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Ecoenzymatic stoichiometry at the extremes: How microbes cope in an ultra-oligotrophic desert soil

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ABSTRACT

Arid ecosystems are characterized by stressful conditions of low energy and nutrient availability for soil microorganisms. It has been observed that the ecoenzymes needed for the transformation of organic compounds into assimilable products show similar scaling relationships in different habitats (logarithmic C:N:P scaling ratios ~1:1:1). In this study in Cuatro Ciénegas Basin (CCB) in the Chihuahuan desert of México, we report among the lowest ecoenzymatic activities yet quantified in soil. Nevertheless, activities for both organic N and organic P acquisition enzymes scale with C acquisition with a slope of ~1.0, indicating that the soil microbial communities of this ultra-oligotrophic desert ecosystem follow the global ecoenzymatic stoichiometry patterns. CCB soil microbial communities were co-limited by C and either by N or P but this co-limitation played out differently in different parts of the CCB as indicated by microbial ecoenzymatic shift to allocate more resources to acquire and immobilize the scarcer nutrient. By extending ecoenzymatic analyses to these ultra-oligotrophic soils, our findings support the broad utility of the approach in illuminating how microbes acquire limiting resources in arid ecosystems.

1. Introduction

Drylands (defined as sites with <500 mm mean annual precipitation; Noy-Meir, 1973) cover more than one-third of the Earth's continental surface and thus constitute the most extensive terrestrial biome on the planet (Pointing and Belnap, 2012). Estimates of carbon storage for dryland regions indicate that they contribute 36% of the total carbon storage worldwide (Campbell et al., 2008). A high proportion of these dryland areas is covered by grasslands, which represent an important pool (8%) of global carbon (C) reservoirs (IPCC, 2001). In desert grasslands, the main inputs of soil organic matter (SOM) are from underground biomass rather than from aerial biomass (Sims and Singh, 1978); the former also represents the principal source of soil nitrogen (N) and phosphorus (P).

Due to the low water availability of desert ecosystems, SOM decomposition is slower than in more humid settings (i.e. tropical or temperate forest ecosystems; Burke et al., 1998). Thus, arid ecosystems are usually characterized by stressful conditions of low energy and nutrient availability for soil microorganisms (Schimel et al., 2007) and N and P availability often limit primary productivity as well as microbial activity (López-Lozano et al., 2012).

Additionally, in soils derived from geologic substrata with low apatite content (as is the case for the soil in our study), both organic P and occluded P are the dominant forms in the soil (Walker and Syers, 1976; Perroni et al., 2014a) but these are relatively unavailable to plants. Therefore, P availability in these soils depends on mineralization of organic P fractions by soil microorganisms (Walker and Syers, 1976; Cross and Schlesinger, 2001), making microbial P limitation especially relevant for soil carbon processing in desert regions.

Most soil organic compounds are transformed or metabolized by microbes (Bradford et al., 2013), mainly by heterotrophic microorganisms that produce extracellular enzymes (ecoenzymes) that cleave organic molecules to allow C, N, and P assimilation (Waring et al., 2014). Ecoenzyme biosynthesis responds to environmental signals such as low nutrient availability to meet microbial nutrient demands; additionally, ecoenzymes can also enter the soil after cell lysis (Rilling et al., 2007; Sinsabaugh et al., 2009).

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After the recognition that ecoenzymes are major drivers of C and nutrient cycling in terrestrial, freshwater, and marine ecosystems, several ecoenzymes have been identified as useful indicators of nutrient deficiency and microbial nutrient demand (Burns, 1982; Nannipieri, 1994; Olander and Vitousek, 2000; Schimel and Weintraub, 2003; Renella et al., 2006; Sinsabaugh et al., 2009; Sinsabaugh and Follstad, 2012; Sinsabaugh et al., 2012; Waring et al., 2014). These enzymes are: β-1,4-glucosidase (BG) and cellobiohydrolase (CBH) as indicators of energy (C) demand; β-1,4-N-acetylglucosaminidase (NAG) and leucine aminopeptidase (LAP) as indicators of N demand; and acid or alkaline phosphatase (AP), as indicator of P demand (Schimel and Weintraub, 2003). These enzymes catalyze terminal reactions that produce assimilable molecules containing C, N, and P from high molecular weight organic compounds (Sinsabaugh et al., 2009).

Soil microorganisms acclimate to stress by reassigning key resources (i.e. energy, C, N, and P) to acquisition mechanisms rather than growth (Schimel et al., 2007). While it has also been reported that the ratios of C:N:P in microbial biomass are relatively constrained across ecosystems relative to variability in environmental nutrient availability (Cleveland and Liptzin, 2007), these ratios in the soil microbial biomass could nevertheless indicate how allocation shifts alter nutrient demand. For example, higher biomass C:N ratios likely reflect a greater overall investment in C-rich structural cellular material (Paul and Clark, 1996) while lower N:P ratios may reflect the higher allocation to P-rich ribosomes (Elser et al., 2003). Because ecoenzymatic activities reflect the microbial cell's response to meet its metabolic nutrient demands in response to environmental nutrient availability, ratios of commonly measured ecoenzymatic activities can be used to assess how the microbial community invests in energy relative to multiple nutrient acquisition under *in situ* conditions as it copes with resource limitation.

According to Sinsabaugh et al. (2009), ecoenzyme activity involves an intersection of Ecological Stoichiometry Theory (EST) with the Metabolic Theory of Ecology (MTE), offering promise to improve our understanding of energy and nutrient controls on microbial community metabolism (Sinsabaugh et al., 2012). This intersection can be understood via the Threshold Elemental Ratio (TER), that defines the element ratio at which growth shifts between nutrient limitation (represented by N and P, at high C:N or C:P) and energy (represented by C, lower C:N or C:P; Sterner and Elser, 2002; Frost et al., 2006). Additionally, under EST, organisms can be characterized with respect to their strength of stoichiometric homeostasis, e.g. the degree to which their biomass elemental composition shifts in response to the elemental composition of its diet or environmental resource supplies (Sterner and Elser, 2002). When the stoichiometric composition of the organism does not vary with changes in resource stoichiometry, the organism is considered strictly homeostatic. The growth of such strictly homeostatic organisms is strongly regulated by the most limiting nutrient and such an organism would be expected to respond with shifts in ecoenzymes that contribute to this homeostasis. In contrast, when the stoichiometry of the organism changes proportionately with the stoichiometry of the resource, the organisms are characterized as weakly or non-homeostatic; such adjustments may dampen the immediate impacts of nutrient limitation on growth but require a capacity for extensive storage (Sterner and Elser, 2002).

