Response of soil extracellular enzyme activity and stoichiometry to short-term warming and phosphorus addition in desert steppe

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Abstract

Background. P is regarded as one of the major limiting factors in grassland ecosystems. Soil available phosphorus deficiency could affect soil extracellular enzyme activity, which plays an essential role in microbial metabolism. Yet, it is still unclear how soil available phosphorus affects soil extracellular enzyme activity and microbial nutrient limitation of desert grassland in the context of climate warming.

Methods. The study carried out a short-term open-top chambers (OTCs) experiment in desert steppe to examine the effects of warming, P addition and their interaction on soil properties, the activities of soil extracellular enzyme s and stoichiometries.

Results. The findings demonstrated that soil acquisition enzyme stoichiometry of C: N: P was 1.2:1:1.5 in this experiment region, which deviated from the global mean scale (1:1:1). Warming increased soil AN (ammonium nitrogen and nitrate nitrogen) contents, decreased MBC (microbial biomass carbon) and MBN (microbial biomass nitrogen). Phosphorus addition raised the soil available phosphorus contents and MBP (microbial biomass phosphorus). Soil extracellular enzyme activities and stoichiometries in desert grassland are largely impacted by soil AN, MBC: MBP, and MBN: MBP. These results revealed that the changes of soil available nutrients and stoichiometries induced by short-term warming and P addition could influence soil microbial activities and alleviate soil microbial carbon and phosphorus limitation. Our findings highlight the critical role played by soil available phosphorus in regulating soil extracellular enzyme activity and microbial nutrient limitation of desert grassland. It will be better to comprehend the microbiological mechanisms underlying these events with further research on the soil microbial communities.

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Introduction

Phosphorus (P), one of the most significant mineral elements, is crucial for plant development, substance synthesis and energy metabolism (Sharma et al.,2020), Yet, due to the effects of climate change, human activities, land use patterns and other reasons, there has been a severe shortage of soil available P worldwide (Hinsinger, 2001; Hou et al., 2020). P has evolved into a major limiting factor for plant growth in grassland ecosystems (Du et al., 2020), which may have negative effects on primary productivity and other ecological processes (Tang et al.,2018; Hou et al.,2020). Since 1850-1900, the temperature of the atmosphere has risen by around 1.1 °C, and it is predicted to reach or exceed 1.5-2.0 °C in the future (IPCC, 2021). The availability of nutrients in the soil may change as a result of climate warming (Feike et al.,2012; Hu et al., 2022), which requires soil microbes to secrete extracellular enzymes in response to the variation of soil nutrients (Raiesi and Salek-Gilani,2018; Cui et al.,2019a), this is because the activity of soil extracellular enzymes regulates the decomposition of soil organic matter and the cycling of nutrients, which in turn affects the function of ecosystem services (Güsewell, 2004; Hu et al., 2016). Soil extracellular enzyme secretion is frequently the rate-limiting step of microbial metabolism (Nannipieri et al.,2018). In this process, the temperature affects the soil microbial activity (Zi et al.,2018). According to the studies, global warming has the potential to change soil available phosphorus by altering mean annual temperature and precipitation(Hou et al.,2018),

76 and also have an impact on phosphorus fixation, mineralization, and weathering by indirectly
77 affecting plant growth and soil microbial community structure (Delgado-Baquerizo et al.,2013).
78 Evaluating the degree of nutrient availability and limitation of the microbial environment can be
79 achieved by analyzing soil C, N, and P acquisition enzyme activities and stoichiometries
80 (Moorhead et al., 2013; Dong et al., 2019).
81   The previous findings demonstrated that climate change can increase the availability of
82 nutrients by accelerating the microbial breakdown of soil organic matter (Bardgett et al., 2008).
83 The demand for carbon and phosphorus from microbes may change as a result of soil short-term
84 warming, leading to greater microbial phosphorus limitation (Zheng et al.,2020). The addition of
85 P enhanced the soil available phosphorus, reduced phosphorus limitation, and increased carbon
86 sequestration (Manzoni et al.,2012; Wang et al., 2022). P addition had both enhancing and
87 inhibitory impacts on the soil extracellular enzyme activity (Jing et al.,2016; Wang et al., 2020a),
88 even the input rates of various phosphorus fertilizers affected soil acquiring enzyme activities
89 (Waring et al.,2014; Tian et al.,2016). These findings imply that potential mechanisms
90 influencing soil extracellular enzyme activity and microbial metabolism induced by soil
91 available P in the context of climate change are controversial.
92   A typical fragile grassland ecosystem, the desert steppe is especially vulnerable to climate
93 change (Zuo et al.,2020), which is important to sustain ecological security and socioeconomic
94 development (Kang et al.,2007). The degradation of desert grasslands and the decline of
95 ecosystem stability and service functions may be driven by climate change, overgrazing, and
96 unreasonable exploitation (Reynolds et al.,2007; Deng et al., 2014; Zhang et al.,2019). Given the
97 importance of the soil available nutrients for primary production and other ecological processes
98 in grassland ecosystems (Tang et al., 2018), understanding the relationship between soil
99 available phosphorus, soil extracellular enzyme activity, and its stoichiometry under warming
100 conditions can help provide insight into the role of soil available phosphorus in controlling
101 ecological processes, which is crucial to actively respond to climate warming and develop
102 effective conservation strategies for desert grassland (Liu et al.,2017; Wang et al.,2021). Here,
103 we set up a field experiment to investigate the response of soil extracellular enzyme activity and
104 its stoichiometry to warming and P addition in desert grassland, we sought to address the
105 following two issues: (ⅰ) how soil extracellular enzyme activities, microbial nutrient limitation
106 reacts to short-term warming and P addition; and (ⅱ) what are the major factors affecting soil
107 extracellular enzyme activities and stoichiometries. Based on the previous studies (Waring et
108 al.,2014; Zheng et al., 2020), we hypothesized that (ⅰ) the degree of nutrient limitation of soil
109 microorganisms was closely related to the content of soil available phosphorus; (ⅱ) warming
110 and P addition would significantly reduce soil extracellular enzyme activity in desert grassland.

111 **Materials & Methods**

112 **Study site**

113 The experiment was conducted on a desert steppe in Eastern Yanchi County (37°04′-38°10 N,
114 106°03′-107°04′ E), Ningxia Hui Autonomous Region, northwest China. It is located on the
115 southwest edge of the Mu Us Desert, which has a semiarid continental monsoon climate.

116 According to meteorological data from the Yanchi Meteorological Station, the average annual
117 temperature is 8.8°C, and the average annual precipitation is 298.15mm, with the majority of the
118 precipitation falling between July and September (1980-2021). The habitat type is the desert
119 steppe. The soil type is classified as Arenosol (IUSS, 2015), with 4.12 $g·kg^{-1}$ of soil organic
120 matter, 0.40 $g·kg^{-1}$ of total nitrogen, 0.32 $mg·kg^{-1}$ of total phosphorus, 2.30 $mg·kg^{-1}$ of available
121 phosphorus, and pH 8.57. The dominant species in the region are *Agropyron mogolicum*,
122 *Lespedeza potaninii*, *Caragana korshinskii*, *Stipa bungeana*, and *Polygala tenuifolia Willd*.

123 **Experimental Design**
124 We conducted a randomized split-plot design with two temperature treatments (CK, control; W,
125 warming) as the main plot and three P addition levels (0 $g·m^{-2}·yr^{-1}$, 5 $g·m^{-2}·yr^{-1}$, and 10 $g·m^{-2}·yr^{-1}$
126 ^{1}) as the subplot in April 2022. There were six treatments: P_0, P_5, P_{10}, WP_0, WP_5, and WP_{10}, each
127 replicated four times, for a total of 24 treatment plots. A PVC sheet was put into the soil at a
128 depth of 0.8 m to divide each subplot from the main plot, which was spaced 3 m apart to provide
129 a buffer zone. The phosphorus fertilizer was supplied by triple superphosphate [Ca (H_2PO_4)
130 $_2·H_2O$], which was evenly distributed to the treatment plots before rainfall from early June to
131 August. Although the rate of phosphorus fertilizer input in this study is higher than the rate at
132 which phosphorus is deposited in the atmosphere of northern China (Zhu et al., 2016), it meets
133 with the current agricultural fertilization in China (Cui et al.,2020; Guo et al., 2022).
134 Based on the meteorological monitoring of the study site from 1980 to 2021 (Figure S1) and
135 the existing research foundation of the team (Ma et al.,2019; Ma et al.,2021), we modified the
136 experimental warming device. OTCs (Open Top Chambers) were used as a passive temperature
137 rise device in this investigation. The stainless steel and high-transmittance glass material (5mm
138 thick) were used to construct a regular octagonal prism structure, which has a substrate area of
139 5.6 m^2 and a vertical height of 1.8 m. They were installed in the sample plot permanently to
140 avoid disruption. The air and soil temperatures at 15cm above and below ground were
141 automatically recorded every half-hour using HOBO MX2302A and HOBO MX2201 data
142 loggers, respectively (Onset Computer Corporation, Bourne, Massachusetts, USA).

