

Differences of soil enzyme activities and its influencing factors under different flooding conditions in Ili Valley, Xinjiang

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Background: Wetland is a special ecosystem formed by the interaction of land and water. The moisture content variation will greatly affect the function and structure of wetland internal system.

Method: In this paper, three kinds of wetlands with different flooding levels (*Phragmites australis* wetland (long-term flooding), *Calamagrostis epigeios* wetland (seasonal flooding) and *Ditch millet* wetland (rarely flooded)) in Ili Valley of Xinjiang China was selected as research area. The changes of microbial biomass carbon, soil physical and chemical properties in wetlands were compared, and redundancy analysis was used to analyze the correlation between soil physical and chemical properties, microbial biomass carbon and enzyme activities (soil sucrase, catalase, amylase and urease). The differences of soil enzyme activities and its influencing factors under different flooding conditions in Ili Valley were studied and discussed.

Result: The results of this study were the following: (1) The activities of sucrase and amylase in rarely flooded wetlands and seasonally flooded wetlands were significantly higher than those in long-term flooded wetlands; the difference of catalase activity in seasonal flooded wetland was significant and the highest. (2) Redundancy analysis showed that soil organic carbon, dissolved organic carbon, total phosphorus and soil microbial biomass carbon had significant effects on soil enzyme activity ($p < 0.05$). (3) The correlation between soil organic carbon and the sucrase activity, total phosphorus and the catalase activity was the strongest; while soil organic carbon has a significant positive correlation with invertase, urease and amylase activity, with a slight influence on catalase activity. The results of this study showed that the content of organic carbon, total phosphorus and other soil fertility factors in the soil would be increased and the enzyme activity would be enhanced if the flooding degree was changed properly.

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16

17 Abstract

18 **Background:** Wetland is a special ecosystem formed by the interaction of land and water.
19 The moisture content variation will greatly affect the function and structure of wetland internal
20 system.21 **Method:** In this paper, three kinds of wetlands with different flooding levels (*Phragmites*
22 *australis* wetland (long-term flooding), *Calamagrostis epigeios* wetland (seasonal flooding) and
23 *Ditch millet* wetland (rarely flooded)) in Ili Valley of Xinjiang China was selected as research
24 area. The changes of microbial biomass carbon, soil physical and chemical properties in
25 wetlands were compared, and redundancy analysis was used to analyze the correlation between
26 soil physical and chemical properties, microbial biomass carbon and enzyme activities (soil
27 sucrase, catalase, amylase and urease). The differences of soil enzyme activities and its
28 influencing factors under different flooding conditions in Ili Valley were studied and discussed.29 **Result:** The results of this study were the following: (1) The activities of sucrase and
30 amylase in rarely flooded wetlands and seasonally flooded wetlands were significantly higher
31 than those in long-term flooded wetlands; the difference of catalase activity in seasonal flooded
32 wetland was significant and the highest. (2) Redundancy analysis showed that soil organic
33 carbon, dissolved organic carbon, total phosphorus and soil microbial biomass carbon had
34 significant effects on soil enzyme activity ($p < 0.05$). (3) The correlation between soil organic
35 carbon and the sucrase activity, total phosphorus and the catalase activity was the strongest;
36 while soil organic carbon has a significant positive correlation with invertase, urease and
37 amylase activity, with a slight influence on catalase activity. The results of this study showed
38 that the content of organic carbon, total phosphorus and other soil fertility factors in the soil

39 would be increased and the enzyme activity would be enhanced if the flooding degree was
40 changed properly.

41 **Keywords:** Wetland; flooding conditions; soil enzyme activity; soil physical and chemical
42 properties; microbial biomass carbon.

43

44 **Introduction**

45 Wetlands are ecological systems with unique biological characteristics, soil and hydrology
46 (Jenkinson DS., 1991). Its soil is immersed in water, and there are many different kinds of
47 animals, plants and microorganisms with wetland characteristics. So wetland is not only a natural
48 landscape with affluent ecological diversity in nature, but also an important environment for
49 human survival (Lu Xiaoyi, 2004; Garken Jumaken ette et al., 2018). According to research,
50 water is an important environmental factor, which plays an important role in maintaining the
51 stability of structure and function in wetland ecosystem and affecting the biogeochemical cycle
52 in wetland (Yu Xianmin, 1999). Therefore, in recent years, the effects of water on wetland soil
53 factors and plant growth have been studied widely. Studies have found that plants can adapt to
54 the stress of different flooding environments by changing their height, the stem diameter, and
55 population density (Tan Xuejie et al., 2006). Compared with natural exposed soil, the submerged
56 environment promoted the growth and activity of soil microorganisms, enhanced the activity of
57 the soil enzyme (liu Yajun et al., 2017). It can be seen that different water conditions have a
58 profound influence on the growth and reproduction of wetland plants.

59 The Ili Valley belongs to the arid inland river basin wetland, and its total wetland area is
60 about 2.4×10^5 km². There are many types and wide distribution of wetlands in the valley.
61 Because of its natural environmental conditions and special geographical location, Ili Valley has
62 created a wetland landscape with abundant water resources and rich species. At present, Ili
63 Valley is a key protected biodiversity area in China (Galkenjumaken Ett et al., 2014). In recent
64 years, owing to human over-reclamation and the influence of natural factors, the degradation of
65 most wetlands has become more and more serious, especially the fluctuation of water content.
66 However, the change of water conditions will have a great impact on the process of soil carbon
67 accumulation and decomposition (Wan Zhongmei, 2009). Besides, with the degradation of
68 wetlands, the content of soil organic carbon decreased significantly, and the activity of soil
69 enzymes also changed (Chen Yanxin, 2018). There are few reports on this aspect in the Ili
70 Valley. This study takes Ili valley wetland with different flooding levels as the research object. It
71 analyzes the change of soil enzyme activity of wetland under different flooding conditions, and it
72 discusses the relationship between water and soil factors affecting the enzyme activity, which
73 provided a theoretical basis for the study of the mechanism of soil water impact on soil and
74 wetland protection in Ili Valley.

75

76 **Materials & Methods**

77 **Site description**

78 Ili River Valley is located in the northwest direction of Tian Shan Mountains in Xinjiang
79 China, and surrounded by high mountains in the north, east and south, showing the natural
80 geographical features of "three mountains with two valleys". It enjoys the reputation of "Wet
81 Island in the western region" and "Jiang Nan beyond the Great Wall", and is the main
82 transportation route of the ancient Silk Road.