Our main objective in this study was to calculate the soil ecoenzymatic stoichiometry and determine its relation with soil energy (organic carbon) and nutrient availability for the soil microbe community in an extremely oligotrophic desert ecosystem with very low soil organic matter content. For that, we measured soil organic nutrients, nutrients within the microbial biomass, soil ecoenzyme activities, and we estimated microbial homeostasis at the community level in two energy-contrasting soils within the Cuatro Ciénegas Basin, México. We sought to quantify soil and microbial C:N:P ratios together with ecoenzymatic activity to determine the roles of energy and nutrient limitation in affecting microbial metabolism under these stressful conditions. Our hypothesis is that under lower soil C availability, the microorganisms invest more energy in nutrient acquisition rather than on increasing their biomass, by producing more ecoenzymes associated with the scarcer nutrient. This mechanism allows the microbial soil community to maintain nutrient homeostasis in soils with constrained energy availability. Our data shed light on the factors controlling carbon and soil nutrient cycling within and across desert ecosystems and extend the range of our current understanding of ecoenzymatic coupling in soil ecosystems.

2. Methods

2.1. Site description and soil sampling

This study was carried out in a grassland soil in the central region of the Chihuahuan Desert in the Cuatro Ciénegas basin (26°50′N and 102°8′W) in Coahuila, Mexico (740 m a. s.l). The climate is hot and arid; in spite of an average annual temperature of 21 °C, temperatures as high as 45 °C have been reported, mainly in July, as well as temperatures below 0 °C in January (SMN, CONAGUA, 2013). The mean annual precipitation is 253 mm but this is highly variable among years (Fig. 1). The majority of rainfall occurs mainly in summer. In the western side of the basin, Jurassic-era gypsum is the dominant parent material while in the eastern side Jurassic-era sandstones dominate (McKee et al., 1990). According to the world reference base for soil resources (WRB), the dominant soils are *Gypsisols* and *Calcisols* for the western and eastern sides, respectively. In both parts of the basin the grass *Sporobolus airoides* (Torr.) is the dominant plant species (Perroni et al., 2014b).

A sampling site was selected in each side of the basin: Churince (CH), in the western side; 26° 50.561′N; −102° 08.099′W; and Rancho Pozas Azules reserve (PA) in the eastern side; 26° 49.635′N; −102°01.470′W. Total aboveground biomass was 493 ± 61 and 323 ± 9 g m^{-2}; meanwhile total belowground biomass was 751 ± 170 and 289 ± 23 g m^{-2} for the western and the eastern sides, respectively (Tapia-Torres et al., 2015; Montiel-González unpublished data). Additionally, total C, N and P concentrations were 13.4 ± 1.8 mg g^{-1}; 0.9 ± 0.2 mg g^{-1}; 0.09 ± 0.01 mg g^{-1}, for the western side; and 5.9 ± 0.7 mg g^{-1}; 0.6 ± 0.07 mg g^{-1};

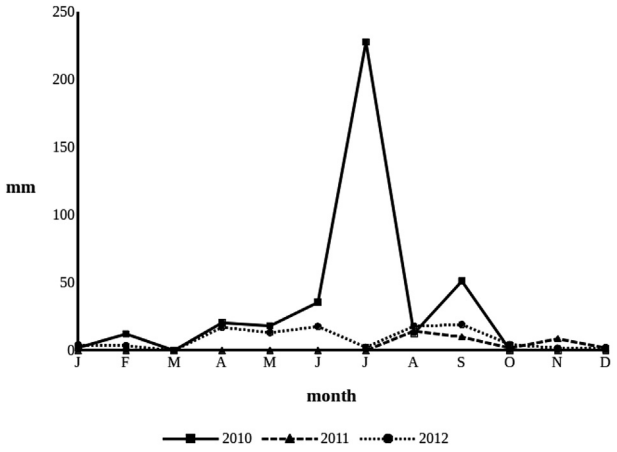

Fig. 1. Monthly rainfall for 2010, 2011 and 2012 year at Cuatro Ciénegas Basin.

0.09 ± 0.01 mg g^{-1} for the eastern side (Tapia-Torres et al., 2015; Montiel-González, unpublished data). Soil moisture contents were 13% and 9% for the western and the eastern side, respectively. The fine soil fraction (silt + clay) was 33% and 41% for the western and the eastern side, respectively. At each site, a 100 by 50 m plot was demarcated, which was then divided into 10 sections, separated from each other by 10 m. A random sampling transect was then selected in each section, with 15-cm top deep soil samples taken from ten sampling points (every 5 m); these were then mixed to form one composite sample. In total, 10 composite samples were taken at each plot in September of 2010, 2011, and 2012. Previous studies conducted on the study site have reported that soil biological activity increases mainly in the months of August and September because most of the annual precipitation is concentrated during these two months; soil moisture also increases (Perroni et al., 2014a; Tapia-Torres et al., 2015). Soil for biogeochemical and enzymatic activity analyses was stored in black plastic bags and refrigerated at 4 °C for laboratory analyses.

2.2. Biogeochemical analyses

Soil pH was measured in deionized water (1:2 w:v) with a Corning™ digital pH meter. To allow nutrient concentrations and enzymatic activities to be corrected for soil sample moisture content, a 100-g subsample was oven-dried at 75 °C to constant weight for soil moisture determination using the gravimetric method.

All C forms were determined with a Total Carbon Analyzer (UIC Mod. CM 5012; Chicago, E.U.A), while N and P forms were determined by colorimetric analyses using a Bran-Luebbe Auto Analyzer III (Norderstedt, Germany). Microbial P was determined by colorimetric analyses using a spectrophotometer (Evolution 201, Thermo Scientific Inc.)

Available, dissolved, and microbial nutrient forms were extracted from moist soil samples. Inorganic nitrogen forms (NH_4^+ and NO_3^-) were extracted with 2 M KCl (Robertson et al., 1999) and determined colorimetrically by the phenol-hypochlorite method. Inorganic phosphorus (IP) was extracted with sodium bicarbonate (pH 8.5) and was determined colorimetrically by the molybdate-ascorbic acid method (Murphy and Riley, 1962).