143 **Soil sampling and measurements**
144 Three soil cores (5 cm in diameter, 15 cm in depth) were collected randomly from each plot after
145 removing any surface debris on August 25, 2022. These soil samples were cleaned of plant roots,
146 stone, or other pollutants, and manually homogenized to one composite sample, then returned to
147 the laboratory immediately. The soil was divided into two parts, one of which was air-dried and
148 sieved through a 2 mm mesh to measure soil organic carbon (SOC), total nitrogen (TN), total
149 phosphorus (TP), and available phosphorus (AP). The other of which was stored at 4 °C to
150 measure soil soluble organic carbon (DOC), ammonium nitrogen (NH_4^+–N), nitrate nitrogen
151 (NO_3^-–N), microbial biomass, and soil C-, N-, P- acquiring enzyme activities.
152 Soil moisture content (SMC) was determined by oven-drying the soil at 105°C for 48 hours. A
153 PHS-3E glass pH electrode (Leici, Shanghai, China) was used to measure the pH of the soil in a
154 suspension of air-dried soil and distilled water (1:5, w/v) (Anderson and Ingram, 1993). A TOC
155 analyzer was used to measure the soil DOC concentration (Vario TOC, Elementar, Hanau,

156   Germany). Soil available nitrogen (NH_4^+–N and NO_3^-–N) was determined by the KCl extraction
157   method with continuous flow analyzer; AP was determined by $NaHCO_3$ extraction with
158   molybdenum antimony anti-colorimetric method; Soil SOC and TN were determined by
159   potassium dichromate external heating method and H_2SO_4-H_2O_2 digestion with Kjeldahl method;
160   Soil TP was determined by $HClO_4$-H_2SO_4 digestion with molybdenum antimony anti-
161   colorimetric method (Nelson, 1996). Microbial biomass (MBC, MBN, MBP) was extracted and
162   analyzed by chloroform fumigation(Brookes et al.,1985; Vance et al.,1987), respectively.
163   **Soil enzyme extraction and vector analysis**
164   Soil C (β-1,4-glucosidase, BG), N (leucine aminopeptidase, LAP, and β-1,4-N-acetylglucosa
165   minidase, NAG), and P acquisition enzyme (alkaline phosphatase, ALP) activities were
166   determined using a modified standard fluorescence technique (Saiya-Cork et al., 2002; German
167   et al.,2011). Soil extracellular enzyme activity (EEA)was expressed in units of nmol g^{-1} h^{-1}
168   (Sinsabaugh et al.,2008; Sinsabaugh et al, 2009; Waring et al.,2014). The ratios of C, N, and P
169   acquisition enzymes were calculated by the following formulae to determine soil extracellular
170   enzyme stoichiometry (EES), respectively. Scatter plots of soil ecoenzymatic stoichiometry were
171   used to identify microbial resource-limited (Hill et al.,2012; Jiang et al., 2022).
172   Soil e C: N = ln (BG) /ln (LAP + NAG)
173   Soil e C: P = ln (BG) /ln (ALP)
174   Soil e N: P = ln (LAP + NAG) /ln (ALP)
175   Soil C, N, and P -acquiring enzyme stoichiometries were assessed using vector analysis.
176   Vector length and vector angle were calculated by the following formulae, respectively
177   (Moorhead et al., 2016). Limitations on N and P are shown by vector angles of less than 45° and
178   greater than 45°, respectively, while a relative carbon limitation is shown by vector length.
179   Relative C limitation =Vector Length= $\sqrt{[\ln(BG)/\ln(LAP + NAG)]^2 + [\ln(BG)/\ln(ALP)]^2}$
180   N/P limitation =Vector angle(º) = Degrees {ATAN2[(ln(BG)/ln(ALP),(ln(BG)/ln(LAP+NAG)]}
181   **Statistical analyses**
182   A two-way analysis of variance using a split-plot design was carried out to investigate the effects
183   of warming, P addition, and their interaction effects on soil extracellular enzyme activity and
184   stoichiometries of desert grassland. One-way ANOVA was used to compare the differences in
185   soil properties, soil extracellular enzyme activity, and stoichiometries of the different treatments.
186   Significant differences were tested using Duncan's multiple comparison tests (P<0.05), and the
187   data for soil enzyme activity had been transformed using a natural logarithm before statistical
188   analysis. Before performing an ANOVA, all data were tested for normality of the residuals and
189   homogeneity of variances in SPSS 25.0 for Windows (SPSS Inc., Chicago, Illinois, USA).
190   Pearson correlation analysis was used to determine the association between soil characteristics,
191   microbial biomass and their stoichiometric. Mantel test was used to explore the correlation
192   between carbon, nitrogen, and phosphorus acquisition enzyme activities and soil environmental
193   factors. RDA(redundancy analysis) was used to explore the corresponding relationships of soil
194   properties, microbial biomass, and extracellular enzyme activity in Canoco 5.0. For the graphics,

195   R 4.2.3 and Origin 2022 (Origin Laboratory Corporation, Northampton, MA, USA) were

196   applied.

Results

198   **Soil temperature and soil moisture content**

199   OTCs had the anticipated warming effect in all temperature-increased treatment plots (Fig. 1),

200   with an average increase of 1.17°C in air temperature 15cm above the ground and 0.65°C in soil

201   temperature 15cm below ground. Warming considerably decreased soil moisture content by

202   13.67%. While P addition significantly increased SMC by 11.20% and 19.52% at P_5 and P_{10},

203   respectively. Soil moisture content was not significantly affected by warming × P addition (Table

204   1).

205   **Soil properties**

206   No significant effects of SOC, TN, or TP by warming (Table. 1). P addition had distinct effects

207   on SOC under different P addition rates, experiencing a decline of 8.02% at P_5 and an increase of

208   2.44% at P_{10}. P addition significantly increased TP and decreased TN. Warming × P addition

209   resulted in a rise of SOC and TP. As for TN, WP_{10} decreased by 4.48%, while WP_5 grew by

210   13.43%. Warming and P addition did not significantly affect SOC: TN, but decreased SOC: TP.

211   Warming × P addition increased SOC: TP at WP_5 while lowering it by 22.59% at WP10. Soil

212   TN: TP decreased by 2.58%, and 29.38% at WP_5 and WP_{10}, respectively.

213   The soil pH was not significantly affected by warming, P addition, and their interactions

214   (Table 1). P addition and warming both lowered soil DOC contents, but their interaction effects

215   significantly increased DOC 12.22% and 15.06% at WP_5 and WP_{10}, respectively. Warming and

216   warming × P addition significantly increased the AN content. P addition decreased AN by 6.67%

217   at P_5 and did not change at P_{10}. The addition of P had a considerable impact on available P

218   content, and the higher the rate of P addition, the greater the increase in soil available P content.

219   Nevertheless, there was no discernible influence of available P by warming. Warming and P

220   addition decreased DOC: AN. Warming × P addition significantly affected DOC: AN, with

221   33.05% decrease in WP_5 and no significant difference in WP_{10}. P addition and warming × P

222   addition had a significant negative impact on DOC: AP and AN: AP.

223   **Soil microbial biomass and their stoichiometry**

224   Compared to the control plots (P_0), all of the treatments led to a reduction in MBC and MBN

225   (Figs. 2A and 2B). Warming and P addition did not significantly affect MBC and MBN, but

226   their interaction significantly decreased them. MBP responded differently from MBC and MBN

227   (Fig. 2C), warming lowered MBP by 9.01%, P addition and warming × P addition considerably

228   increased MBP. Ratios of MBC: MBN, MBC: MBP, and MBN: MBP were significantly reduced

229   by warming and P addition. Warming × P addition increased MBC: MBN (Fig. 2D) and a

230   substantial decrease in MBC: MBP (Fig. 2E) and MBN: MBP (Fig. 2F).

231   **Soil enzyme activity and their stoichiometry**

232   Soil BG was significantly lowered by warming, P addition, and the effects of their interaction

233   (Fig.3A). As for warming × P addition, the activity of BG at WP_5 was higher than that at WP_{10}.

234   LAP+NAG was significantly decreased by P addition (Fig.3B), but not significantly affected by

235 warming and their interaction effects. Soil ALP was significantly reduced by warming, P
236 addition, and warming × P addition (Fig.3C). Warming and P addition reduced ln (BG): ln
237 (LAP+NAG) (Fig.3D), while demonstrating a different trend by Warming × P addition, rising by
238 4.10% at WP_5, and falling by 13.93% at WP_{10}. Regarding ln (BG): ln (ALP) increased at WP_5
239 (Fig. 3E), with no significant impacts from other treatments. Warming, P addition and the
240 interaction effects raised ln (LAP+NAG): ln (ALP) (Fig.3F). In addition, a significant correlation
241 between BG, NAG+LAP, and ALP activity was also discovered (Fig. 4).