83 In addition, the Ili Valley is situated at 80°09'E-84°56'E in the east longitude and 42°14'N-
84 44°50'N in the North latitude, with an altitude of 530~1000 m and an area of 56,400 km². Due to
85 the excellent natural environment and unique geographical position of the Ili Valley, the water
86 resources and mineral resources are quite abundant, and there are various species in the valley.
87 The climate is warm and humid, belonging to the temperate continental climate, with a great
88 temperature difference between day and night. The annual average temperature is 10.4 °C, and
89 the annual average sunshine hours are 2700~3000 hours. The annual average precipitation is
90 approximately 417.6 mm, mainly concentrated in spring and summer, which is 60%~70% of the
91 annual precipitation. With the increase of altitude, the precipitation can be as high as 600 mm in
92 mountainous areas, and the annual average evaporation is about 1260~1900 mm, which is the
93 wettest climate area in Xinjiang China.

94 The Ili River Valley mainly distributes forests, grasslands and meadows. The grassland soil
95 types are mainly gray-calcium soils. The plant species are mostly perennial and cold-tolerant
96 grasses; the forest soil is mainly taupe forest soil, and the tree species are mostly Xinjiang
97 clouds. Cedar, snowy spruce, eucalyptus, etc (Yang Yuhai et al., 2010).

98

99 **Study site and sample collection**

100 The sampling sites were selected in Ili River floodplain wetland and Liberate Bridge
101 National Wetland Park in Zhaosu County, as shown in figure 1. In September 2017, three kinds
102 of wetland soils with different flooding degrees were collected in Wetland Park, i.e. *Ditch millet*
103 *wetland* (DMW), *Calamagrostis epigeios wetland* (CEW) and *Phragmites australis wetland*
104 (PAW). Among them, DMW belongs to the rarely flooded habitat; CEW belongs to the seasonal
105 flooded habitat, with a one-year flooding period of about 2~3 months; while PAW belongs to the
106 long-term flooded habitat, with a one-year flooding period of about 10 months.

107 Three plots (1 m×1 m) were randomly set up in the selected sampling area. Firstly, in each
108 wetland type, plant and its litter on the surface of the plot were removed with a shovel to obtain
109 the three random soil profiles. Afterwards, soil samples of 0~10 cm, 10~20 cm, 20~30 cm,
110 30~40 cm were collected from bottom to top, respectively. And the total of 36 soil samples were
111 collected from three wetland types, the collected samples were sealed in plastic bags and brought
112 back to the laboratory. The samples were divided into two parts, one of which was stored in a
113 sealed bag and stored in a refrigerator for the determination of soil microbial biomass carbon and
114 soil enzyme activity, and the other was placed in a bag and air-dried, ground, and passed through
115 a 0.15 mm sieve to determine the physical and chemical properties of the soil.

116

117

Figure.1

118

119 Analysis of soil properties**120 (1) Soil physical and chemical properties**

121 The content of soil organic carbon (SOC) was measured by a $K_2CrO_7-H_2SO_4$ oxidation
122 procedure (Lu Rukun, 1999). The soil samples were boiled with perchloric acid and sulfuric
123 acid. Afterwards, the total phosphorus (TP) content in soil was determined by colorimetry (Lu
124 Rukun, 1999). The content of easily oxidized organic carbon (EOC) in soil can be obtained by
125 putting potassium permanganate solution into soil sample and then colorimetric method (Blair et
126 al., 1995). The content of dissolved organic carbon (DOC) was determined by colorimetry (Zhan
127 Xinhua et al., 2002). It is known that NH_4^+ in soil leaching solution reacts with hypochlorite and
128 phenol in strong alkaline medium to form water-soluble dye indophenol blue. The content of
129 ammonium nitrogen (NH_4^+-N) can be determined by colorimetry (Zhao Jie et al., 2011).

130 (2) Microbial biomass carbon

131 The content of microbial biomass carbon (MBC) was determined by fumigation of the
132 sample with $CHCl_3$ and extraction with $0.5mol \cdot L^{-1} K_2SO_4$ (Vance, E.D et al., 1987).

133 (3) Enzyme activity

134 Catalase activity was determined by measuring the O_2 absorbed by $KMnO_4$ in the sample
135 added with H_2O_2 solution (Rodríguez-Kabana and Truelove, 1982). The sucrase activity was
136 determined by measuring glucose content after incubation for 24 h at 37 °C with sucrose as a
137 substrate (Guan Songyin, 1986). The amylase activity was measured by colorimetry (Zhou Likai
138 et al., 1980), and it was determined by measuring the amount of glucose produced during
139 hydrolysis. For the determination of urease activity (Guan Songyin, 1986), first one 5 g soil
140 sample was put into a 100 ml quantificational carafe, then add 5ml of 10% urease solution and
141 10ml of citrate buffer (pH 6.7), put the quantificational carafe into incubator for 24 h at 37 °C.
142 Finally, the released ammonium was determined colorimetrically at 578 nm using Indophenol
143 reagent.

144

145 Statistical analysis

146 The processing software (Excel 2010, SPSS 19.0 and CANOCO 4.5) were used to analyze
147 the integrated data. One-way ANOVA method was used to analyze the differences of soil
148 microbial biomass carbon, soil physical, chemical properties and soil enzyme activities in
149 different flooding degrees. The two-way ANOVA method was used to analyze the degree of
150 flooding and the depth of soil layer, and their interaction effects on soil microbial biomass
151 carbon, soil basic physical and chemical properties and soil enzyme activity were discussed. The
152 effects of soil physicochemical properties and microbial biomass carbon on soil enzyme activity
153 were analyzed by RDA sorting. It should be noted that the factors significantly related to soil
154 enzyme activities need to be selected by Monte Carlo analysis before the redundancy analysis.
155 The T-value double sequence diagram of CANOCO can also be used to analyze the single
156 environmental factor affecting soil enzyme activity.

157

158 Results

159 Soil physical and chemical properties

160 The same flooding conditions, the soil physical and chemical properties of soil layers with
161 different depths have certain differences (Table 1). Except for the PAW, the content of soil
162 organic carbon in the DMW and CEW was obviously different among the three soil layers. The
163 content of the SOC of the 0~10 cm soil layer of the CEW was significantly higher than that in
164 10~20 cm, 20~30 cm and 30~40 cm soil layers, while that in the 0~10 cm and the 10~20 cm soil
165 layer of the DMW was significantly higher than that in the 20~30 cm and 30~40 cm soil layers.

