

# Differences of soil enzyme activities and their influencing factors under different flooding conditions in Yili Valley

Zhang Yulu<sup>Corresp., 1, 2</sup>, Cui Dong<sup>Corresp., 1, 2</sup>, Yang Haijun<sup>3</sup>

<sup>1</sup> College of Biology and Geography, Yili Normal University, Yining, Ili Kazakh Autonomous Prefecture, China

<sup>2</sup> Institute of Resources and Ecology, Yili Normal University, Yining, Ili Kazakh Autonomous Prefecture, China

<sup>3</sup> Ministry of Education Key Laboratory of Vegetation Ecology, Institute of Grassland Science, Northeast Normal University, Changchun, China

Corresponding Authors: Zhang Yulu, Cui Dong

Email address: zyq\_2918855109@126.com, cuidongw@126.com

**Background.** Wetland is a special ecosystem formed by the interaction between land and water. Water is an important component of wetland. The change of moisture content will greatly affect the function and structure of wetland internal system.

**Method.** This study takes *Phragmites australis* wetland (long-term flooding), *Calamagrostis epigeios* wetland (seasonal flooding) and Ditch millet wetland (rarely flooded) in Yili Valley of Xinjiang as research objects. The changes of microbial biomass carbon and soil physical and chemical properties in wetlands were compared, and the correlation between soil basic physical and chemical properties, microbial biomass carbon and the enzyme activity (soil sucrase, catalase, amylase and urease) were analyzed by the redundancy analysis. The differences of soil enzyme activities and its influencing factors under different flooding conditions in Yili Valley were studied.

**Result.** The results were as follows:(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; there were no significant difference in catalase activities between rarely flooded wetlands and long-term flooded wetlands; and the catalase activities were highest in seasonal flooded wetlands. (2)The activities of sucrase in seasonally flooded wetlands were 4.91% lower than those in rarely flooded wetlands, and the activities of sucrase in long-term flooded wetlands were 46.04% lower than those in seasonally flooded wetlands; as far as catalase activities in 30-40 cm soil layer is concerned, the rarely flooded wetland was significantly reduced by 18.45% compared with the seasonal flooded wetland, and the seasonal flooding was significantly reduced by 33.20% compared with long-term flooding wetland.(3) Redundancy analysis showed that soil organic carbon, dissolved organic carbon, total phosphorus and soil microbial biomass carbon had significant effects on soil enzyme activity ( $p < 0.05$ ), while ammonium nitrogen and easily oxidized organic carbon had no significant effects on soil enzyme activity; the correlation between soil organic carbon and the sucrase activity, total phosphorus and the catalase activity was the strongest; soil organic carbon was positively correlated with sucrase, urease and amylase activity, but had a slight influence on catalase activity; dissolved organic carbon had a positive correlation with four soil enzyme activities. It has been showed that different flooding conditions can affect the activity of soil enzymes in wetlands. Properly changing the degree of flooding will increase the content of soil fertility factors such as organic carbon and total phosphorus, and enhance the activity of enzymes.

# 1 Differences of soil enzyme activities and their influencing 2 factors under different flooding conditions in Yili Valley

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4 ZHANG Yu-lu<sup>1,2</sup>, CUI Dong<sup>1,2</sup>, Yang Haijun<sup>1,2,3</sup>

5

6 <sup>1</sup> College of Biology and Geography, Yili Normal University, Yining 835000, China7 <sup>2</sup> Institute of Resources and Ecology, Yili Normal University, Yining 835000, China8 <sup>3</sup> Ministry of Education Key Laboratory of Vegetation Ecology, Institute of Grassland Science,  
9 Northeast Normal University, Changchun 130024, China

10

11 Corresponding Author: CUI Dong

12 448 Liberate West Road, Yining City, Xinjiang, Yili Kazakh Autonomous Prefecture, 835000,  
13 China

14 Email address: cuidongw@126.com

15

## 16 Abstract

17 **Background.** Wetland is a special ecosystem formed by the interaction between land and  
18 water. Water is an important component of wetland. The change of moisture content will greatly  
19 affect the function and structure of wetland internal system.