242 **Vector analysis**
243 All the treatments were over the 1:1 line (Fig. 5A), which showed that microbial nutrients were
244 severely phosphorus limited. Warming, P addition, and their interaction effects reduced vector
245 angle (Fig. 5B). Vector length and vector angle were decreased as a result of warming and P
246 addition (Figs. 5C and 5D). Warming × P addition increased vector length by 11.64% at WP_5
247 and decreased by 7.53% at WP_{10}.

248 **Relationships between soil properties and soil extracellular enzyme activities**
249 Pearson correlation showed a significant relationship between soil AP and DOC: AP, AN: AP,
250 MBN: MBP, MBC: MBP. Soil SMC content was significantly related to DOC: AP, AN: AP.
251 Mantel correlation analysis showed that soil total nutrients and stoichiometry had no significant
252 effects on soil EEAs and EES. However, a positive link between soil C and P acquisition enzyme
253 activities and soil available nutrients, microbial biomass, and their stoichiometry was observed,
254 soil N acquisition enzyme activity was closely related to DOC: AN, DCO: AP, and MBP. (Fig.
255 6). By using a redundancy analysis, we further examined the connection between the soil
256 available nutrients, soil EEAs, EES, and vectors (Fig.7). The results revealed that the important
257 factors affecting soil EEAs and EES were AN, MBC: MBP, and MBN: MBP. There had a
258 significant positive correlation between AN and e C: P. e N: P was positively correlated with AP.
259 MBC was positively correlated with e C: N. Vector length was positively correlated with AN,
260 while vector angle was significantly negatively correlated with available P (Fig. 7).

261 # Discussion
262 **Short-term warming**
263 In this study, we conducted a field experiment to investigate the effects of short-term warming
264 and P addition on the soil extracellular enzyme activity and stoichiometries. Short-term warming
265 increased soil AN content (Table 1), and reduced microbial biomass C and N (Fig.2). Soil C-, N-
266 , and P-acquiring enzymes were decreased by warming (Fig. 3), which was consistent with our
267 hypotheses. No significant effect on the soil total nutrients and stoichiometries by warming
268 (Table 1). Correlation analysis and redundancy analysis revealed a significant positive
269 correlation between soil EEAs and soil available nutrients, microbial biomass and their
270 stoichiometry (Figs. 6 and 7), these results were in keeping with the previous studies (Cui et al.,
271 2021; Li et al., 2022b). Further research on soil microbial communities of desert grassland is
272 required to better understand the exact microbiological mechanisms by which microbial biomass
273 controls the soil extracellular enzyme activity.

274   Soil moisture content may also affect soil EEA (Allison and Treseder,2008; Li et al., 2022a).
275   Warming considerably decreased SMC in our study (Table 1), however, SMC had no significant
276   impact on soil EEAs and EES (Figs. 6 and 7), and it was not a limiting factor for soil EEA. The
277   experiment demonstrated that soil microorganisms may adapt to dry, infertile soil conditions by
278   adjusting soil extracellular enzyme activity in response to their demands and the surrounding
279   environment (Gong et al.,2015; Wang et al.,2017). Meanwhile, we discovered that warming
280   slightly raised ln (BG): ln (ALP) and ln (LAP+NAG): ln (ALP), decreased ln (BG): ln
281   (LAP+NAG) (Fig. 3), vector length and vector angle (Fig. 5), it is likely because warming
282   reduced the soil microbial biomass C and N, and consequently influenced the secretion of C- and
283   N-acquiring enzymes to adapt to environmental changes (Turner and Wright, 2014). Taken
284   together, short-term warming changed the activities of soil microbial in desert steppe.

285   **Phosphorus addition**

286   Soil phosphatases typically showed higher activity in P-limited ecosystems, and soil C and N-
287   acquiring enzyme activity reflects microbial demand for energy and nutrients (Turner and
288   Wright, 2014). In our study, P addition lowered soil extracellular enzyme activities (Fig. 3),
289   which had a great impact on the activity of the P acquisition enzyme (Marklein and
290   Houlton,2012; Shi et al.,2021). P addition significantly promoted the soil total P, available P and
291   microbial biomass P content while concurrently reducing DOC, AN, MBC, and MBN contents
292   (Table 1, Fig. 2). Varying alterations were found in MBC, MBN, and MBP, suggesting that soil
293   microorganisms may preferentially fix P (Bünemann et al., 2012), which affected the
294   stoichiometries of soil microbial biomass. Correlation analysis demonstrated a negative
295   correlation between soil C-, P- acquiring enzyme activity and available P, MBP (Fig. 6) . The
296   relationship is most likely explained by the fact that P addition increased plant growth and
297   nutrient uptake while reducing the number of nutrients available for the synthesis of soil
298   enzymes(Wang et al.,2008). Yet, contrary to the results of some research, this might be
299   influenced by the factors like the rate of P addition, type of ecosystem and study site (Colvan et
300   al,2001; Tian et al., 2016; Wang et al., 2020b).

301   In terms of soil extracellular enzyme stoichiometry, ln (BG): ln (LAP+NAG) decreased by P
302   addition, whereas ln (BG): ln (ALP) and ln (LAP+NAG): ln (ALP) increased (Fig. 3), suggesting
303   that P addition decreased microbial demand for carbon and soil microbial demand for alkaline
304   phosphatase production costs in phosphorus-limited soils, and also affects microbial activity
305   associated with C and N cycling enzymes(Allison et al., 2010). P addition decreased vector
306   length and vector angle (Fig. 5), demonstrating that P addition can alleviate both the soil
307   microbial carbon and phosphorus limitation in desert grassland, the result confirmed our
308   hypothesis that soil microbial nutrient limitation of desert grassland is closely related to soil AP
309   content.

310   **The interactive effects of short-term warming and P addition**

311   In this study, warming × P addition significantly reduced soil EEAs (Fig. 3). As for C- acquiring
312   enzyme activity, WP$_5$ was higher than WP$_{10}$, which may be related to the significant increase in
313   soil DOC and AN content. This, in turn, may have caused the low level of P$_5$ addition rate to

314   secrete more C and N acquisition enzymes at higher temperatures, increasing microbial carbon
315   and nitrogen demand (Fig. 2). At WP_{10}, however, there were less available carbon and nitrogen
316   in the soil, and fewer C- acquiring enzymes are secreted by the microorganisms. WP_{10} was
317   consistently slightly higher than WP_5 throughout the changes in N-, and P-acquiring enzyme
318   activities, suggesting that the amount of available phosphorus may be a vital factor (Table 1). P-
319   acquiring enzymes were most significantly impacted by warming × P addition (Fig. 3) . It
320   showed a negative feedback relationship between high available phosphorus content and soil
321   EEA (Allison et al., 2007). The positive connection between the activities of the BG,
322   LAP+NAG, and ALP indicates that these soil enzymes changed significantly under experiment
323   warming and P addition (Fig. 4), and it further illustrates that the microbial acquisition to C, N,
324   and P was altered. About soil EES, warming × P addition resulted in a reduction of ln (BG): ln
325   (ALP) and ln (LAP+NAG): ln (ALP), increased ln (BG): ln (LAP+NAG) at WP_5 and declined at
326   WP_{10} (Fig. 3). Combining the results of the redundancy analysis and correlation analysis, the
327   primary factors that influenced the soil EEAs and EES were the soil AN, MBC: MBP, and MBN:
328   MBP. It can be shown that the interaction between warming and P addition had an impact on soil
329   physicochemical properties, which altered the stoichiometry of soil extracellular enzymes (Zheng
330   et al.,2015; Zhang et al.,2018).
331   Additionally, the natural log ratio of soil C-N-P- acquiring enzymes in this study was 1.2:1:1.3
332   (Table S1), which differs from the global mean scale of 1:1:1 (Sinsabaugh et al., 2009),
333   demonstrating that desert grassland in the study region was largely C and P limitation. This is
334   partially consistent with the findings of the previous study, demonstrating that desert grassland
335   was severely P-limited in the temperate grassland of northern China (Peng and Wang, 2016).
336   The ratio of soil C: N: P acquisition enzymes adjusted to 1.3:1:1.2 at WP_5, it is shown that a low
337   phosphorus addition rate causes both soil microbial carbon and phosphorus limitation in the
338   region, while the addition of P_{10} alleviated it. Also, previous research had revealed that adding P
339   may boost soil microbial nitrogen demand, and lead to an increase in N-acquiring enzyme
340   activity (Wang et al., 2020b). Nevertheless, it did not occur in our study, and this issue may need
341   to be confirmed by further research. Vector analysis, however, revealed that relative carbon
342   limitation increased at WP_5 and reduced at WP_{10}. As for phosphorus limitation, a higher level of
343   phosphorus addition rate (P_{10}) was beneficial to alleviate phosphorus limitation in the region.
344   Warming × P addition changed the soil available nutrient contents and their stoichiometries,
345   while soil nutrient stoichiometry influences microbial C, N, and P metabolism by regulating soil
346   elemental balance(Cui et al., 2019b). In this process, the secretion of soil microbial enzymes in
347   P-deficiency areas of desert grassland was stimulated to adapt to nutrient limitation (Schimel et
348   al.,2007; Xiao et al.,2018). From the above results, in this study, soil available nutrients,
349   microbial biomass, and their stoichiometry are the key factors contributing to microbial nutrient
350   limitation in desert grassland.