166 For total phosphorus (TP), there were no significant differences between the DMW and
167 PAW in the three soil layers, and the TP content of the CEW decreased gradually with the
168 increase of soil depth. In terms of easily oxidized organic carbon (EOC), there were no
169 significant differences between the CEW and PAW in the three soil layers. The EOC content in
170 0~10 cm soil layer was the highest, while that in 30~40 cm soil layer was the lowest in the
171 DMW. The content of DOC in wetlands under three flooding conditions showed a decreasing
172 trend with the increase of soil depth.

173 Two-way ANOVA shows that except $\text{NH}_4^+\text{-N}$, different flooding conditions and soil layers
174 have significant effects on the soil physical and chemical properties (Table 2). Among them, the
175 effect of flooding conditions on the physical and chemical properties of soil was greater than that
176 of soil depth. Only the F value of TP in different soil depths was higher than that of F value in
177 different flooding conditions, indicating that different soil conditions had a stronger effect on TP
178 content. At the same time, TP and SOC are also significantly affected by the interaction between
179 different flooding conditions and the soil depth, but the interaction has no significant effect on
180 EOC, DOC and $\text{NH}_4^+\text{-N}$. Different flooding conditions in the same soil layer have different
181 effects on the soil physical and chemical properties (Table 1). By comparing the average values
182 in (Table 1), showed that the contents of TP, DOC and $\text{NH}_4^+\text{-N}$ in wetlands with different
183 flooding conditions are $\text{PAW} > \text{CEW} > \text{DMW}$; the EOC content in wetlands with different
184 flooding conditions is $\text{DMW} > \text{CEW} > \text{PAW}$. And the SOC content in the CEW and DMW was
185 significantly higher than that in the PAW.

186

187

Table 1

188

189

Table 2

190

191

192 Soil microbial biomass carbon

193 There were differences in soil microbial biomass carbon at different soil depths under the
194 same flooding conditions (Figure 2). The MBC content in 0~10 cm and 10~20 cm soil layers of
195 the DMW and the CEW was significantly higher than that in 20~30 cm and 30~40 cm soil layers;
196 the MBC content in 0~10 cm soil layers of the PAW was significantly higher than that in 30~40

197 cm soil layers. The MBC in the wetland with three flooding conditions showed a trend of
198 decreasing with the increase of soil depth.

199 The results of two-way ANOVA showed that different flooding conditions and soil layers
200 had significant effects on soil microbial biomass carbon, and the interaction between them also
201 significantly affected soil microbial biomass carbon (Table 3). The content of MBC decreased
202 gradually with the increase of flooding degree. The F value of MBC in different soil layers is far
203 greater than that in different flooding conditions, which indicates that different soil depth has a
204 deeper impact on the MBC than the flooding condition. The soil depth is indeed one of the
205 important factors affecting the change of MBC content.

206

207

Figure.2

208

209

Table 3

210

Differences of soil enzyme activities

211 The activities of enzymes in different soil layers are different (Table 4). For the sucrose, the
212 sucrose activity of wetlands with three flooding degrees decreased significantly with the increase
213 of soil depth. Among them, the sucrose activity of 0-10 cm and 10~20 cm soil layers in the
214 DMW and CEW was significantly higher than that of 20~30 cm and 30~40 cm soil layers; the
215 sucrose activity of 0~10 cm soil layers in the PAW was significantly higher than that of 10~20
216 cm, 20~30 cm and 30~40 cm soil layers. There was no significant difference in the catalase
217 activity between the DMW and PAW, but the catalase activity decreased with the increase of soil
218 depth in the CEW. The amylase activity of the different soil layers was not significantly different
219 in the PAW; the amylase activity in the DMW decreased at first and then increased with the
220 increase of soil depth; the amylase activity in the CEW decreased with the increase of soil depth,
221 and the amylase activity in 0-10 cm soil layer was the strongest. There was no significant
222 difference in the urease activity among the three wetlands of flooding conditions.

223 According to Two-way ANOVA of flooding conditions and soil depth on soil enzyme
224 activity (Table 5). Except the sucrose, the soil depth had no significant effect on other soil
225 enzyme activities. Different flooding conditions have significant effects on the activities of the
226 sucrose and amylase. The F values of sucrose, amylase and urease activities in different flooding
227 conditions were higher than those in different soil depths, which indicated that the effects of
228 different flooding conditions on soil enzyme activities were greater than those at different soil
229 depths, and flooding conditions were one of the important factors affecting soil enzyme activities.
230 According to the average comparison in Table 4, the activities of sucrose and urease decreased
231 gradually with the increase of flooding degree. Among them, the activities of sucrose in the
232 CEW significantly decreased by 4.91% compared with the DMW, and the activities of sucrose in
233 the PAW significantly decreased by 46.04% compared with the CEW.

234 Although the flooding conditions had no significant effect on the catalase activity in 0~10
235 cm, 10~20 cm and 20~30 cm soil layers, the effects of different flooding conditions were
236

237 extremely significant on the catalase activity in 30~40 cm soil layers. As far as the catalase
238 activity in 30~40 cm soil layers was concerned, the DMW was significantly reduced by 18.45%
239 compared with the CEW, and the CEW was significantly reduced by 33.20% compared with the
240 PAW. The activities of sucrase and amylase in the DMW and CEW were significantly higher
241 than those in the PAW, while the catalase activity was the highest in the CEW.

242

243

Table 4

244

245

Table 5

246

247 **Correlation analysis between soil enzyme activity and soil physical-chemical factors,** 248 **microbial biomass carbon**

249 Redundancy analysis (RDA) was used to analyze the relationship between soil physical and
250 chemical factors, microbial biomass carbon and soil enzyme activities in wetlands under
251 different flooding conditions (Fig. 3). The results showed that the first two sorting axis together
252 explained 52.6% of the change of soil enzyme activity, of which the contribution rate of the first
253 sorting axis (RDA 1) was 47.6% and that of the second sorting axis (RDA 2) was 5%. This
254 indicated that most of the information between soil physical and chemical factors, microbial
255 biomass carbon and soil enzyme activities could be reflected by these two axes, and was mainly
256 determined by the first sorting axis. According to the redundancy analysis (Fig. 3), the arrow
257 lines of SOC, DOC and TP are the longest, which together with the importance sorting results of
258 Table 6 shows that SOC, DOC, TP and MBC can explain the changes of soil enzyme activities
259 very well. The angles are small and the directions are the same between SOC and the sucrose, TP
260 and catalase, which indicates that there are significant positive effects between SOC and the
261 sucrase activity, TP and the catalase activity. SOC may be the dominant factor affecting the
262 sucrase activity in Ili Valley, and TP is an important factor affecting the catalase activity.