Dissolved nutrients were extracted with deionized water after shaking for 45 min and then filtering through a Millipore 0.42-μm filter (Jones and Willett, 2006). Previous to acid digestion, one aliquot of the filtrate was used to determine dissolved ammonium (DNH_4^+) and IP in deionized water extract. Total dissolved nitrogen (TDN) was digested by the macro-Kjendahl method. Total dissolved P (TDP) was also acid digested and determined by colorimetry. Total dissolved carbon (TDC) was measured with an Auto Analyzer of carbon (TOC CM 5012) module for liquids (UIC-COULOMETRICS). Inorganic dissolved carbon (IDC) was determined in an acidification module CM5130. Dissolved organic carbon (DOC), nitrogen (DON) and phosphorous (DOP) were calculated as the difference between total dissolved forms and inorganic dissolved forms.

Microbial C (C_{mic}), N (N_{mic}) and P (P_{mic}) concentrations were determined by the chloroform fumigation extraction method (Vance et al., 1987). Fumigated and non-fumigated samples were incubated for 24 h at 25 °C and constant moisture. C_{mic} was extracted from fumigated and non-fumigated samples with 0.5 M K_2SO_4, filtered through Whatman No. 42 filters (Brookes et al., 1985). C concentration was measured from each extract as total and inorganic concentration by the method described before. C_{mic} was calculated by subtracting the extracted carbon in non-fumigated samples from that of fumigated samples and dividing it by a K_{EC} value (extractable part of microbial biomass C) of 0.45 (Joergensen, 1996). N_{mic} was extracted with the same procedure used for C_{mic} but the extract was filtered through a Whatman No. 1 paper. The filtrate was acid digested and determined as TN by Macro-Kjeldahl method (Brookes et al., 1985). N_{mic} was calculated as for C_{mic}, but divided by a K_{EN} value (extractable part of microbial biomass N after fumigation) of 0.54 (Joergensen and Mueller, 1996). P_{mic} was extracted using $NaCO_3$ 0.5 M at pH 8.5. After this, the fumigation-extraction technique involving chloroform was performed (Cole et al., 1978). P_{mic} was calculated as for C_{mic} and N_{mic} and converted using a K_P value (extractable part of microbial biomass P after fumigation) of 0.4 (Lathja et al., 1999). P_{mic} was determined colorimetrically by the molybdate-ascorbic acid method using an Evolution 201 Thermo Scientific Inc. spectrophotometer (Murphy and Riley, 1962). Finally, C_{mic}, N_{mic} and P_{mic} values were normalized on a dry soil basis.

2.3. Ecoenzyme activity analyses

We measured the activities of four ecoenzymes with assay techniques reported by Tabatabai and Bremner (1969); Eivazi and Tabatabai, (1977; 1988) and Verchot and Borelli (2005). The potential activities of β-1,4-glucosidase (BG), cellobiohydrolase (CBH), β-1,4-N-acetylglucosaminidase (NAG), and alkaline phosphatase (AP) were quantified colorimetrically using ρ-nitrophenol (ρNP) substrates. For all enzymes, we used 2 g of fresh soil and 30 ml of modified universal buffer (MUB) pH 9 for ecoenzyme extraction. After that, three replicates and one control (sample without substrate) per sample were prepared. Additionally three substrate controls (substrate without sample) were included per assay. We centrifuged the tubes after the incubation period and then 750 μl of supernatant was diluted in 2 ml of deionized water and measured for the absorbance of ρ-nitrophenol (ρNP) at 410 nm on an Evolution 201 Thermo Scientific Inc, spectrophotometer. Ecoenzyme activities were expressed as nanomoles of ρNP formed per gram of soil dry weight per hour (nmol ρNP [g SDW]$^{-1}$ h^{-1}).

2.4. Data analyses

Soil biogeochemistry and ecoenzymatic data were subjected to a repeated measures analysis of variance (RM-ANOVA; Von Ende, 1993). Site (CH and PA) was considered as between-subject factor and year (2010, 2011 and 2012), and their interaction, were considered as within-subject factors. When RM-ANOVA indicated significant factor effects, mean comparisons were performed with Tukey's multiple comparisons test (Von Ende, 1993). Ecoenzyme activities were normalized to units per μg of available organic carbon (OC) using the DOC data corresponding to each sample. Unlike previous work in which the total organic carbon (TOC) value was used for ecoenzyme activity normalization (Sinsabaugh et al., 2009, 2010), we used DOC as we consider it to be a better indicator of soil C availability. However, specifically for the purpose of comparing our results with previously reported global patterns, ecoenzyme activities were also normalized to units per mg total organic carbon (TOC) using observed correlations between DOC and TOC from Cuatro Ciénegas samples.

Data were $\log_e$-transformed prior to regression analysis to conform to the conventions of stoichiometric analyses and to normalize variance (Sterner and Elser, 2002; Sinsabaugh and Follstad, 2012). After that, relationships between ecoenzyme activities were calculated with type II regression using SMATR (SMATR, R Development Core Team, 2007).

2.5. Stoichiometric homeostasis and threshold elemental ratio

We used Equation (1) to calculate the degree of community-level microbial C:N and C:P homeostasis (H′) by soil microorganisms (Sterner and Elser, 2002)

$$H' = 1/m \quad (1)$$

In Equation (1), m is the slope of $\log_e$ C:N_R (resources) versus $\log_e$ C:N_B (microbial biomass) or slope of $\log_e$ C:P_R versus $\log_e$ C:P_B scatterplot. $H' >> 1$ represents strong stoichiometric homeostasis, while $H' \approx 1$ represents weak or no homeostasis (Sterner and Elser, 2002).

To connect measured ecoenzyme activity with Ecological Stoichiometry Theory (EST) and the Metabolic Theory of Ecology (MTE), we followed Sinsabaugh et al. (2009) to calculate the TER for C:N and C:P using the following equations:

$$TER_{C:N} = ((BG/NAG)B_{C:N})/n_o \quad (2)$$

$$TER_{C:P} = ((BG/AP)B_{C:P})/p_o \quad (3)$$

where $TER_{C:N}$ and $TER_{C:P}$ are the threshold ratios (dimensionless), BG/NAG is the ecoenzymatic activity ratio for β-1,4-glucosidase and β-1,4-N-acetylglucosaminidase, BG/AP is the ecoenzymatic ratio for β-1,4-glucosidase and alkaline phosphatase, $B_{C:N}$ and $B_{C:P}$ are the C:N or C:P ratios of the microbial biomass (respectively), and n_0 and p_0 are the dimensionless normalization constants for N and P respectively. These normalization constants p_0 and n_0 are the intercepts in the SMA regressions for $\log_e$(BG) vs $\log_e$(NAG) and $\log_e$(BG) vs $\log_e$(AP) respectively. For a more detailed analysis of the derivation of the equations, see Sinsabaugh et al. (2009).