Conclusions

352   In this study, we investigated how soil C, N, and P acquisition enzyme activities and
353   stoichiometries responded to short-term warming and P addition in desert grassland. The results

354 showed that warming did not affect soil available P but altered soil AN, MBC, and MBN. P
355 addition significantly raised soil available phosphorus contents, which had positive effects on the
356 activities of BG, LAP+NAG, and ALP. For the different rates of P addition, P_{10} was preferable
357 to P_5 for eliminating the relative carbon and phosphorus limitation. Warming and P addition
358 slightly raised ln (BG): ln (ALP) and ln (LAP+NAG): ln (ALP), decreased ln (BG): ln
359 (LAP+NAG). About the interaction of warming and P addition, resulting in a reduction of ln
360 (BG): ln (ALP) and ln (LAP+NAG): ln (ALP), increased ln (BG): ln (LAP+NAG) at WP_5 and
361 declined at WP_{10}. The primary factors that influenced the soil EEAs and EES were the soil AN,
362 MBC: MBP, and MBN: MBP. In conclusion, Short-term warming, P addition, and their
363 interaction significantly affected soil extracellular enzymes and stoichiometries, which in turn
364 changed microbial resource acquisition techniques in desert steppe. More extensive research on
365 soil microbial communities is required to confirm the potential role that soil available
366 phosphorus may play in actively adapting to global warming and microbial nutrient constraint.
367

368 **References**

369 Allison SD, Treseder KK.2008. Warming and drying suppress microbial activity and carbon
370   cycling in boreal forest soils. Global Change Biology 14:2898–2909. DOI 10.1111/j.1365-
371   2486.2008.01716.x
372 Allison VJ, Condron LM, Peltzer DA, Richardson, S.J., Turner, B.L .2007. Changes in enzyme
373   activities and soil microbial community composition along carbon and nutrient gradients at
374   the Franz Josef chronosequence, New Zealand. Soil Biology & Biochemistry 39:1770–
375   1781. DOI 10.1016/j.soil bio.2007.02.006
376 Allison SD, Weintraub MN, Gartner TB, Waldrop MP .2010. Evolutionary-Economic Principles
377   as Regulators of Soil Enzyme Production and Ecosystem Function. In: Shukla, G., Varma,
378   A. (eds) Soil Enzymology. Soil Biology, vol 22. Springer, Berlin, Heidelberg. DOI
379   10.1007/978-3-642–14225-3_12
380 Anderson JM, Ingram JSI .1993. Tropical Soil Biology and Fertility: A Handbook of Methods.
381   CAB International Wallingford, UK, Wallingford, p 221. DOI 10.2307/2261129
382 Bardgett RD, Freeman C, Ostle NJ .2008. Microbial contributions to climate change through
383   carbon cycle feedbacks. The ISME Journal 2(8): 805–814. DOI 10.1038/ismej.2008.58
384 Brookes PC, Landman A, Pruden G, Jenkinson DS .1985. Chloroform fumigation and the release
385   of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in
386   soil. Soil Biology & Biochemistry 17: 837–842. DOI 10.1016/0038-0717(85)90144-0
387 Bünemann E K, Oberson A, Liebisch F, Keller F, Annaheim KE, Huguenin-Elie O, Frossard E
388   .2012. Rapid microbial phosphorus immobilization dominates gross phosphorus fluxes in
389   grassland soil with low inorganic phosphorus availability. Soil Biology & Biochemistry
390   51:84–95. DOI 10.1016/ j.soilbio. 2012. 04.012
391 Colvan S, Syers J, O'Donnell A .2001. Effect of long-term fertilizer use on acid and alkaline
392   phosphormonoesterase and phosphodiesterase activities in managed grassland. Biology and
393   Fertility of Soils 34: 258–263. DOI 10.1007/ s003740100411

394   Cui H, Sun W, Delgado-Baquerizo M, Song W, Ma JY, Wang K, Ling X .2020. Phosphorus
395   addition regulates the responses of soil multifunctionality to nitrogen over-fertilization in a
396   temperate grassland. Plant and Soil 473:73-87. DOI 10.1007/ s11104-020-04620-2
397   Cui YX, Bing HJ, Fang LC, Jiang M, Shen GT, Yu JL, Wang X, Zhu H, Wu YH, Zhang XC.
398   2021. Extracellular enzyme stoichiometry reveals the carbon and phosphorus limitations of
399   microbial metabolisms in the rhizosphere and bulk soils in alpine ecosystems. Plant and Soil
400   458(1–2):7–20. DOI 10.1007/ s11104-019-04159-x
401   Cui YX, Fang LC, Guo XB, Wang X, Wang YQ, Zhang YJ, Zhang XC .2019a. Responses of soil
402   bacterial communities, enzyme activities, and nutrients to agricultural-to-natural ecosystem
403   conversion in the Loess Plateau, China. Journal of Soils and Sediments 19(3):1427–1440.
404   DOI 10.1007/s11368-018-2110-4
405   Cui YX, Fang, LC, Guo XB, Han F, Ju WL, Ye LP, Wang X, Tan WF, Zhang XC .2019b.
406   Natural grassland as the optimal pattern of vegetation restoration in arid and semi-arid
407   regions: Evidence from nutrient limitation of soil microbes. Science of the Total
408   Environment 648:388–397. DOI 10.1016/ j.scitotenv.2018.08.173
409   Deng L, Sweeney S, Shangguan Z-P . 2014. Grassland responses to grazing disturbance: plant
410   diversity changes with grazing intensity in a desert steppe. Grass and Forage Science
411   69:524–533. DOI 10.1111 /g- fs.12065
412   Delgado-Baquerizo M, Maestre F T, Gallardo A, Bowker M, Wallenstein M, Quero J, Ochoa
413   V, Gozalo B, García-Gómez M, Soliveres S, García-Palacios P, Berdugo M, Valencia E,
414   Escolar C, Arredondo T, Barraza-Zepeda C, Bran D, Carreira J, Chaieb M, Conceição A,
415   Derak M, Eldridge D, Escudero A, Espinosa C... 2013. Zaady E. Decoupling of soil nutrient
416   cycles as a function of aridity in global drylands. Nature 502(7473): 672-676. DOI
417   10.1038/nature12670
418   Dong CC, Wang W, Liu HY, Xu XT, Zeng H .2019. Temperate grassland shifted from nitrogen
419   to phosphorus limitation induced by degradation and nitrogen deposition: Evidence from soil
420   extracellular enzyme stoichiometry. Ecological Indicators 101: 453–464. DOI 10.1016/
421   j.ecolind.2019.01.046
422   Du E, Terrer C, Pellegrini AFA, Ahlström A, van Lissa CJ, Zhao X, Xia N, Wu X, Jackson RB
423   .2020. Global patterns of terrestrial nitrogen and phosphorus limitation. Nature Geoscience
424   13(3): 221–226. DOI 10.1038/s41561-019-0530-4
425   Feike AD, Elise P, Jack AM, Dana MB, Yolima C, Daniel RL, Ronald FF, David GW .2012.
426   Climate change alters stoichiometry of phosphorus and nitrogen in a semiarid grassland.
427   New Phytologist 196(3): 807–815. DOI 10.1111/j.1469-8137.2012.04349.x
428   German DP, Weintraub MN, Grandy AS, Lauber CL, Rinkes ZL, Allison SD .2011. Optimization
429   of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biology and
430   Biochemistry 43: 1387–1397. DOI 10.1016/ j.soilbio.2011.03.017
431   Guo N, Xie MY, Fang Z, Jiao F, Han XY .2022. Divergent responses of plant biomass and
432   diversity to short-term nitrogen and phosphorus addition in three types of steppe in Inner
433   Mongolia, China. Ecological Processes 11(1): 32. DOI 10.1186/s13717-022-00376-y

434   Güsewell S .2004. N : P ratios in terrestrial plants: variation and functional significance. New
435   Phytologist 164: 243–266. DOI 10.1111/j.1469-8137.2004.01192.x

436   Hill BH, Elonen CM, Seifert LR, May AA, Tarquinio E .2012. Microbial enzyme stoichiometry
437   and nutrient limitation in US streams and rivers. Ecology Indicators 18: 540–551. DOI
438   10.1016 /j.ecolin- d. 2012.01.007