263 A single environmental factor analysis was carried out for the environmental factors
264 affecting soil enzyme activity by using the T-value double-sequence diagram of CANOCO 4.5
265 (Fig. 4). As shown in (Fig. 4A), the arrows of sucrase, urease and amylase all fall on the solid
266 line circle of SOC, indicating that SOC has a significant positive correlation with the sucrase,
267 urease and amylase activities, that is to say, the activities of sucrase, urease and amylase increase
268 with the increase of SOC content. The arrow of catalase passes through the solid line circle of
269 SOC, which shows that there is a positive correlation between SOC and CAT. (Fig. 4B) shows
270 that four soil enzymes pass through the solid line circle of DOC, which indicates that there are a
271 positive correlation between DOC and the activities of the four soil enzymes. (Fig. 4C) shows
272 that most of the four soil enzymes fall outside the solid line circle and dotted line circle of NH_4^+ -
273 N, indicating that there was no significant relationship between NH_4^+ -N and the activities of the
274 four soil enzymes.

275 Different environmental factors have different effects on soil enzyme activity (Table 6). The
276 effects of different environmental factors on soil enzyme activities were $\text{SOC} > \text{DOC} > \text{TP} >$

277 MBC > NH⁴⁺-N > EOC. Among them, the effects of SOC, DOC, TP and MBC were significant
278 on soil enzyme activity, especially the effect of SOC on soil enzyme activity was extremely
279 significant. And SOC had the greatest effect on soil enzyme activity, accounting for 45% of the
280 total explanations ($F = 27.82, p < 0.01$). The effects of NH⁴⁺-N and EOC were not significant on
281 soil enzyme activity ($p > 0.05$).

282

283

Figure.3

284

285

Table 6

286

287

Figure.4

288

289 **Discussion**

290 **Effects of different flooding conditions on soil enzyme activities**

291 Soil enzyme is a kind of proteins with special catalytic ability, which mainly comes from
292 the decomposition of soil microorganisms, animal and plant secretions and residues (Guan
293 Songyin, 1986). Soil moisture has a significant correlation with soil microbial activity and type,
294 and different water conditions will directly affect the existence and activity of soil enzyme
295 activity (Wan Zhongmei et al., 2005). It indicates that the degree of flooding did significantly
296 affect the activity of soil enzymes. The results showed that the activities of sucrase and amylase
297 were closely related to the degree of flooding, and decreased gradually with the increase of
298 flooding degree (Wan Zhongmei et al., 2008; Zhou Xiaoming, 2018), which was consistent with
299 the effect of flooding degree on sucrase and amylase activities, and the activities of sucrase and
300 amylase in the rarely flooded and seasonal flooded wetlands were significantly higher than those
301 in the long-term flooded wetlands. The phenomenon may be due to the increase of soil moisture,
302 which leads to the decrease of soil permeability, restricts the growth of soil microorganisms,
303 greatly slows down the decomposition of soil humus, and thus reduces the activity of soil
304 enzymes. Studies have shown that soil moisture can affect soil microbial biomass by changing
305 soil oxygen content (Rousk J et al., 2009). Therefore, it is also possible that in an environment
306 with sufficient substrate and moist soil, increasing soil water will affect the availability of
307 oxygen, thus affecting the growth of soil microorganisms and plant roots, resulting in the
308 decrease of enzyme activity (Guenet et al., 2012).

309 In this study, although there is no significant correlation between flooding conditions and
310 catalase, urease activity, as a whole, urease activity in very few flooded wetlands is much higher
311 than that in perennial flooded wetlands, which may be because the deeper the soil layer in the
312 wetland, the less the water content, the more conducive to the accumulation of soil organic
313 matter and the improvement of enzyme activity. This is similar to the results of other researchers
314 (Xu Jingjing et al., 2017). In this study, the catalase activity did not change significantly with the
315 increase of the water content, which was consistent with the result that the catalase activity did

316 not change significantly with the water gradient in non-rhizosphere soil (Tian Youhua et al.,
317 2012).

318

319 **Effects of microbial biomass carbon and soil physical-chemical properties on soil enzyme** 320 **activities**

321 Soil microbial biomass is an essential indicator for soil quality. On the one hand, it is highly
322 sensitive and reflects small changes in soil before total carbon changes (Powlson D S, Brookes P
323 C, Christensen B T., 1987). On the other hand, it also reflects the process of nutrient transfer and
324 the energy cycle (Doran JW., 1994). Previous studies have shown that the carbon contents of soil
325 microbial biomass and soil enzyme activities in the treatment of underwater are higher than those
326 in the treatment of natural bareness (Liu Yajun et al., 2017). (Table 6) shows that soil microbial
327 biomass carbon is significantly correlated with soil enzyme activity, which is closely related to
328 soil microbial biomass carbon and soil enzyme activity obtained by the predecessors. With the
329 change of water gradient, soil enzyme activity is positively correlated with microbial biomass
330 carbon (Wan Zhongmei et al., 2008). This is significantly related to the microbial biomass
331 carbon and soil enzyme activity in this study, and the results are consistent with the law of
332 decrease with the increase of water content (Table 6). This phenomenon is due to the fact that the
333 soil aeration of rarely flooded wetlands is good, the vegetation grows luxuriantly, accelerates the
334 decomposition of soil humus, provides a large number of carbon sources for the metabolism
335 process of soil microorganisms, and leads to the increase of microbial biomass carbon (Jia G et
336 al., 2005).