3. Results

As expected for a desert ecosystem, annual precipitation varied considerably during the study period (2010, 2011 and 2012). The highest annual precipitation was observed in 2010 with 379 mm, following by two dry years (36 mm and 102 mm for 2011 and 2012, respectively; Fig. 1).

3.1. Dynamics of soil and microbial nutrients

During all three years, soil samples in the western, Churince end of the basin (CH) always had higher DOC concentrations than in the eastern, Pozas Azules end (PA). Among years, 2010 showed the highest DOC concentration and 2012 showed the lowest concentration (Tables 1 and 2). DON concentration also varied among years but did not differ between sites; 2012 had the highest values and the lowest concentrations were observed in 2011 (Tables 1 and 2). NH_4^+ concentration was only different between sites in 2010, with CH having a higher concentration than PA (Tables 1 and 2). In the rainy year (2010), PA had higher DOP concentration than CH. Meanwhile, in 2011 and 2012 the patterns were inverted: CH showed higher concentration than PA (Tables 1 and 2). Meanwhile, DOC:DON, DOC:DOP, and DON:DOP ratios did not differ significantly among sites and years (Tables 1 and 2).

For microbial nutrients, during the three years, soil C_{mic} concentrations in CH were always higher than in PA. Among years, highest concentrations occurred in 2010 while 2011 had the lowest concentrations (Tables 1 and 2). However, N_{mic} concentration was higher in CH than in PA regardless of year (Tables 1 and 2). C:P_{mic} and N:P_{mic} ratios, both only showed site differences in 2010, when CH had a higher value than PA (Tables 1 and 2). In contrast, P_{mic} and C:N_{mic} ratios were not different for any of the comparisons (Tables 1 and 2).

3.2. Ecoenzymatic stoichiometry

NAG activity differed significantly among years but not between sites, decreasing over time; 2010 showed the highest activity and 2012 the lowest activity. In the same way, CBH differed significantly among years but not between sites; 2011 showed the highest activity followed by 2010 and the lowest activity was observed in 2012. In the rainy year (2010), PA had higher BG activity than CH but in 2011 the pattern was reversed, as CH showed higher activity. Meanwhile, in 2012 BG activity did not show any difference between sites (Tables 1 and 2). Similarly, the rainy year also had higher AP activity than the other two years (2011 and 2012). CH had higher AP activity than PA but only in 2010 (Tables 1 and 2).

CBH:NAG ratio was highest in 2010 relative to the other two years but the sites did not differ for this ratio (Tables 1 and 2). However, CH had higher BG:NAG ratio than PA but only in 2011. Samples from PA had higher BG:AP ratio than those from CH, again only in 2010 (Tables 1 and 2). On the other hand, PA showed higher NAG:AP ratio than CH during all three sampling years (Tables 1 and 2).

For most of the model II regressions analyzed, the results showed differences between sites (CH vs PA) in 2010, as PA had steeper slopes than CH for ln(BG) vs ln(NAG) and ln(CBH) vs ln(NAG). Meanwhile, CH had a steeper slope for ln(NAG) vs ln(AP) than PA (Table S1). In contrast, there were no differences in regression slopes between sites in 2011. Meanwhile, in 2012, a difference in slope was observed between sites only for ln(CBH) vs ln(AP), as PA showed a steeper slope than CH (Table S1). Additionally, when all the data were analyzed together, the ecoenzymatic stoichiometry of these soils for organic N and organic P acquisition both scale with C acquisition with a slope of ~1 that follows the global ecoenzymatic stoichiometry pattern but in a range that is one order of magnitude lower than previous observations (Fig. 2; Sinsabaugh et al., 2009).

3.3. Stoichiometric homeostasis and threshold elemental ratios

To test for the strength of stoichiometric homeostasis, we analyzed for associations between microbial biomass elemental ratios and those in soil resources. When all the data were analyzed together by site, the relationships between log C:N_R and log C:N_B, and between log C:P_R and log C:P_B were not different from zero ($p > 0.05$), indicating strong community-level elemental homeostasis in both sites (CH and PA) regardless of year (Fig. 3).

Based on the microbial C:N:P stoichiometric values and parameters generated from the enzymatic data, estimated $TER_{C:N}$ values in PA were higher than in CH in 2010 and 2012 although no differences between sites were observed in 2011 (Fig. 4A). We observed the opposite pattern for $TER_{C:P}$, as $TER_{C:P}$ in CH exceeded that for PA but only in 2010; there were no statistically significant differences in 2011 and 2012 although in both of these years $TER_{C:P}$ values were higher in CH than in PA (Fig. 4B).

4. Discussion

The soil ecoenzymatic activities reported in this study (Fig. 2) are among the lowest enzyme levels yet quantified in soil (Acosta-Martínez et al., 2003; Bastida et al., 2006; Sinsabaugh et al., 2008; Abdalla and Langer, 2009; Bell and Henry, 2011; Hortal et al., 2013), reflecting the aridity and ultraoligotrophic nature of the site (Elser et al., 2005). Intriguingly, the ecoenzymatic stoichiometry of these soils nevertheless follows the global ecoenzymatic stoichiometry patterns (Fig. 2). While we found differences in the slopes of the ecoenzymatic stoichiometry regressions between sites when the results were analyzed by sampling year (Table S1), the model II regression results indicated no differences between sites when all the data for the three years were analyzed together by site (Table S2). Previous studies have shown that the slopes of ecoenzymatic regressions can differ significantly by habitat (e. g. soil vs.

Table 1
Means of soil nutrients and ecoenzyme activities quantified in two grasslands during three consecutive years (2010, 2011, 2012) in the Cuatro Ciénegas Basin, Coahuila, Mexico.