439   Hinsinger P .2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-
440   induced chemical changes: A review. Plant and Soil237:173–195. DOI 10.1023
441   /A:10133516175- 32

442   Hou EQ, Chen CR, Luo YQ, Zhou GY, Kuang YW, Zhang YG, Heenan M, Lu XK, Wen DZ
443   .2018. Effects of climate on soil phosphorus cycle and availability in natural terrestrial
444   ecosystems. Global Change Biology 24(8): 3344–3356. DOI 10.1111/gcb.14093

445   Hou EQ, Luo YQ, Kuang YW, Chen CR, Lu XK, Jiang LF, Luo XZ, Wen DZ .2020. Global
446   meta-analysis shows pervasive phosphorus limitation of aboveground plant production in
447   natural terrestrial ecosystems. Nature Communications 11(1):637. DOI 10.1038/s41467-
448   020-14492-w

449   Hu N, Li H, Tang Z, Li ZF, Li GC, Jiang Y, Hu XM, Lou YL .2016. Community size, activity and
450   C: N stoichiometry of soil microorganisms following reforestation in a Karst region.
451   European Journal of Soil Biology73:77–83. DOI 10.1016/j.ej-sobi. 2016.01.007

452   Hu WJ, Tan JR, Shi XR, Lock TR, Kallenbach RL, Yuan ZY .2022. Nutrient addition and
453   warming alter the soil phosphorus cycle in grasslands: A global meta-analysis. Journal of
454   Soils and Sediments 22(10): 2608–2619. DOI 10.1007 /s11368-022-03276-y

455   Allan RP, Hawkins E, Bellouin N, Collins B .2021. IPCC, 2021: Summary for Policymakers.
456   In: Masson-Delmotte, V, Zhai P, Pirani A, Connors SL, Péan C, Berger S, Caud N, Chen
457   Y, Goldfarb L, Gomis MI, Huang M, Leitzell K, Lonnoy E, Matthews JBR, Maycock
458   TK, Waterfield T, Yelekçi O, Yu R, Zhou B (eds.) Climate Change 2021: The Physical
459   Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the
460   Intergovernmental Panel on Climate Change. Cambridge University Press, pp. 3–32. DOI
461   10.1017/9-781 009157896.001

462   IUSS (2015) World Reference Base for Soil Resources .2014. Update 2015. FAO, Rome, Italy.

463   Jiang C, Zhu B, Zeng H .2022. Soil extracellular enzyme stoichiometry reflects the unique
464   habitat of karst tiankeng and helps to alleviate the P-limitation of soil microbes. Ecological
465   Indicators 144: 109552. DOI 10.1016/ j.ecolind. 2022.109552

466   Jing X, Yang XX, Ren F, Zhou HK, Zhu B, He JS .2016.Neutral effect of nitrogen addition and
467   negative effect of phosphorus addition on topsoil extracellular enzymatic activities in an
468   alpine grassland ecosystem. Applied Soil Ecology 107:205–213. DOI
469   10.1016/j.apsoil.2016.06.004

470   Kang L, Han XG, Zhang ZB, Sun OJ .2007. Grassland ecosystems in China: Review of current
471   knowledge and research advancement. Philosophical Transactions of the Royal Society of
472   London. Series B, Biological sciences362:997–1008. DOI 10.1098/rstb. 2007. 2029

473 Li JW, Xie JB, Zhang Y, Dong LB, Shangguan ZP, Deng L .2022a. Interactive effects of
474   nitrogen and water addition on soil microbial resource limitation in a temperate desert
475   shrubland. Plant and Soil 475(1–2): 361–378. DOI 10.1007/ s11104-022-05371-y
476 Li YZ, Zhou HK, Chen WJ, Wu Y, Qiao LL, Yan ZR, Liu GB, Xue S .2022b. Long-term
477   warming does not affect soil ecoenzyme activity and original microbial nutrient limitation
478   on the Qinghai—Tibet Plateau. Soil Ecology Letters 4(4): 383–398. DOI 10.1007/s42832-
479   021-0116-0
480 Liu Y, Jiang M, Lu XG, Lou YJ, Liu B .2017. Carbon, Nitrogen and Phosphorus contents of
481   wetland soils in relation to environment factors in Northeast China. Wetlands 37(1): 153–
482   161. DOI 10.1007/s13157-016-0856-2
483 Ma Y, Wang N, Jia H, Cao B. 2019. Evaluation of a modified open-top chamber simulation
484   system on the study of elevated CO_2 concentration effects. Journal of Environmental
485   Sciences 10, 307–315. DOI 10.7515/JEE182070
486 Ma Y, Xie Y, Ha R, Cao B, Song L. 2021. Effects of Elevated CO_2 on Photosynthetic
487   Accumulation, Sucrose Metabolism-Related Enzymes, and Genes Identification in Goji
488   Berry (Lycium barbarum L.). Frontiers in Plant Science 12, 643555. DOI 10.3389/ fpls.
489   2021.643555
490 Manzoni S, Taylor P, Richter A, Porporato A, Agren GI .2012. Environmental and stoichiometric
491   controls on microbial carbon-use efficiency in soils. New Phytologist 196:79–91. DOI 10.
492   1111/j. 1469- 8137. 2012. 04225.x
493 Marklein AR, Houlton BZ .2012. Nitrogen inputs accelerate phosphorus cycling rates across a
494   wide variety of terrestrial ecosystems. New Phytologist 193: 696–704. DOI 10.1111/j.1469-
495   8137.2011.03967.x
496 Moorhead DL, Rinkes ZL, Sinsabaugh RL, Weintraub MN .2013. Dynamic relationships between
497   microbial biomass, respiration, inorganic nutrients and enzyme activities: informing enzyme-
498   based decomposition models. Frontiers in Microbiology 4:223. DOI 10.3389/ fmicb.2013.
499   00223
500 Moorhead DL, Sinsabaugh RL, Hill BH, Weintraub MN .2016. Vector analysis of ecoenzyme
501   activities reveal constraints on coupled C, N and P dynamics. Soil Biology and
502   Biochemistry 37: 1–7. DOI 10. 1016/ j.soilbio.2015.10.019
503 Nannipieri P, Trasar-Cepeda C, Dick RP .2018. Soil enzyme activity: a brief history and
504   biochemistry as a basis for appropriate interpretations and meta-analysis. Biology and
505   Fertility Soils 54 (1): 11–19. DOI 10.1007/s00374-017-1245-6
506 Nelson DW, Sommers LE .1996. Total Carbon, Organic Carbon, and Organic Matter. 9,961–
507   1010. DOI 10.2136/SSSABOOKSER5.3.C34
508 Peng XQ, Wang W .2016. Stoichiometry of soil extracellular enzyme activity along a climatic
509   transect in temperate grasslands of northern China. Soil Biology and Biochemistry 98:74–
510   84. DOI 10.1016/ j.soil- bio.2016.04.008
511 Raiesi F, Salek-Gilani S .2018. The potential activity of soil extracellular enzymes as an indicator
512   for ecological restoration of rangeland soils after agricultural abandonment. Applied Soil
513   Ecology 126: 140–147. DOI 10.1016/ j.apsoil.2018.02.022

514 Reynolds JF, Smith DMS, Lambin EF, Turner BL, Mortimore M, Batterbury SPJ, Downing TE,
515   Dowlatabadi H, Fernández RJ, Herrick JE, Huber-Sannwald E, Jiang H, Leemans R, Lynam
516   T, Maestre FT, Ayarza M, Walker B .2007. Global Desertification: Building a Science for
517   Dryland Development. Science 316: 847–851. DOI 10.1126/ science.1131634

518 Saiya-Cork KR, Sinsabaugh RL, Zak DR .2002. The effects of long-term nitrogen deposition on
519   extracellular enzyme activity in an Acer saccharum forest soil. Soil Biology & Biochemistry
520   34: 1309–1315. DOI 10.1016/S0038-0717(02) 00074-3

521 Schimel J, Balser TC, Wallenstein M .2007. Microbial stress-response physiology and its
522   implications for ecosystem function. Ecology 88: 1386–1394. DOI 10.1890/06-0219

523 Sharma AK, Muhlroth A, Jouhet J, Marechal E, Alipanah L, Kissen R, Brembu T, Bones AM,
524   Winge P .2020. The Myb-like transcription factor phosphorus starvation response (PtPSR)
525   controls conditional P acquisition and remodeling in marine microalgae. New Phytologist
526   225:2380–2395. DOI 10. 1111/ nph.16248

527 Shi JY, Gong JR, Baoyin T, Luo QP, Zhai ZW, Zhu CC, Yang B, Wang B, Zhang ZH, Li XB
528   .2021. Short-term phosphorus addition increases soil respiration by promoting gross
529   ecosystem production and litter decomposition in a typical temperate grassland in northern
530   China. Catena 197:104952. DOI 10.1016/ j.catena.2020.104952