337 There was a certain relationship between soil organic carbon and soil enzyme activity (Wan
338 Zhongmei et al., 2009). Soil organic matter is the main source of enzyme substrate, and the
339 content of organic matter greatly affects the activity of soil enzyme. It can be seen from table 4
340 and showed that the activities of sucrase and amylase basically decrease with the increase of soil
341 depth. This may be because plant roots are mostly concentrated on the surface of the soil.
342 Vigorous root activity promotes the turnover of litter on the ground. The high content of organic
343 matter in the soil leads to the increase of the respiration intensity of microorganisms, which is
344 convenient for the reproduction of microorganisms, so that the higher soil enzyme activity is
345 accumulated on the surface of the soil (Wan Zhongmei et al., 2008). It can be seen from Figure 3
346 that there is a significant positive correlation between soil organic carbon and sucrase, urease and
347 amylase activities; there is a positive correlation between soil oxidizable organic carbon and
348 sucrase, urease, catalase and amylase activities. This shows that most of the soil enzymes are
349 sensitive to the change of soil active organic carbon composition, which is consistent with the
350 previous research results (Xiao Ye et al., 2015). The soil organic matter in the degraded wetland
351 of Napa sea in Northwest China changed from original swamp to swamp grassland, the
352 cultivated land decreased gradually, and the activity of soil enzyme showed the same trend (Lu
353 Mei et al., 2004). These results indicate that the soil enzyme activity is closely related to soil
354 nutrients such as soil organic matter, and the change of soil enzyme activity can better reflect the
355 degree of soil degradation.

356 In this study, total phosphorus has a significant positive effect on the activities of four soil
357 enzymes, and has the strongest correlation with the activities of catalase, which can better
358 explain the changes of catalase activity, indicating that total phosphorus is the main factor
359 affecting the activities of urease, catalase and sucrase through direct or indirect effects (Liu
360 Guang shen et al., 2003). Previous studies have shown that the relationship between ammonium
361 nitrogen and soil enzyme activity is not significant, and with the increase of soil depth, it first
362 decreases and then increases (Xu Jing et al., 2017). The content of oxidized organic carbon in
363 different wetland types decreased with the increase of soil depth (Xiao Ye et al., 2015). This is
364 similar to the conclusion that the relationship between ammonium nitrogen, easily oxidized
365 organic carbon and soil enzyme activity is not significant, and decreases with the increase of soil
366 depth. This may be because the increase of water content can adjust the physical structure of soil,
367 improve the effectiveness of soil nutrients, and facilitate the transfer of easily oxidized organic
368 carbon and ammonium nitrogen in the soil (Wang Jie et al., 2014). It can be seen that the
369 decrease of wetland water will lead to the loss of soil nutrients, which will cause a large area of
370 wetland degradation.

371

372

373 **Conclusions**

374 The results show that soil organic carbon (SOC), dissolved organic carbon (DOC) and total
375 phosphorus (TP) had significant effects on soil enzyme activity in wetland, while ammonium
376 nitrogen (NH_4^{+-}N), easily oxidized organic carbon (EOC) and microbial biomass carbon (MBC)
377 had no significant effects on soil enzyme activity. Among them, the correlation between soil
378 organic carbon and the sucrase activity, total phosphorus and the catalase activity were the
379 strongest, indicating that soil organic carbon is the main factor affecting sucrase activity, and
380 total phosphorus is an important factor affecting catalase activity. Soil organic carbon had a
381 significant positive correlation with sucrase, urease and amylase activity, but had a slight
382 influence on catalase activity. Dissolved organic carbon had a positive correlation with four soil
383 enzyme activities. It can be seen that the activity of soil enzyme in wetland is related closely to
384 soil organic carbon and dissolved organic carbon.

385 Compared with the soil enzyme activities, it was found that the activities of sucrase and
386 urease in wetland were in the order of rarely flooded wetlands > seasonal flooded wetlands >
387 long-term flooded wetlands with the increase of flooding degree. The activity of amylase in
388 rarely flooded wetland and seasonal flooded wetlands was significantly higher than that in long-
389 term flooded wetlands. The activity of catalase in seasonal flooded wetlands was the highest. All
390 of these indicate that the humid environment will inhibit the survival of plants and
391 microorganisms in the soil, hinder the decomposition of organic matter, and lead to the decrease
392 of enzyme activity. In conclusion, the soil enzyme activity is closely related to soil nutrients such
393 as soil organic matter and water, and the change of soil enzyme activity can better reflect the
394 degree of soil degradation.

395

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534 **Figure Interpretation**

535 **Figure.1**

536 **Diagram of wetland sampling point in Ili Valley.**

537 *DMW: Ditch millet wetland; CEW: Calamagrostis epigeios wetland; PAW: Phragmites australis wetland*

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539 **Figure.2**

540 **Soil microbial biomass carbon content in wetland soil of different layers under different flooding conditions.**

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542

543 **Figure.3**

544 **Redundancy analysis of the effect of soil physical and chemical properties on soil enzyme activity**

545 The quadrant of arrow in the figure represents the positive and negative correlation between different factors
546 and the sorting axis, the hollow arrow represents several soil enzymes, the solid arrow represents
547 environmental variables, and the cosine value of corresponding angle represents the correlation between
548 environmental variables and soil enzymes. With the smaller the cosine value, the greater the correlation. Solid
549 line represents the factors significantly related to the soil enzyme activity ($p < 0.05$).

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551 **Figure.4**

552 **The T-value for a single factor influencing varied of soil enzyme activities in wetland.**

553 The quadrant of the arrow in the figure represents the positive and negative correlation between different
554 factors and the sorting axis. The arrow represents several soil enzymes, and the solid triangle represents
555 environmental variables.

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Table 1 (on next page)

Soil basic physical and chemical properties of different soil thickness under different flooding conditions.

The values are average (standard error). The different letters of the same column data represent significant differences among different soil layers of the same wetland ($p < 0.05$). Table 4 is the same.