Parameters	Year					
	2010		2011		2012	
	Churince	Pozas Azules	Churince	Pozas Azules	Churince	Pozas Azules
pH	8.5	8.4	8.6	8.6	8.8	8.9
DOC[a]	12.89	5.06	26.92	8.16	31.63	13.43
DON[b]	9.76	9.54	4.34	7.66	12.50	9.34
DOP[c]	1.69Ba	2.56Aa	2.04Aa	1.13Bb	2.43Aa	2.09Aa
Ammonium (NH_4^+)	5.78Aa	1.69Ba	3.06Ab	1.64Aa	1.69Ab	1.73Aa
C mic[d]	382	371	163	108	165	116
N mic[e]	39.73Aa	15.36Ba	14.33Ab	13.8Ba	14.34Ab	5.88Bb
P mic[f]	1.49	3.10	3.4	1.95	3.3	2.65
BG[g]	190Ba	330Aa	160Aa	60Bb	30Ab	40Ab
CBH[h]	140	130	190	200	2	2
NAG[i]	120	118	29	67	23	20
AP[j]	326Aa	158Ba	137Ab	133Aa	94Ab	38Aa
DOC:DON	1.5	1.01	5.9	1.22	4.0	4.1
DOC:DOP	8.6	5.6	14.3	18.7	12.2	9.1
DON:DOP	6.1	3.0	6.2	6.6	4.6	2.7
$C:N_{mic}$	31	28	9	8	20	24
$C:P_{mic}$	173Aa	74Ba	28Ab	49Aa	44Ab	37Aa
$N:P_{mic}$	20.1Aa	2.82Ba	3.1Ab	6.61Aa	3.33Ab	1.58Aa
BG:NAG	1.74Ab	2.92Aa	10.58Aa	0.98Ba	1.51Ab	3.8Aa
NAG:AP	0.42	0.82	0.199	0.519	0.284	0.743
NAG:CBH	2.5	0.68	0.19	0.238	6.59	8.61
BG:AP	0.58Ba	2.26Aa	1.57Aa	0.55Ab	0.28Aa	1.22Ab

ns = not significant. Values followed horizontally by a different online letter (A and B) indicate that means are significantly different ($P < 0.05$) between sites (Churince and Pozas Azules) within sampling year (2010, 2011 and 2012); whereas different lowercase letters (a, b and c) vertically indicate that means are significantly different ($P < 0.05$) among sampling dates within a site. Nutrients and C_{mic}, N_{mic}, P_{mic}, in μg g^{-1}, enzymes in nmol ρ-NP g^{-1} SDW h^{-1}.

[a] Dissolved organic carbon.
[b] Dissolved organic nitrogen.
[c] Dissolved organic phosphorous.
[d] Microbial carbon.
[e] Microbial nitrogen.
[f] Microbial phosphorus.
[g] β-1,4-Glucosidase.
[h] Cellobiohydrolase.
[i] β-1,4-N-acetylglucosaminidase.
[j] Alkaline phosphatase.

lotic sediment vs. lentic sediment) (Sinsabaugh et al., 2009, 2012; Sinsabaugh and Follstad, 2012). However, within these habitats, C acquisition enzymes have similar scaling relationships with N and P acquisition enzymes. The N and P ecoenzyme scaling coefficient values for soils have been reported as 1.09 and 1.16 (Sinsabaugh et al., 2009); our results indicated very similar values ranging from 0.96 to 1.19 and 1.12–1.49, respectively. While our N acquisition values are similar to those previously reported by Sinsabaugh et al. (2009) for data dominated by temperate soils, our P acquisition values are more similar to those reported in more weathered tropical soil (1.18; Waring et al., 2014) and consistent with the previously documented strong P limitation of the Cuatro Ciénegas ecosystem. Nevertheless, despite the low overall soil ecoenzymatic activity in this arid ecosystem, activities for organic N acquisition and organic P acquisition both scaled with C acquisition with a slope of ~1.0 (Fig. 2), indicating that the soil microbial communities exhibit similar patterns of allocation to nutrient acquisition despite the diverse community composition and conditions exhibited by the two regions of the ultra-oligotrophic Cuatro Ciénegas valley.

4.1. Microbial nutrient limitation in desert soils

Consistent with known effects of water availability on nutrient cycling in arid and semiarid ecosystems (Schimel and Parton, 1986; Bell et al., 2014), we observed differences in nutrient dynamics among years in concert with high interannual variation in moisture input. Due to the effects of Hurricane Alex (June 25–July 2nd 2010), 2010 was the wettest year in the last 40 years (379 mm), followed by the two driest years in the same period (36 and 102 mm respectively; Fig. 1). The low rainfall in both dry years (2011–2012) appeared to favor nutrient limitation of overall ecosystem productivity, as reflected in decreased available soil and microbial nutrient concentrations, as well as in lower absolute levels of ecoenzymatic activity (Table 1). On the other hand, increases in soil nutrient transformations and microbial activity in the year with higher water availability (2010) allowed us to observe more clearly different forms of nutrient limitation on microbial activity. This annual variability has been reported before for other grassland within the Chihuahuan desert (Bell et al., 2014). Thus, in the following, we focus on soil microbial nutrient status and enzymatic allocation patterns during this highly productive year.

Despite conditions of strong nutrient and water limitation, CCB soil microbial communities adjust physiologically to process low N and P resources, an inference supported by the soil community homeostasis analyses, in which the relationships between log $C:N_R$ and log $C:N_B$, and between log $C:P_R$ and log $C:P_B$ in both regions of the valley had slopes not significantly different from zero (Fig. 3). Theses physiological adjustments can be reflected in the degree of ecoenzyme expression. Generally, ecoenzyme expression is related to the quality of available organic matter and nutrient demands of the microbial biomass (Sinsabaugh et al., 2009; Sinsabaugh and Follstad, 2012). Based on ecoenzyme stoichiometry, the microbes of the two edaphically contrasting regions of the Cuatro Ciénegas valley exhibited different patterns of nutrient limitation in 2010. Soil microbes in the western side of the Cuatro Ciénegas valley exhibited lower BG:NAG ratios (Tables 1 and 2) as well as lower

Table 2
F-ratios and significant levels of the repeated measures ANOVA for soil variables quantified in two grasslands during three years (2010, 2011 and 2012) in Cuatro Ciénegas Basin, Coahuila Mexico.