531 Sinsabaugh RL, Hill BH, Shah JJF .2009. Ecoenzymatic stoichiometry of microbial organic
532   nutrient acquisition in soil and sediment. Nature 462:795–798. DOI 10.1038/ nature08632

533 Sinsabaugh RL, Lauber CL, Weintraub MN, Ahmed B, Allison SD, Crenshaw C, Contosta AR,
534   Cusack D, Frey S, Gallo ME, Gartner TB, Hobbie SE, Holland K, Keeler BL, Powers JS,
535   Stursova M, Takacs-Vesbach C, Waldrop MP, Wallenstein MD, Zak DR, Zeglin LH .2008.
536   Stoichiometry of soil enzyme activity at global scale. Ecology Letters 11(11):1252–1264.
537   DOI 10.1111/j.1461-0248. 2008.01245.x

538 Tang ZY, Xu WT, Zhou GY, Bai YF, Li JX, Tang XL, Chen DM, Liu Q, Ma WH, Xiong GM,
539   He HL, He NP, Guo YP, Guo Q, Zhu JL, Han WX, Hu HF, Fang JY, Xie ZQ .2018.
540   Patterns of plant carbon, nitrogen, and phosphorus concentration in relation to productivity
541   in China's terrestrial ecosystems. Proceedings of the National Academy of Sciences of the
542   United States of America 115 (16):4033–4038. DOI 10.1073/pnas.1700295114

543 Tian J, Wei K, Condron LM, Chen Z, Xu Z, Chen L .2016. Impact of land use and nutrient
544   addition on phosphatase activities and their relationships with organic phosphorus turnover
545   in semi-arid grassland soils. Biology and Fertility of Soils 52: 675–683. DOI
546   10.1007/s00374-016-1110-z

547 Turner BL, Wright SJ .2014. The response of microbial biomass and hydrolytic enzymes to a
548   decade of nitrogen, phosphorus, and potassium addition in a lowland tropical rain forest.
549   Biogeochemistry 117: 115–130. DOI 10.1007/ s10533-013-9848-y

550 Vance ED, Brookes PC, Jenkinson DS .1987. An extraction method for measuring soil microbial
551   biomass C. Soil Biology &Biochemistry 19:703–707. DOI 10.1016/ 0038-0717(87)90052-6

552 Wang C, Mori T, Mao Q, Zhou K, Wang Z, Zhang Y, Mo H, Lu X, Mo J .2020a. Long-term
553   phosphorus addition downregulates microbial investments on enzyme productions in a

554   mature tropical forest. Journal of Soils and Sediments 20(2):921–930. DOI 10.1007/s11368-
555   019-02450-z

556 Wang M, Gong Y, Lafleur P, Wu Y .2021. Patterns and drivers of carbon, nitrogen and
557   phosphorus stoichiometry in Southern China's grasslands. Science of The Total
558   Environment 785:147201. DOI 10.1016/ j.scito- tenv.2021.147201

559 Wang QK, Wang SL, Liu YX .2008. Responses to N and P fertilization in a young Eucalyptus
560   dunnii plantation Microbial properties, enzyme activities and dissolved organic matter.
561   Applied Soil Ecology 40:484–490. DOI 10.1016/ j.apsoil. 2008.07.003

562 Wang R, Cao Y, Wang H, Dijkstra FA, Jiang J, Zhao R, Ma W, Li T, Dorodnikov M, Wang Z,
563   Sardans J, Peñuelas J .2020b. Exogenous P compounds differentially interacted with N
564   availability to regulate enzymatic activities in a meadow steppe. European Journal of Soil
565   Science 71(4): 667–680. DOI 10.1111/ejss. 12906

566 Wang R, Dorodnikov M, Dijkstra FA, Yang S, Xu Z, Li H, Jiang Y .2017. Sensitivities to
567   nitrogen and water addition vary among microbial groups within soil aggregates in a
568   semiarid grassland. Biology and Fertility of Soils 53:129–140. DOI 10.1007/s00374-016-
569   1165-x

570 Wang X, Cui Y, Wang Y, Duan C, Niu Y, Sun R, Shen Y, Guo X, Fang L .2022. Ecoenzymatic
571   stoichiometry reveals phosphorus addition alleviates microbial nutrient limitation and
572   promotes soil carbon sequestration in agricultural ecosystems. Journal of Soils and
573   Sediments 22(2): 536–546. DOI 10.1007/s11368-021-03094-8

574 Waring BG, Weintraub SR, Sinsabaugh RL .2014. Ecoenzymatic stoichiometry of microbial
575   nutrient acquisition in tropical soils. Biogeochemistry 117:101–113. DOI 10.1007/s10533-
576   013-98 49-x

577 Zhang K, Su Y, Yang R .2019. Variation of soil organic carbon, nitrogen, and phosphorus
578   stoichiometry and biogeographic factors across the desert ecosystem of Hexi Corridor,
579   northwestern China. Journal of Soils and Sediments 19:49–57. DOI 10.1007/ s11368-018-
580   2007-2

581 Zhang XY, Yang Y, Zhang C, Niu SL, Yang H, Yu GR, Wang HM, Blagodatskaya E, Kuzyakov
582   Y, Tian DS, Tang YQ, Liu S, Sun XM .2018. Contrasting responses of phosphatase kinetic
583   parameters to nitrogen and phosphorus additions in forest soils. Functional Ecology 32:106–
584   116. DOI 10.1111/1365-2435.12936

585 Zheng HF, Liu Y, Chen YM, Zhang J, Li HJ, Wang LF, Chen QM .2020. Short-term warming
586   shifts microbial nutrient limitation without changing the bacterial community structure in an
587   alpine timberline of the eastern Tibetan Plateau. Geoderma 360:113985. DOI 10.1016/j.geod-
588   erma.2019.113985

589 Zheng MH, Huang J, Chen H, Wang H, and Mo JM .2015. Responses of soil acid phosphatase
590   and beta-glucosidase to nitrogen and phosphorus addition in two subtropical forests in
591   southern China. European Journal of Soil Biology 68:77–84. DOI 10.1016/ j. ejsobi. 2015.
592   03.010

593 Zhu JX, Wang QF, He NP, Smith MD, Elser JJ, Du JQ, Yuan GF, Yu GR, Yu Q .2016.
594   Imbalanced atmospheric nitrogen and phosphorus depositions in China: Implications for

595   nutrient limitation. Journal of Geophysical Research: Biogeo sciences 121(6): 1605–1616.
596   DOI 10.1002 /2016JG003393
597   Zi HB, Hu L, Wang CT, Wang GX, Wu PF, Lerdau M, Ade LJ .2018. Responses of soil bacterial
598   community and enzyme activity to experimental warming of an alpine meadow. European
599   Journal of Soil Science 69:429–438. DOI 10.1111/ ejss.12547
600   Zuo XA, Cheng H, Zhao SL, Yue P, Liu XP, Wang SK, Liu LX, Xu C, Luo WT, Knops JMH,
601   Medina-Roldán E. 2020. Observational and experimental evidence for the effect of altered
602   precipitation on desert and steppe communities. Global Ecology and Conservation 21:
603   e00864. DOI 10.1016/j.gecco.2019.e00864

Warming, P addition and their interaction effects on soil properties, nutrients contents and stoichiometries.

SMC, soil moisture content. SOC, soil organic carbon. TN, soil total nitrogen. TP, soil total phosphorus. DOC, dissolved organic carbon. AN, soil ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N). AP, soil available phosphorus. The results of two-way split-plotANOVAs for the treatments are shown in the table (* $P < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and "—" indicate no significance). Lowercase letters show significant differences among the treatments by Duncan's test at $p < 0.05$.