20 **Method.** This study takes *Phragmites australis* wetland (long-term flooding), *Calamagrostis*  
21 *epigeios* wetland (seasonal flooding) and Ditch millet wetland (rarely flooded) in Yili Valley of  
22 Xinjiang as research objects. The changes of microbial biomass carbon and soil physical and  
23 chemical properties in wetlands were compared, and the correlation between soil basic physical  
24 and chemical properties, microbial biomass carbon and the enzyme activity (soil sucrose,  
25 catalase, amylase and urease) were analyzed by the redundancy analysis. The differences of soil  
26 enzyme activities and its influencing factors under different flooding conditions in Yili Valley  
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28 **Result.** The results were as follows:(1) the activities of sucrose and amylase in rarely  
29 flooded wetlands and seasonally flooded wetlands were significantly higher than those in long-  
30 term flooded wetlands; there were no significant difference in catalase activities between rarely  
31 flooded wetlands and long-term flooded wetlands; and the catalase activities were highest in  
32 seasonal flooded wetlands. (2)The activities of sucrose in seasonally flooded wetlands were 4.91%  
33 lower than those in rarely flooded wetlands, and the activities of sucrose in long-term flooded  
34 wetlands were 46.04% lower than those in seasonally flooded wetlands; as far as catalase  
35 activities in 30-40 cm soil layer is concerned, the rarely flooded wetland was significantly  
36 reduced by 18.45% compared with the seasonal flooded wetland, and the seasonal flooding was  
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38 analysis showed that soil organic carbon, dissolved organic carbon, total phosphorus and soil  
39 microbial biomass carbon had significant effects on soil enzyme activity ( $p < 0.05$ ), while  
40 ammonium nitrogen and easily oxidized organic carbon had no significant effects on soil enzyme

41 activity; the correlation between soil organic carbon and the sucrase activity, total phosphorus  
42 and the catalase activity was the strongest; soil organic carbon was positively correlated with  
43 sucrase, urease and amylase activity, but had a slight influence on catalase activity; dissolved  
44 organic carbon had a positive correlation with four soil enzyme activities. It has been showed  
45 that different flooding conditions can affect the activity of soil enzymes in wetlands. Properly  
46 changing the degree of flooding will increase the content of soil fertility factors such as organic  
47 carbon and total phosphorus, and enhance the activity of enzymes.

48 **Keywords:** Yili valley; wetland; flooding conditions; differences in enzyme activity;  
49 microbial biomass carbon.

50

## 51 Introduction

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

67 The Yili Valley belongs to the arid inland river basin wetland, and its total wetland area is  
68 about  $2.4 \times 10^5$  km<sup>2</sup>. There are many types and wide distribution of wetlands in the valley. Due to  
69 the natural environmental conditions and special geographical location, the Yili Valley has  
70 created a wetland landscape with abundant water resources and rich species, which is a key  
71 biodiversity area in China (Galkenjumaken Ett et al., 2014). In recent years, owing to human  
72 over-reclamation and the influence of natural factors, the degradation of most wetlands has  
73 become more and more serious, especially the fluctuation of water content. However, the change  
74 of water conditions will have a great impact on the process of soil carbon accumulation and  
75 decomposition (Wan Zhongmei, 2009). Besides, with the degradation of wetlands, the content of  
76 soil organic carbon decreased significantly, and the activity of soil enzymes also changed (Chen  
77 Yanxin, 2018). At present, there are few reports on this aspect in the Ili Valley. This study takes  
78 Yili valley wetland with different flooding degrees as the research object. It analyzes the change  
79 of soil enzyme activity of wetland under different flooding conditions, and it discusses the  
80 relationship between water and soil factors affecting the enzyme activity, which provided a

81 theoretical basis for the study of the mechanism of soil water impact on Soil and wetland  
82 protection in Yili Valley.

