOS | 0.00 | 0.995 | 1.72 | 0.186 | 1.00 | 0.371 |

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