Parameters	Source of variation		
	Between subject	Within subjects	
	Site	Date	Date X site
pH	0.1 (0.7)^{ns}	65 (<0.000001)	2.3 (0.11)^{ns}
DOC[a]	19.89 (0.0003)	7.85 (0.001)	1.58 (0.229)^{ns}
DON[b]	0.29 (0.59)^{ns}	5.97 (0.006)	3.27 (0.051)^{ns}
DOP[c]	0.14 (0.70)^{ns}	2.7 (0.07)^{ns}	4.2 (0.02)
Ammonium (NH_4^+)	17.5 (0.0005)	13.7 (0.00003)	13.9 (0.00003)
C_{mic}[d]	4.73 (0.04)	52.9 (<0.00001)	0.4 (0.67)^{ns}
N_{mic}[e]	14.9 (0.001)	13.19 (0.00005)	5.82 (0.006)
P_{mic}[f]	0.12 (0.72)^{ns}	0.57 (0.52)^{ns}	3.11 (0.056)^{ns}
BG[g]	2.02 (0.17)^{ns}	68.6 (<0.00001)	17.8 (<0.00001)
CBH[h]	0.03 (0.85)^{ns}	83.8 (<0.00001)	0.1 (0.89)^{ns}
NAG[i]	1.68 (0.21)^{ns}	50 (<0.00001)	2.79 (0.07)^{ns}
AP[j]	12.27 (0.002)	16.7 (<0.00001)	3.79 (0.03)
DOC:DON	3.8 (0.08)^{ns}	3.4 (0.053)^{ns}	2.6 (0.1)^{ns}
DOC:DOP	0.014 (0.90)^{ns}	1.98 (0.16)^{ns}	0.41 (0.66)^{ns}
DON:DOP	0.79 (0.39)^{ns}	2.14 (0.13)^{ns}	0.93 (0.040)^{ns}
C:N_{mic}	0.8 (0.38)^{ns}	1.6 (0.21)^{ns}	0.7 (0.48)^{ns}
C:P_{mic}	7.21 (0.04)	24.67 (0.001)	10.16 (0.003)
N:P_{mic}	49.3 (0.002)	14.78 (0.002)	19.8 (0.0007)
BG:NAG	4.1 (0.057)^{ns}	3.7 (0.03)	11.1 (0.0001)
NAG:AP	8.31 (0.012)	1.32 (0.28)^{ns}	0.09 (0.911)^{ns}
NAG:CBH	0.003 (0.95)^{ns}	7.36 (0.01)	0.43 (0.65)^{ns}
BG:AP	4 (0.06)^{ns}	1.95 (0.16)^{ns}	8.29 (0.0016)

ns = not significant.
[a] Dissolved organic carbon.
[b] Dissolved organic nitrogen.
[c] Dissolved organic phosphorous.
[d] Microbial carbon.
[e] Microbial nitrogen.
[f] Microbial phosphorus.
[g] β-1,4-Glucosidase.
[h] Cellobiohydrolase.
[i] β-1,4-N-acetylglucosaminidase.
[j] Alkaline phosphatise.

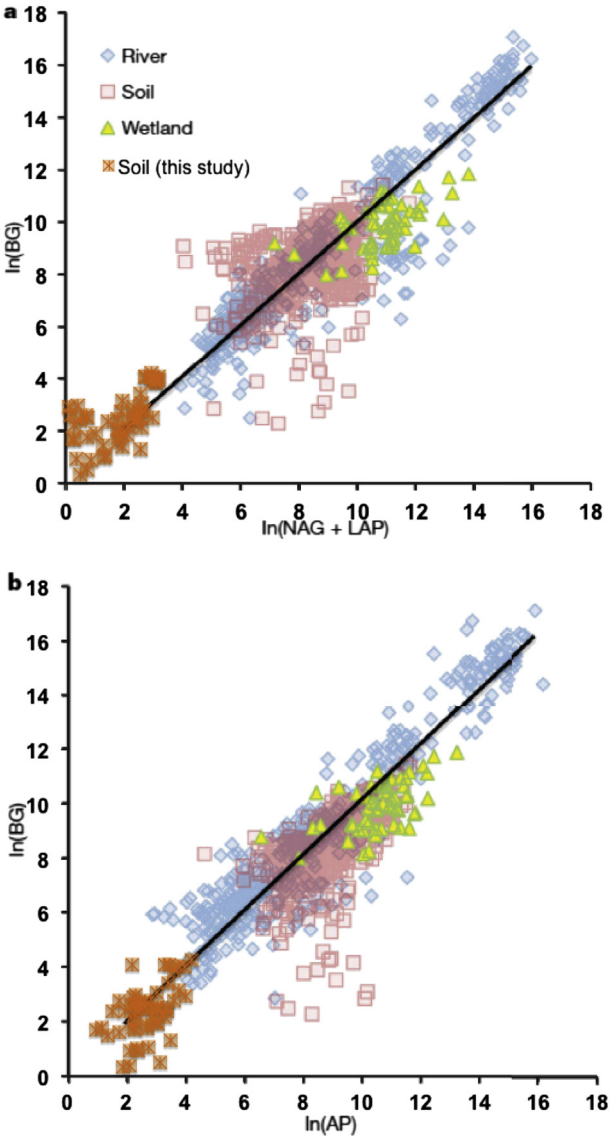

Fig. 2. Global patterns of organic nitrogen acquisition activity and organic phosphorus acquisition activity in relation to carbon acquisition. The figure of Sinsabaugh et al. (2009) with data for terrestrial (red squares) and wetland (green triangles) soils and river (blue circles) sediments was modified and our data for Cuatro Cienegas soil were superimposed (orange stars). N acquisition for river, soil and wetland soils and sediments was measured by the potential activities of β-1,4-N-acetylglucosaminidase (NAG) and leucine aminopeptidase (LAP) (Sinsabaugh et al., 2009). In this study, N acquisition was measured by the potential activity of β-1,4-N-acetylglucosaminidase (NAG) (a); P acquisition was measured as phosphatase (AP) activity (b); β-1,4-glucosidase (BG) represented C acquisition. Enzyme activities for Cuatro Ciénegas (CH and PA) soil were among the lowest data yet quantified; however, they followed the same global ecoenzymatic stoichiometry patterns. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

$B_{C:N}/R_{C:N}$ ratios (where $B_{C:N}$ is C:N ratio of microbial biomass and $R_{C:N}$ is the C:N ratio of labile organic matter; Sinsabaugh et al., 2009; Sinsabaugh and Follstad, 2012), indicative of N limitation of the soil microbial community. This interpretation of a greater role of N limitation in CH is consistent with the shallower slope for ecoenzymatic regressions in Churince than Pozas Azules for ln(BG) vs ln(NAG) (0.85 and 1.14 respectively for CH and PA) and ln(CBH) vs ln(NAG) (0.67 and 1.17 respectively; Table S1), indicative of disproportionate allocation to N-acquisition enzymes relative to energy/C enzymes in Churince. These site-specific differences in C/N enzyme coupling in the two lobes of the CCB valley point to the importance of characterizing spatial variation in soil enzyme patterns, especially where edaphic conditions influence the geochemical composition of the soils.