83

## 84 **Materials & Methods**

### 85 **Experimental site**

86 Yili River Valley is located in the northwest direction of Tian shan Mountains in Xinjiang,  
87 surrounded by high mountains in the north, east and south, showing the natural geographical  
88 features of "three mountains with two valleys". It enjoys the reputation of "wet island in the  
89 western region" and "jiangnan beyond the Great Wall", and is the main transportation route of  
90 the ancient Silk Road. In addition, the Yili Valley is situated at 80°09'E—84°56'E in the east  
91 longitude and 42°14'N—44°50'N in the North latitude, with an altitude of 530—1000 m and an  
92 area of 56,400 km<sup>2</sup>. Because of the excellent natural environment and unique geographical  
93 position of the Yili Valley, the water resources and mineral resources are quite abundant, and  
94 there are various species in the valley. The climate is warm and humid, belonging to the  
95 temperate continental climate, with a great temperature difference between day and night. The  
96 annual average temperature is 10.4°C, and the annual average sunshine hours are 2700—3000  
97 hours. The annual average precipitation is about 417.6 mm, mainly concentrated in spring and  
98 summer, which is 60%~70% of the annual precipitation. With the increase of altitude, the  
99 precipitation can be as high as 600 mm in mountainous areas, and the annual average  
100 evaporation is about 1260~1900 mm. The Yili Valley is the wettest climate area in Xinjiang. Yili  
101 Valley mainly distributes forest, grassland, meadow and other vegetation types, among which the  
102 grassland soil type is mainly sierozem, the plant species are mostly perennial and cold-resistant  
103 gramineae. The forest soil is mainly grayish brown forest soil, and most of the tree species are  
104 Xinjiang spruce, Xueling spruce, Miye poplar et al. (Yang Yuhai et al., 2010).

105

### 106 **Sampling point setting and field sample collection**

107 The sampling sites were selected in Yili River floodplain wetland and Liberate Bridge  
108 National Wetland Park in Zhaosu County, as shown in figure 1. Firstly, three wetland soils with  
109 different flooding degrees were collected from Ditch millet wetland (DMW), Calamagrostis  
110 epigeios wetland (CEW) and Phragmites australis wetland (PAW). Among them, DMW belongs  
111 to rarely flooded habitat, CEW belongs to seasonal flooded habitat. The flooding period of one  
112 year is about 2-3 months, while PAW belongs to long-term flooded habitat, and the flooding  
113 period of one year is about 10 months.

114 Three 1 m×1 m plots were randomly set up in the selected sample plots. In each wetland  
115 type, plant and litter on the surface of the plot were removed with a shovel to obtain three  
116 random soil profiles, and then soil samples of 0-10, 10-20, 20-30, 30-40 cm were collected from  
117 bottom to top respectively. A total of 36 soil samples was collected from three wetland types.  
118 The samples were sealed and brought back to the laboratory in plastic bags, and then the samples  
119 were divided into two soil samples, one of which was stored in a sealed bag and stored in a  
120 refrigerator for the determination of soil microbial biomass carbon and soil enzyme activity, and

121 the other soil sample was put into the bag for the determination of soil basic physical and  
122 chemical properties after air drying, grinding and 0.15 mm sieving.

123

124

### Figure.1

125

## 126 **Research method**

### 127 **(1) Soil basic physical and chemical properties**

128 The content of soil organic carbon (SOC) can be calculated by heating and boiling with  
129 potassium dichromate and then titrated with ferrous sulfate solution. The soil samples were  
130 boiled with perchloric acid and sulfuric acid, and then the total phosphorus (TP) content in soil  
131 was determined by colorimetry (Lu Rukun, 2000); the content of easily oxidized organic carbon  
132 (EOC) in soil can be obtained by putting potassium permanganate solution into soil sample and  
133 then colorimetric method (Blair et al., 1995); the content of dissolved organic carbon (DOC) was  
134 determined by colorimetry (Zhan Xinhua et al., 2002). It is known that  $\text{NH}_4^+$  in soil leaching  
135 solution reacts with hypochlorite and phenol in strong alkaline medium to form water-soluble  
136 dye indophenol blue. The content of ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ) can be determined by  
137 colorimetry (Zhao Jie et al., 2011).

### 138 **(2) Soil microbial biomass carbon**

139 In the determination of microbial biomass carbon (MBC) content in wetland soil, firstly, the  
140 fumigated and unfumigated soils were extracted with potassium sulfate solution. Next, the  
141 content of organic carbon in the two extracts was determined by soluble carbon-nitrogen  
142 analyzer. Finally, soil microbial biomass carbon can be calculated by dividing the difference of  
143 carbon content by the conversion coefficient (Yu Shu et al., 2007).