Treatment		pH	SWC	SOC	TN	TP	SOC:TN	SOC:TP	TN:TP	DOC	AN	AP	DOC:AN	DOC:AP	AN:AP
Control	P_0	8.55±0.11a	9.73±0.41b	6.98±1.39ab	0.67±0.12ab	0.34±0.02c	10.46±0.62a	20.27±3.45a	1.94±0.33a	20.79±1.50abc	6.75±0.52cd	3.58±0.39d	3.09±0.21a	5.83±0.26a	1.9±0.17a
	P_5	8.53±0.09ab	10.82±0.93a	6.42±0.79b	0.55±0.12b	0.4±0.04abc	11.92±0.98a	16.18±0.51bc	1.37±0.16bc	17.77±1.27c	6.30±0.56d	30.43±1.89b	2.83±0.26a	0.59±0.07c	0.21±0.02c
	P_{10}	8.48±0.11ab	11.63±1.20a	7.15±1.34ab	0.62±0.1ab	0.48±0.09a	11.47±0.58a	14.87±2.19c	1.29±0.13c	18.38±2.19c	6.73±0.34cd	41.60±2.80a	2.74±0.36a	0.44±0.07d	0.16±0.01c
Warming	P_0	8.39±0.05b	8.40±0.26c	7.05±0.99ab	0.66±0.13ab	0.37±0.08bc	10.77±0.68a	19.3±3.27ab	1.8±0.36ab	19.75±3.47bc	7.61±1.40bc	3.80±0.48d	2.62±0.34a	5.21±0.7a	1.99±0.15a
	P_5	8.44±0.05ab	9.43±0.46b	8.34±0.9a	0.76±0.13a	0.41±0.07abc	11.11±1.35a	20.56±2.33a	1.89±0.43a	23.33±2.64ab	11.26±0.78a	19.73±3.15c	2.07±0.11b	1.2±0.21b	0.58±0.07b
	P_{10}	8.53±0.11ab	9.59±0.22b	7.33±0.41ab	0.64±0.06ab	0.47±0.05ab	11.48±0.88a	15.69±2.06bc	1.37±0.17bc	23.92±2.66a	8.19±0.82b	46.90±6.78a	2.95±0.52a	0.52±0.12cd	0.18±0.03c
Warming		—	*	—	—	—	—	—	—	**	**	—	—	*	*
P addition		—	**	—	—	**	—	*	*	—	*	***	*	***	***
Warming × P		—	—	—	—	—	—	—	—	*	**	***	**	***	*

Figure 1

Air temperature at 15cm above the ground and soil temperature at 15cm below the ground in the warming plots (inside of the OTCs) and the control plots (outside of the OTCs).
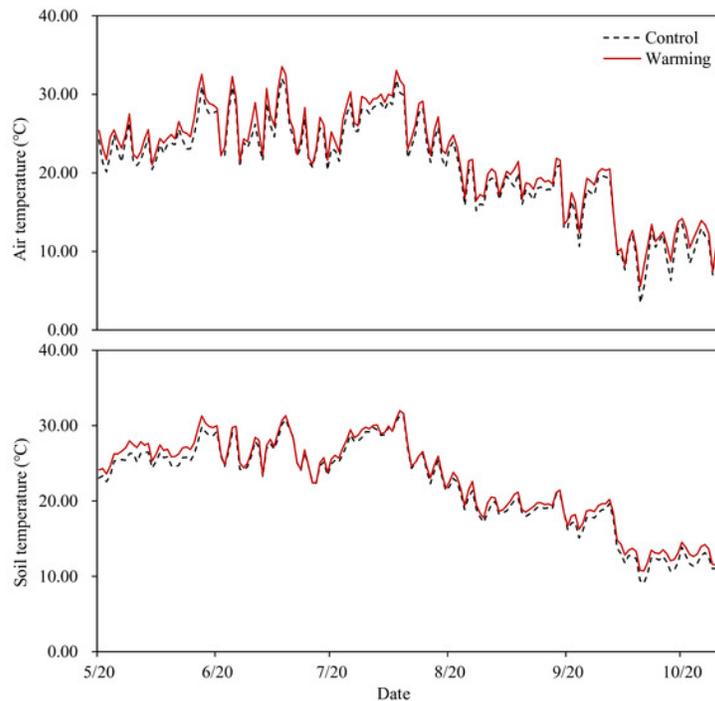
Figure 2

Warming, P addition and the interaction effects on soil microbial biomass and stoichiometries.

MBC, microbial biomass carbon. MBN, microbial biomass nitrogen. MBP, microbial biomass phosphorus. The results of two-way split-plot ANOVAs for the treatments are shown in the figures (* P < 0.05, ** p < 0.01, *** p < 0.001, and ns indicate no significance), lowercase letters above the multi-factor group histograms show significant differences among the treatments by Duncan's test at p < 0.05. Error bars show means ± SE (n=4).
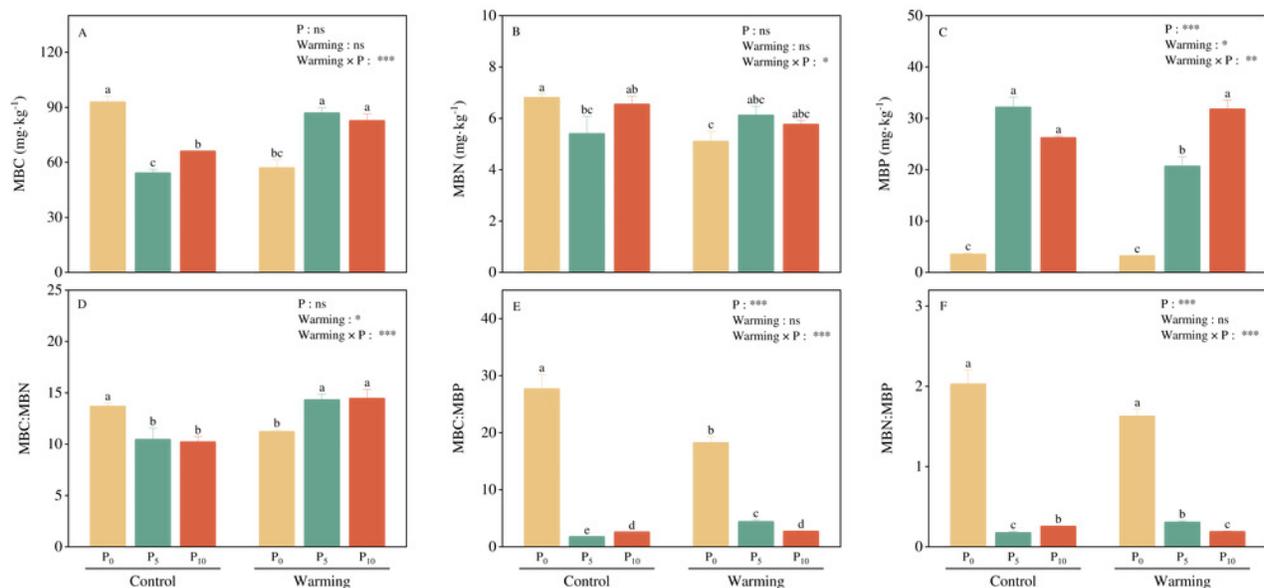
Figure 3

Warming, P addition and the interaction effects on soil extracellular enzyme activities and stoichiometries.

BG, β-1,4-glucosidase. LAP, leucine aminopeptidase. NAG, β-1,4-N-acetylglucosaminidase. ALP, alkaline phosphatase. The results of two-way split-plot ANOVAs for the treatments are shown in the figures (* $P < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and ns indicate no significance), lowercase letters above the multi-factor group histograms show significant differences among the treatments by Duncan's test at $p < 0.05$. Error bars show means ± SE (n=4).
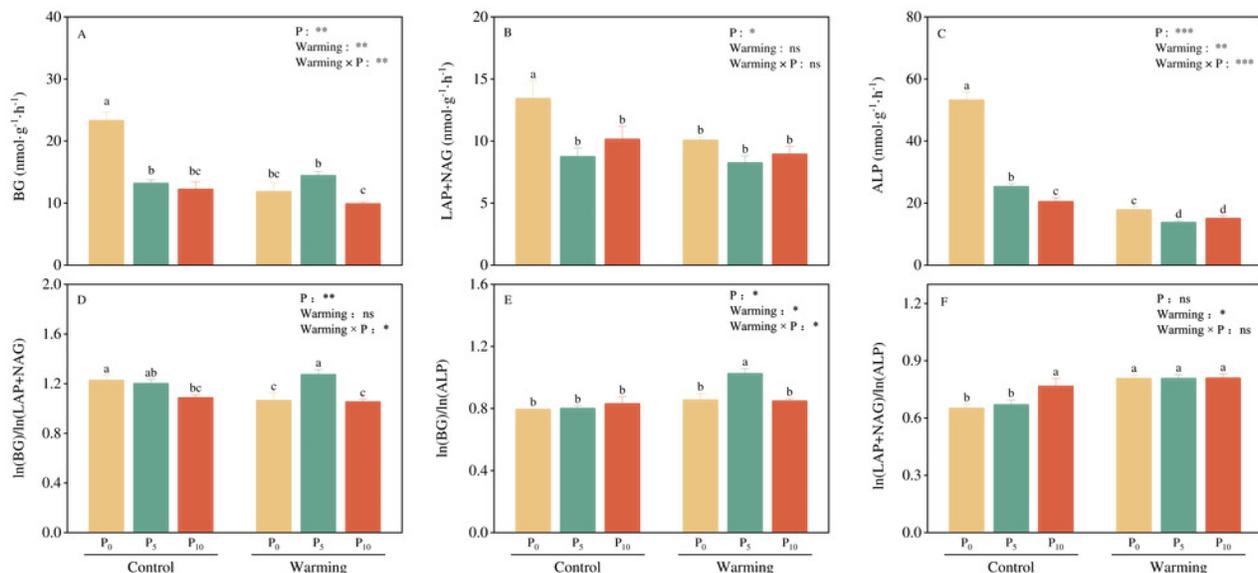
Figure 4

Regression analysis of the soil C, N, and P acquisition enzyme activities.