1 **Table 1**2 **Soil basic physical and chemical properties of different soil thickness under different flooding conditions.**

Wetland Type	Soil Layer (cm)	SOC ($g \cdot kg^{-1}$)	TP ($g \cdot kg^{-1}$)	EOC ($mg \cdot kg^{-1}$)	DOC ($mg \cdot kg^{-1}$)	NH ₄ ⁺ -N ($mg \cdot kg^{-1}$)
DMW	0-10	27.17 a (7.02)	8.15 a (0.57)	7.39 a (0.95)	219.30 a (3.53)	6.99 a (0.25)
	10-20	16.43 ab (6.42)	7.35 a (0.50)	4.11 b (0.85)	199.72 ab (5.87)	6.68 a (0.34)
	20-30	6.57 b (2.29)	6.61 a (0.67)	4.63 b (0.38)	173.28 b (6.42)	6.59 a (0.31)
	30-40	5.79 b (1.98)	6.29 a (0.50)	3.48 b (0.04)	145.87 c (11.29)	6.48 a (0.29)
CEW	0-10	31.37 a (11.01)	8.30 a (0.06)	6.15 a (1.68)	241.81 a (4.27)	7.20 a (0.20)
	10-20	15.06 ab (6.89)	7.85 ab (0.36)	4.09 a (0.20)	214.40 ab (2.94)	6.95 a (0.10)
	20-30	11.18 ab (7.50)	7.23 b (0.42)	4.52 a (0.56)	181.11 b (6.42)	6.78 a (0.08)
	30-40	3.32 b (0.29)	7.20 b (0.23)	3.25 a (1.33)	140.00 c (20.51)	6.76 a (0.08)
PAW	0-10	11.66 a (5.21)	8.72 a (0.64)	5.07 a (3.00)	256.50 a (36.38)	7.22 a (0.25)
	10-20	4.49 a (2.26)	8.39 a (0.56)	3.68 a (0.55)	237.90 a (38.33)	7.02 a (0.15)
	20-30	1.66 a (0.49)	7.88 a (0.88)	3.15 a (0.79)	206.57 a (33.46)	6.97 a (0.24)
	30-40	1.67 a (0.88)	7.82 a (0.47)	3.02 a (2.07)	158.60 a (22.17)	6.84 a (0.32)

3 The values are average (standard error). The different letters of the same column data represent significant
 4 differences among different soil layers of the same wetland ($p < 0.05$). Table 4 is the same.

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Table 2 (on next page)

A two-way ANOVA for the effects of different flooding conditions and soil layers on soil basic physicochemical properties .

1 **Table 2**2 **A two-way ANOVA for the effects of different flooding conditions and soil layers on soil basic**3 **physicochemical properties.**

Influence		SOC	TP	EOC	DOC	NH ₄ ⁺ -N
Factor		(g•kg ⁻¹)	(g•kg ⁻¹)	(mg • kg ⁻¹)	(mg • kg ⁻¹)	(mg • kg ⁻¹)
Soil Layer	<i>F</i>	4.29	4.40	0.50	2.02	1.30
	<i>P</i>	0.03	0.00	0.00	0.00	0.29
Flooding Conditions	<i>F</i>	7.70	3.38	1.72	10.87	3.06
	<i>P</i>	0.00	0.02	0.01	0.00	0.05
Interaction	<i>F</i>	0.55	0.34	0.45	0.13	0.28
	<i>P</i>	0.03	0.02	0.84	0.99	0.94

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Table 3 (on next page)

A two-way ANOVA for the effects of different flooding conditions and different soil layers on soil microbial biomass carbon .

1 **Table 3**

2 **A two-way ANOVA for the effects of different flooding conditions and different soil layers on soil**
3 **microbial biomass carbon.**

Influence Factor		MBC
Soil Layer	<i>F</i>	2192.83
	<i>P</i>	0.00
Flooding Conditions	<i>F</i>	465.36
	<i>P</i>	0.00
Interaction	<i>F</i>	75.75
	<i>P</i>	0.00

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Table 4 (on next page)

Soil enzyme activities of different soil thickness under different flooding conditions .

1 **Table 4**2 **Soil enzyme activities of different soil thickness under different flooding conditions.**

Wetland Type	Soil Layer (cm)	Sucrase (mg·(g·24h) ⁻¹)	Catalase (mg/g)	Amylase (mgC ₆ H ₁₂ O ₆ /(g·h))	Urease (mg/g)
DMW	0-10	1.57 a (0.27)	1.72 a (0.51)	14.52 a (0.22)	23.91 a (10.63)
	10-20	1.43 a (0.31)	1.90 a (0.30)	6.60 b (0.23)	13.74 a (7.38)
	20-30	0.93 ab (0.16)	1.91 a (0.26)	5.75 b (0.51)	12.01 a (7.01)
	30-40	0.47 b (0.08)	2.16 a (0.05)	6.47 b (0.53)	5.86 a (2.62)
CEW	0-10	2.19 a (0.24)	2.10 a (0.02)	13.29 a (3.41)	20.04 a (12.13)
	10-20	1.16 b (0.36)	2.03 a (0.06)	8.76 ab (2.53)	12.98 a (7.36)
	20-30	0.44 c (0.03)	1.96 a (0.05)	5.84 b (0.24)	7.96 a (2.61)
	30-40	0.40 c (0.03)	1.76 b (0.04)	5.83 b (0.59)	7.23 a (2.43)
PAW	0-10	1.21 a (0.38)	1.97 a (0.24)	10.45 a (3.27)	7.81 a (2.62)
	10-20	0.39 b (0.03)	1.92 a (0.25)	6.17 a (0.81)	6.10 a (2.28)
	20-30	0.34 b (0.02)	1.72 a (0.30)	6.21 a (0.26)	4.74 a (2.41)
	30-40	0.32 b (0.02)	1.17 a (0.40)	5.92 a (0.68)	6.86 a (3.10)

3

Table 5 (on next page)

A two-way ANOVA for the effects of flooding conditions and soil layers on soil enzyme activities.

1 **Table 5**2 **A two-way ANOVA for the effects of flooding conditions and soil layers on soil enzyme activities.**

Influence		Sucrase	Catalase	Amylase	Urease
Factor					
Soil Layer	<i>F</i>	7.78	1.25	0.74	1.58
	<i>P</i>	0.03	0.30	0.49	0.23
Flooding	<i>F</i>	21.08	0.60	12.13	1.69
	<i>P</i>	0.00	0.62	0.00	0.20
Interaction	<i>F</i>	2.18	1.11	0.59	0.35
	<i>P</i>	0.04	0.38	0.74	0.90

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Table 6 (on next page)

Significance rank and significance test of soil physicochemical factors and microbial biomass carbon in explanation .