### 144 **(3) Soil enzyme activity**

145 Because the enzymatic product ammonia reacts with phenol sodium hypochlorite to form  
146 blue indigo phenol, the activity of urease can be measured by colorimetry using urea as matrix  
147 (Ding Lei et al., 2011); adding hydrogen peroxide solution to the soil sample and titrating excess  
148 hydrogen peroxide with potassium permanganate solution can calculate the consumption of  
149 hydrogen peroxide solution and determine the activity of catalase (Guan Songyin, 1986). The  
150 reducing sugar produced by enzyme hydrolysis of sucrose and 3, 5-dinitrosalicylic acid will form  
151 orange solution, and the sucrase activity can be determined by colorimetry (Xiao Ye, 2015); the  
152 amylase activity was measured by colorimetry (Zhou Likai et al., 1980), and soil amylase  
153 activity was determined by measuring the amount of glucose produced during hydrolysis.

154

## 155 **Statistical analysis**

156 Excel 2010, SPSS 19.0 and CANOCO 4.5 software were used to analyze the integrated  
157 data. One-way ANOVA method was used to analyze the differences of soil microbial biomass  
158 carbon, soil physical and chemical properties and soil enzyme activities in different flooding  
159 degrees, and Duncan table was used to analyze the significance of the existing data. Two-way  
160 ANOVA method was used to analyze the effects of flooding degree, the soil depth and their

161 interaction on soil microbial biomass carbon, soil basic physical and chemical properties and soil  
162 enzyme activities. Using RDA sorting, the effects of soil physicochemical properties and  
163 microbial biomass carbon on soil enzyme activity were analyzed. It should be noted that the  
164 factors significantly related to soil enzyme activities need to be selected by Monte Carlo analysis  
165 before the redundancy analysis. The T-value double sequence diagram of CANOCO 4.5 can also  
166 be used to analyze the single environmental factor affecting soil enzyme activity.

167

## 168 **Results**

### 169 **Soil basic physical and chemical properties under different flooding conditions**

170 Under the same flooding conditions, the basic physical and chemical properties of different  
171 soil thickness are different (Table 1). Except for the PAW, the content of soil organic carbon in  
172 the DMW and CEW was obviously different among the three soil layers. The content of the SOC  
173 of the 0-10 cm soil layer of the CEW was significantly higher than that in 10-20cm, 20-30cm and  
174 30-40cm soil layers, while that in the 0-10cm and the 10-20cm soil layer of the DMW was  
175 significantly higher than that in the 20-30cm and 30-40cm soil layers. For total phosphorus (TP),  
176 there were no significant differences between the DMW and PAW in the three soil layers, and  
177 the TP content of the CEW decreased gradually with the increase of soil depth. In terms of easily  
178 oxidized organic carbon (EOC), there were no significant differences between the CEW and  
179 PAW in the three soil layers. The EOC content in 0-10 cm soil layer was the highest, while that  
180 in 30-40 cm soil layer was the lowest in the DMW. The content of DOC in wetlands under three  
181 flooding conditions showed a decreasing trend with the increase of soil depth.

182 Two-way ANOVA shows that except  $\text{NH}_4^+\text{-N}$ , different flooding conditions and soil layers  
183 have significant effects on the soil basic physical and chemical properties (Table 2). Among  
184 them, the effect of flooding conditions on the basic physical and chemical properties of soil was  
185 greater than that of soil depth. Only the  $F$  value of TP in different soil depths was higher than  
186 that of  $F$  value in different flooding conditions, indicating that different soil conditions had a  
187 stronger effect on TP content. At the same time, TP and SOC are also significantly affected by  
188 the interaction between different flooding conditions and the soil depth, but the interaction has  
189 no significant effect on EOC, DOC and  $\text{NH}_4^+\text{-N}$ . Different flooding conditions in the same soil  
190 layer have different effects on the soil basic physical and chemical properties (Table 1). By  
191 comparing the average values in (Table 1), it can be found that the contents of TP, DOC and  
192  $\text{NH}_4^+\text{-N}$  in wetlands with different flooding conditions are  $\text{PAW} > \text{CEW} > \text{DMW}$ ; the EOC  
193 content in wetlands with different flooding conditions is  $\text{DMW} > \text{CEW} > \text{PAW}$ . And the SOC  
194 content in the CEW and DMW was significantly higher than that in the PAW.