A converse pattern was observed for P limitation. While previous studies of P concentrations in the Cuatro Ciénegas valley have shown no differences in soil total P concentration (TP) between the western and eastern sides (Perroni et al., 2014a), P availability may nevertheless differ due to the high reactivity of PO_4^-, as P may be strongly bound by calcium and magnesium ions in alkaline soils such as those at Cuatro Ciénegas (Perroni et al., 2014a). For example, near-zero concentrations of dissolved inorganic P (the most available P fraction) have been recorded for grassland soil at Cuatro Ciénegas (López-Lozano et al., 2012; Perroni et al., 2014a), as well as very low concentrations in various aquatic systems in the valley (ca. 0.60 μM total dissolved P; Elser et al., 2005). Therefore, soil P likely represents a critical nutrient constraint within this valley but its role in limiting microbial activity may vary in different sites in the valley. The lower $B_{C:P}/R_{C:P}$ ratio (where $B_{C:P}$ is C:P ratio of microbial biomass and $R_{C:P}$ is the C:P ratio of labile organic matter) in the eastern side (Pozas Azules) of the Cuatro Ciénegas valley is consistent with enhanced P limitation in PA. Therefore soil microbes of Pozas Azules should need to invest disproportionately in P acquisition. Thus, the higher AP activity in the site with lower apparent P limitation (Churince) was unexpected (Table 1). This might be explained by differences in the substrates targeted by different phosphatase enzymes. Among the variety of phosphatase enzyme classes, phosphomonoesterases (PM; often called acid or

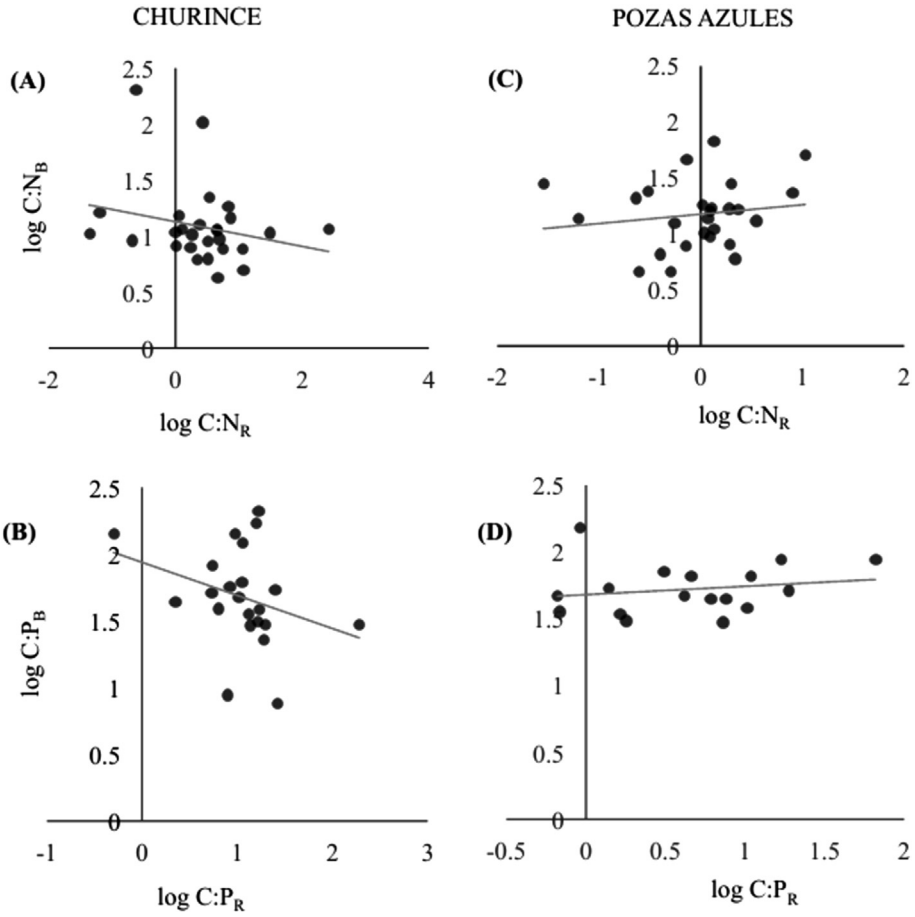

Fig. 3. Soil microbial community homeostasis related with nitrogen (N; panels on top) and phosphorus (P; panels on down) acquisition. A and B) Data from Churince (western side of the valley), C and D) Data from Pozas Azules (eastern side of the valley). Indicating similar patterns of soil microbial community stoichiometry among sites in all cases "strong homeostasis" (slopes are not different to zero: A) $p = 0.154$, B) $p = 0.47$, C) $p = 0.22$, D) $p = 0.44$). The equations for each panel, is as follow: A) $y = -0.1097x + 1.1272$, $R2 = 0.06$. B) $y = -0.2482x + 1.9382$, $R2 = 0.10$. C) $y = 0.0839x + 1.1767$, $R2 = 0.024$. D) $y = 0.0626x + 1.6809$, $R2 = 0.03$.

alkaline phosphatases, AP) that mineralize P from inositol phosphates, nucleotides, phosphoproteins, and sugar phosphates (Turner et al., 2005) are the most frequently evaluated. However, phosphodiesterases (PD), that mineralize P from nucleic acids, phospholipids and other diester phosphates (Turner et al., 2002), are also relevant in sites with low P concentrations (such as Cuatro Ciénegas valley). Phosphate diesters are the main input of organic P (P_O) to soils, but typically constitute only a small fraction of soil P_O (Turrion et al., 2010). In contrast to AP activity, the PD activity was significantly higher in Pozas Azules than in Churince (252 ± 16 and 162 ± 19 nmol ρNP [g SDW]$^{-1}$ h^{-1} respectively, $p < 0.05$; Tapia-Torres unpublished data). These ecoenzyme activity patterns between sites suggest different preferences and capacities of the microbial community to mineralize diverse phosphorus substrates and therefore suggest different microbial strategies for P acquisition between the western and eastern sides of the valley.