1 **Table 6**2 **Significance rank and significance test of soil physicochemical factors and microbial biomass carbon in**
3 **explanation.**

environmental Factor	Sorting of Importance	Degree of Interpretation (%)	Importance (<i>F</i> value)	Significance (<i>P</i> value)
SOC	1	45	27.824	0.002
DOC	2	13.3	5.209	0.012
TP	3	8.9	3.337	0.032
MBC	4	5.1	2.728	0.050
NH ₄ ⁺ -N	5	8.5	3.139	0.114
EOC	6	4.7	1.284	0.296

4

Figure 1

Diagram of wetland sampling point in Ili Valley

DMW: Ditch millet wetland; CEW: *Calamagrostis epigeios* wetland; PAW: *Phragmites australis* wetland

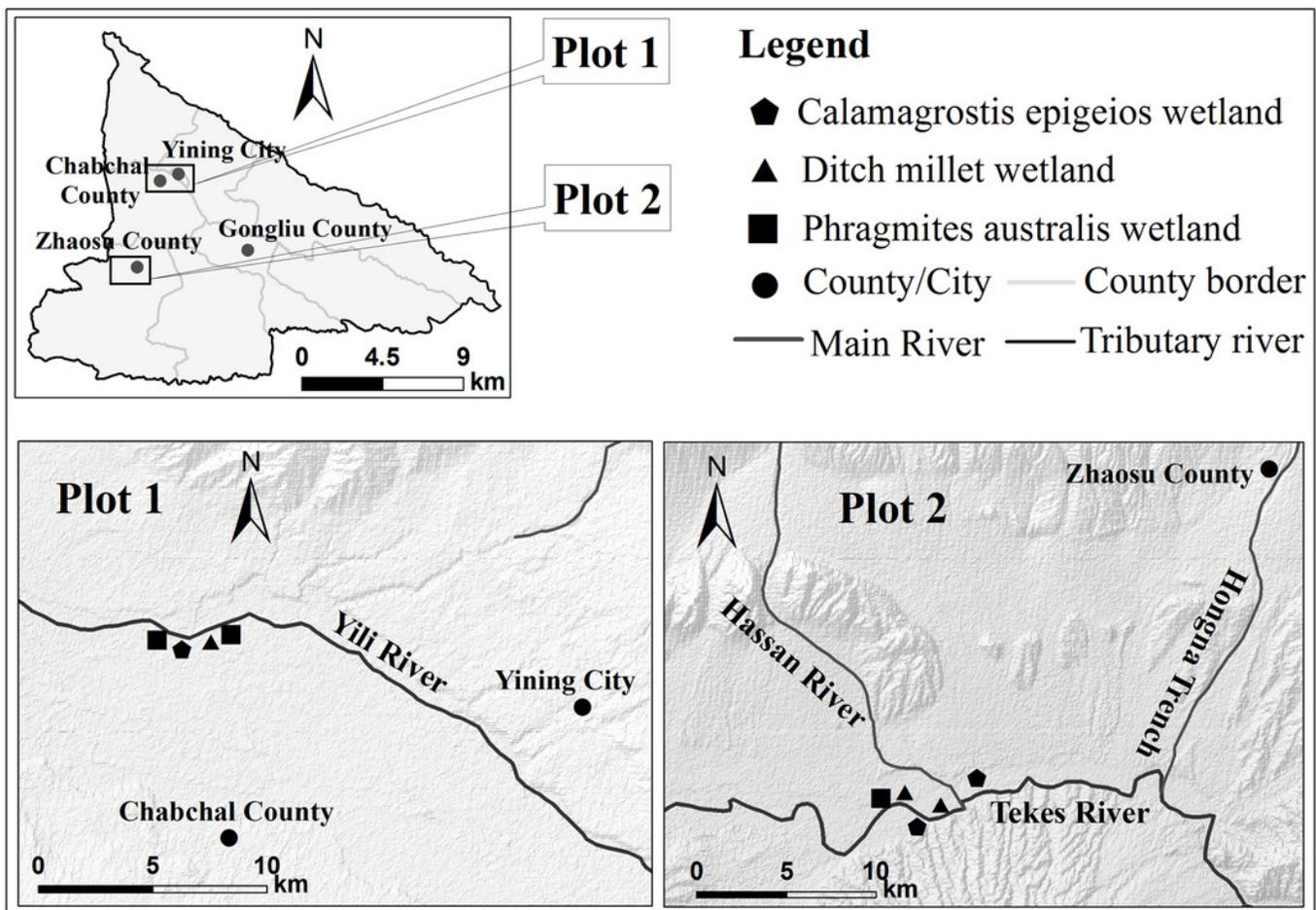


Figure 2

Soil microbial biomass carbon content in wetland soil of different layers under different flooding conditions.