BG, β-1,4-glucosidase. LAP, leucine aminopeptidase. NAG, β-1,4-N-acetylglucosaminidase. ALP: alkaline phosphatase. All data were natural log(ln)-transformed.
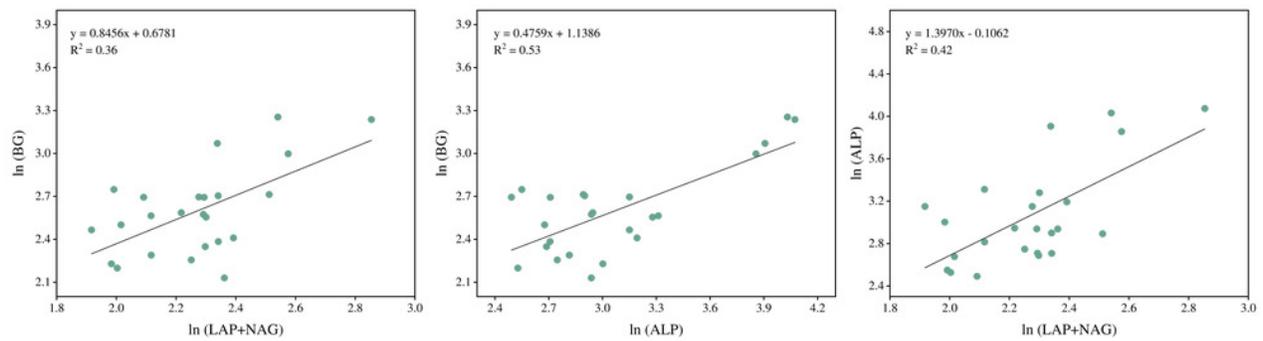
Figure 5

Scatter plots of the eco-enzyme stoichiometry of relative proportions of C/C+P to C/C+N(A). Regression analysis of vector length and vector angle(B). Warming, P addition and the interaction effects on vector length(C) and vector angle(D).

The results of two-way split-plot ANOVAs for the treatments are shown in the figures (* $P <$ 0.05, ** $p < 0.01$, *** $p < 0.001$, and ns indicate no significance), lowercase letters above the multi-factor group boxplots show significant differences among the treatments by Duncan's test at $p < 0.05$. Error bars show means ± SE (n=4).
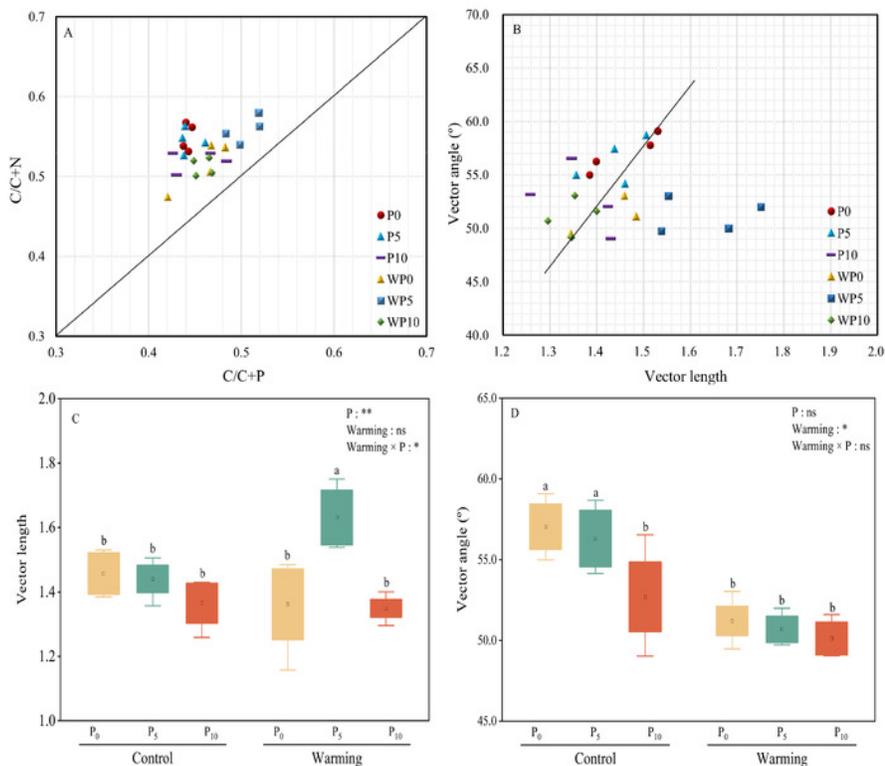
Figure 6

Correlation analysis on soil properties, soil extracellular enzyme activities and stoichiometries.

SOC, soil organic carbon. TN, total nitrogen. TP, total phosphorus. SMC, soil moisture content.

DOC, dissolved organic carbon. AN, soil ammonium nitrogen (NH_4^+-N) and nitrate nitrogen

(NO_3^--N). AP, soil available phosphorus. MBC, microbial biomass carbon. MBN, microbial

biomass nitrogen. MBP, microbial biomass phosphorus. C acquisition enzyme, β-1,4-

glucosidase. N acquisition enzyme, leucine aminopeptidase and β-1,4-N-

acetylglucosaminidase. P acquisition enzyme, alkaline phosphatase.
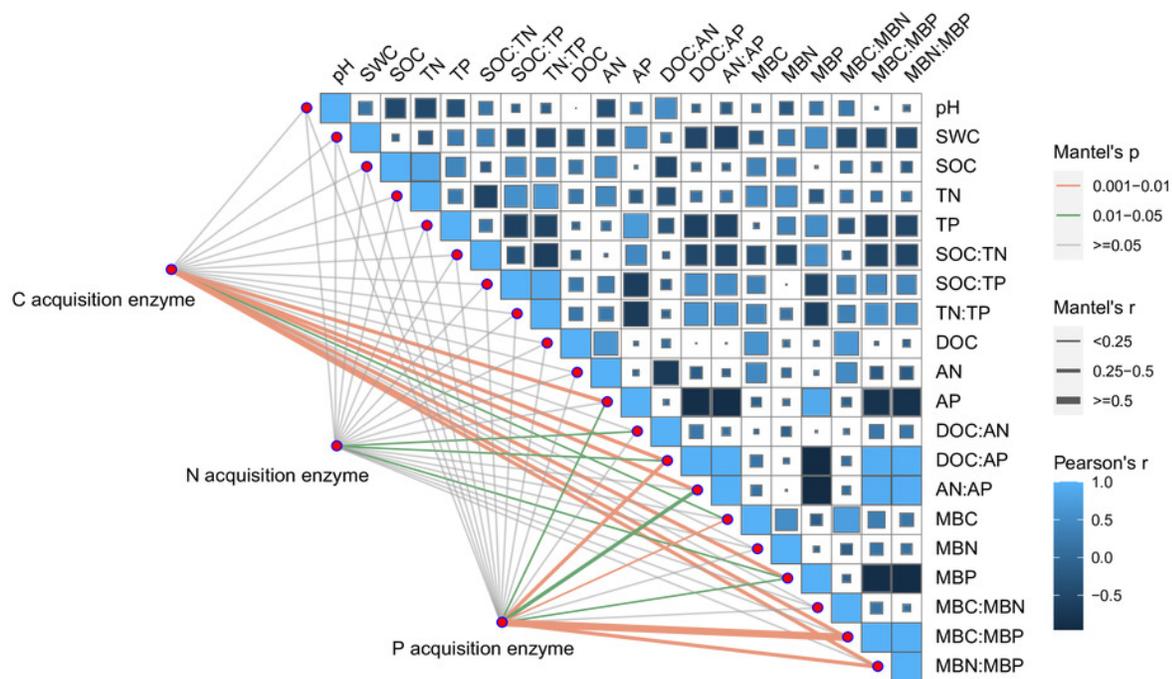
Figure 7

Redundancy analysis (RDA) on soil properties, soil extracellular enzyme activities and stoichiometries, vector length, and vector angle.

SMC, soil moisture content. DOC, dissolved organic carbon. AN, soil ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N). AP, soil available phosphorus. MBC, microbial biomass carbon. MBN, microbial biomass nitrogen. MBP, microbial biomass phosphorus. BG, β-1,4-glucosidase. LAP, leucine aminopeptidase. NAG, β-1,4-N-acetylglucosaminidase. ALP, alkaline phosphatase. e C: N, e C: P, and e N: P represent ln (BG): ln (LAP + NAG), ln (BG): ln (ALP) and ln (LAP +NAG): ln (ALP), respectively. VL, vector length. VA, vector angle.