We next combined our ecoenzymatic data and elemental composition data to estimated microbial TER values to better understand microbial metabolic limitation at the community level in this arid and ultra-oligotrophic site. Notably, we found pronounced site-specific contrasts. For example, estimated $TER_{C:N}$ was lower in the western side (Churince) than in eastern side (Pozas Azules) of the Cuatro Ciénegas valley, while $TER_{C:P}$ had the opposite pattern (higher in the western side than in eastern side of the valley; Fig. 4). The lower $TER_{C:N}$ and $TER_{C:P}$ observed in the sites with N or P limitation, respectively, likely reflect shifts in the soil microbial community that modulate their sensitivity to nutrient limitation. If the C:N or C:P ratio of the organic matter being consumed is greater than the TER for that element, it suggests nutrient limitation (Sterner and Elser, 2002). In the eastern side (Pozas Azules) of the valley, Montiel-González (unpublished data) observed a total organic C:P ratio of 64.7 ± 5, this value is significantly greater ($p < 0.05$) than the $TER_{C:P}$ estimated in this work (19.4 ± 3.4, Fig. 4), which supports the idea of stronger microbial P limitation in the eastern side of the Cuatro Ciénegas valley, reflecting the effects of edaphic substrata.

Overall, our work helps in understanding of how resources are allocated to enzymatic activity by the microbial community depending on soil resource availability. However, the nutrient limitation patterns at the community level are not yet clear. Liebig's Law of the Minimum, which states that only one element limits the growth of organisms at any given time, is widely used in ecology (Danger et al., 2008) but its applicability is not always clear for highly diverse microbial communities (as is the case for the microbial community in CCB, López-Lozano et al., 2012). Indeed, it has been observed in microbial communities that some organisms can adjust their effort to collect various nutrients by allocating more resources to scarce, and less effort to abundant, nutrients (Bloom et al., 1985). Therefore, at a community scale, the concept of the minimum has been replaced by the broader view that C, N and/or P co-limit community production by differentially impacting different members of the community (Sinsabaugh et al., 2010). Our

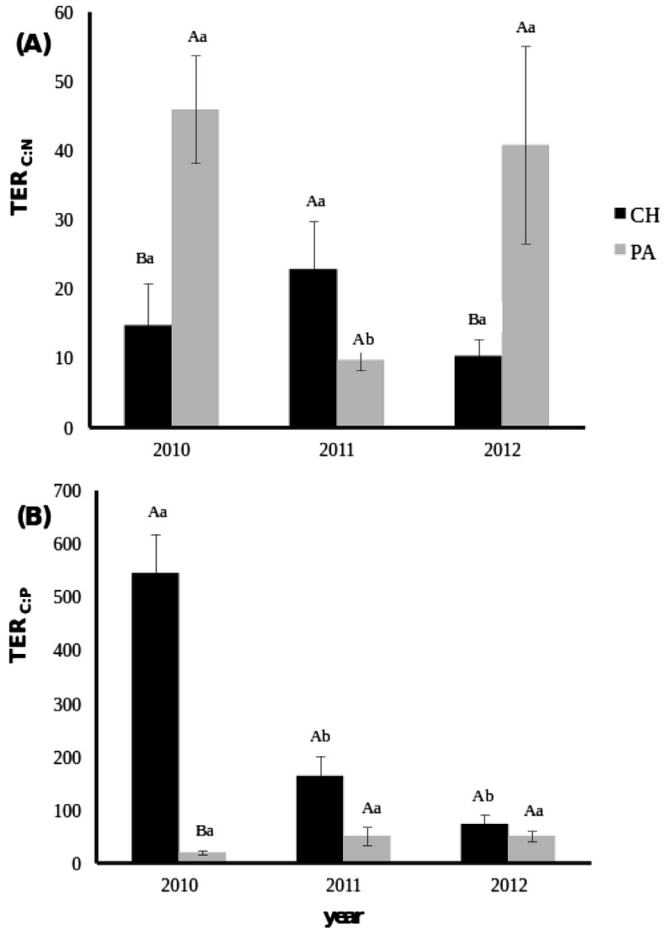

Fig. 4. Threshold Elemental Ratio (TER)C:N and (TER)C:P (A and B, respectively) of soil microbial community during three consecutive years (2010, 2011 and 2012). Different uppercase letter (A and B) indicate that means are significantly different ($P < 0.05$) between sites (Churince and Pozas Azules) within sampling year (2010, 2011 and 2012); whereas different lowercase letters (a, b and c) vertically indicate that means are significantly different ($P < 0.05$) among sampling dates within a site.

data are consistent with an interpretation that the soil microbial communities of this ultra-oligotrophic desert ecosystem are co-limited by C and either by N (Churince, western side of the valley) or by P (Pozas Azules, eastern side of the valley). This C limitation is observed in a high BG/DOC ratio, indicating a high production of enzyme per unit of available carbon in both sites. Our data also suggest that members of these microbial communities allocated more for acquiring the scarcer nutrient, consistent with a variety of emerging views about how energy and material flows are coupled in soil ecosystems (Danger et al., 2008).

4.2. Conclusions

The soil ecoenzymatic activities reported in this study are among the lowest enzyme levels yet quantified in soil. However, activities for organic N acquisition and organic P acquisition both scaled with C acquisition with a slope of ~1.0, indicating that the soil microbial communities exhibit similar patterns of allocation to nutrient acquisition despite contrasting edaphic conditions and community composition in different regions of the Cuatro Cienegas valley. We also showed that the soil microbial communities of this ultra-oligotrophic desert ecosystem were co-limited by C and either by N (Churince, western side of the valley) or by P (Pozas Azules, eastern side of the valley). Nevertheless, our results indicated strong community-level elemental homeostasis in both sites (CH and PA) that is must be maintained by differential investment in enzymes according to the scarcer nutrient. Our findings support the broad generality of the ecoenzymatic approach in microbial resource ecology and illustrate how enzyme responses support balanced resource acquisition by microbes experiencing diverse geochemical and hydrologic conditions.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2015.04.007.

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