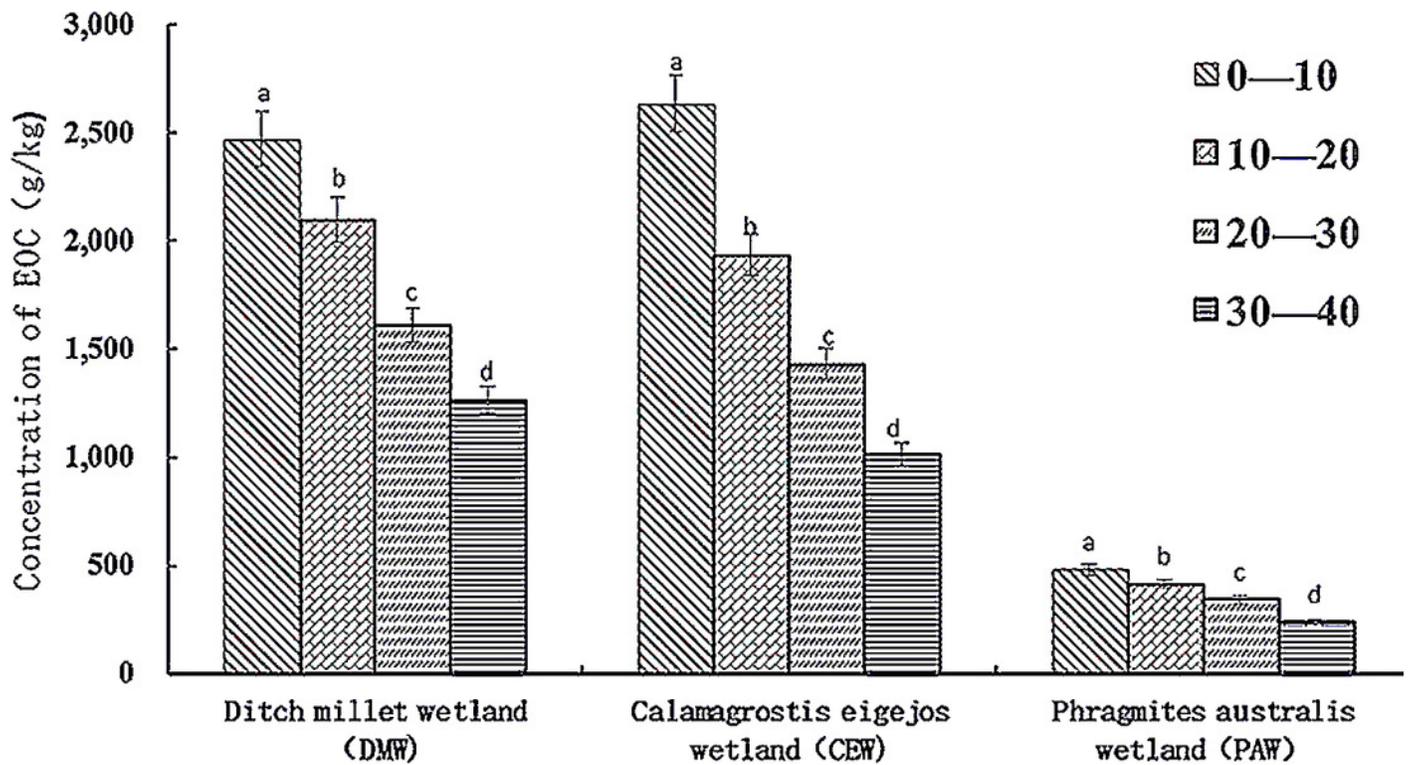


Figure 3

Redundancy analysis of the effect of soil physical and chemical properties on soil enzyme activity

The quadrant of arrow in the figure represents the positive and negative correlation between different factors and the sorting axis, the hollow arrow represents several soil enzymes, the solid arrow represents environmental variables, and the cosine value of corresponding angle represents the correlation between environmental variables and soil enzymes. With the smaller the cosine value, the greater the correlation. Solid line represents the factors significantly related to the soil enzyme activity ($p < 0.05$).

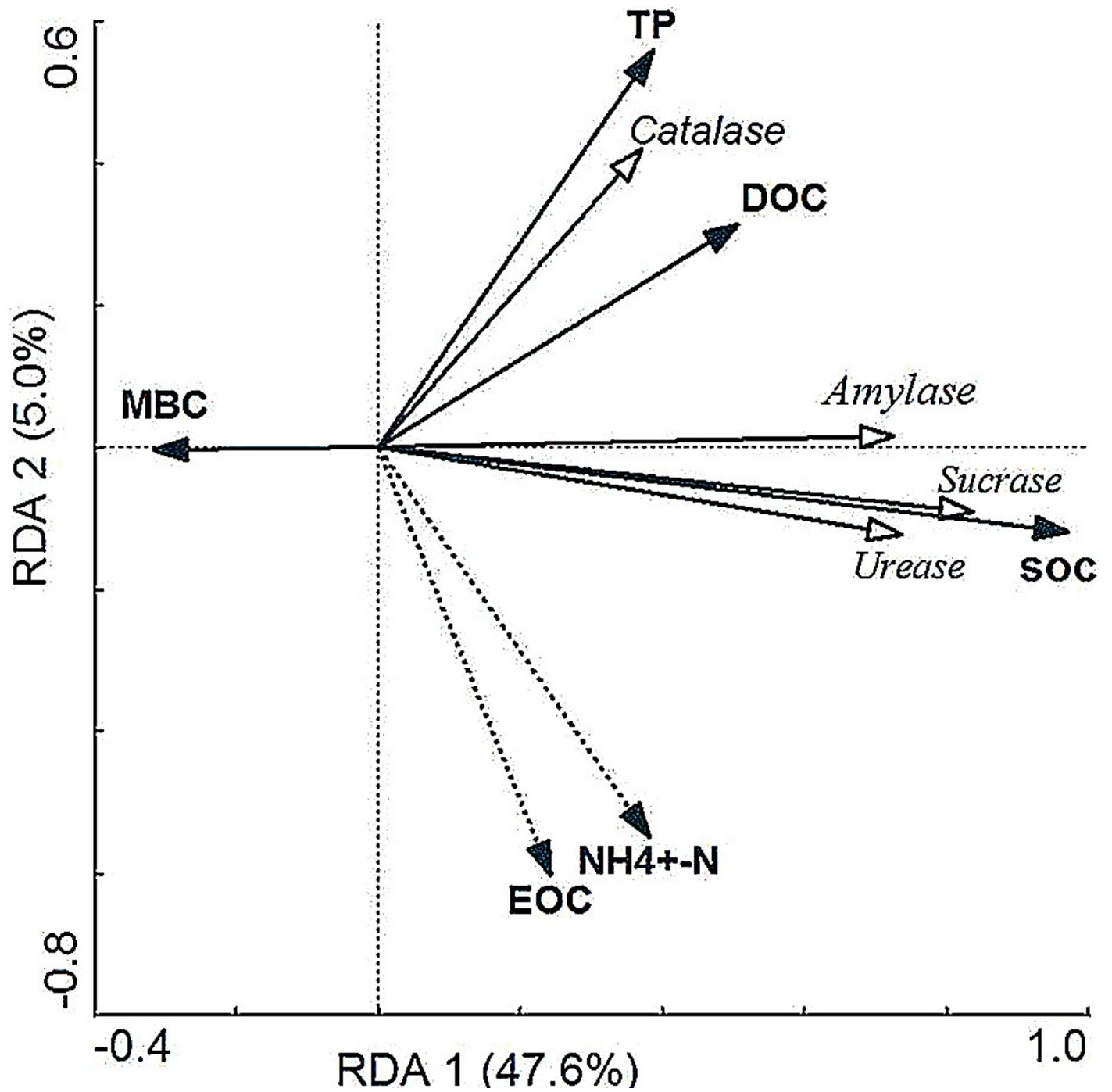


Figure 4

The T-value for a single factor influencing varied of soil enzyme activities in wetland.

The quadrant of the arrow in the figure represents the positive and negative correlation between different factors and the sorting axis. The arrow represents several soil enzymes, and the solid triangle represents environmental variables.