195

196

#### **Table 1**

197

198

#### **Table 2**

199

200

201

**202 Soil microbial biomass carbon (MBC) under different flooding conditions**

203 There were differences in soil microbial biomass carbon at different soil depths under the  
204 same flooding conditions (Fig. 2). The MBC content in 0-10 cm and 10-20 cm soil layers of the  
205 DMW and the CEW was significantly higher than that in 20-30 cm and 30-40 cm soil layers; the  
206 MBC content in 0-10 cm soil layers of the PAW was significantly higher than that in 30-40 cm  
207 soil layers. The MBC in wetlands under three flooding conditions showed a decreasing trend  
208 with the increase of soil depth.

209 The results of Two-way ANOVA showed that different flooding conditions and soil layers  
210 had significant effects on soil microbial biomass carbon, and the interaction between them also  
211 significantly affected soil microbial biomass carbon (Table 3). The content of MBC decreased  
212 gradually with the increase of flooding degree. The *F* value of MBC in different soil layers is far  
213 greater than that in different flooding conditions, which indicates that different soil depth has a  
214 deeper impact on the MBC than the flooding condition. The soil depth is indeed one of the  
215 important factors affecting the change of MBC content.

216

217 **Figure.2**

218

219 **Table 3**

220

**221 Differences of soil enzyme activities in wetlands under different flooding conditions**

222 The activities of enzymes in different soil layers are different (Table 4). For the sucrose, the  
223 sucrose activity of wetlands with three flooding degrees decreased significantly with the increase  
224 of soil depth. Among them, the sucrose activity of 0-10 cm and 10-20 cm soil layers in the DMW  
225 and CEW was significantly higher than that of 20-30 cm and 30-40 cm soil layers; the sucrose  
226 activity of 0-10 cm soil layers in the PAW was significantly higher than that of 10-20 cm, 20-30  
227 cm and 30-40 cm soil layers. There was no significant difference in the catalase activity between  
228 the DMW and PAW, but the catalase activity decreased with the increase of soil depth in the  
229 CEW. The amylase activity of the different soil layers was not significantly different in the PAW;  
230 the amylase activity in the DMW decreased at first and then increased with the increase of soil  
231 depth; the amylase activity in the CEW decreased with the increase of soil depth, and the  
232 amylase activity in 0-10 cm soil layer was the strongest. There was no significant difference in  
233 the urease activity among the three wetlands of flooding conditions.

234 According to Two-way ANOVA of flooding conditions and soil depth on soil enzyme  
235 activity (Table 5), except the sucrose, the soil depth had no significant effect on other soil  
236 enzyme activities. Different flooding conditions have significant effects on the activities of the  
237 sucrose and amylase. The *F* values of sucrose, amylase and urease activities in different flooding  
238 conditions were higher than those in different soil depths, which indicated that the effects of  
239 different flooding conditions on soil enzyme activities were greater than those at different soil  
240 depths, and flooding conditions were one of the important factors affecting soil enzyme activities.

241 According to the average comparison of table 4), the activities of sucrase and urease decreased  
242 gradually with the increase of flooding degree. Among them, the activities of sucrase in the  
243 CEW significantly decreased by 4.91% compared with the DMW, and the activities of sucrase in  
244 the PAW significantly decreased by 46.04% compared with the CEW. Although the flooding  
245 conditions had no significant effect on the catalase activity in 0-10 cm, 10-20 cm and 20-30 cm  
246 soil layers, the effects of different flooding conditions were extremely significant on the catalase  
247 activity in 30-40 cm soil layers. As far as the catalase activity in 30-40 cm soil layers was  
248 concerned, the DMW was significantly reduced by 18.45% compared with the CEW, and the  
249 CEW was significantly reduced by 33.20% compared with the PAW. The activities of sucrase  
250 and amylase in the DMW and CEW were significantly higher than those in the PAW, while the  
251 catalase activity was the highest in the CEW.

252

253

**Table 4**

254

255

**Table 5**

256

**257 Correlation analysis of soil physical and chemical factors, microbial biomass carbon and  
258 soil enzyme activity**

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

273 A single environmental factor analysis was carried out for the environmental factors  
274 affecting soil enzyme activity by using the T-value double-sequence diagram of CANOCO 4.5  
275 (Fig. 4). As shown in (Fig. 4A), the arrows of sucrase, urease and amylase all fall on the solid  
276 line circle of SOC, indicating that SOC has a significant positive correlation with the sucrase,  
277 urease and amylase activities, that is to say, the activities of sucrase, urease and amylase increase  
278 with the increase of SOC content. The arrow of catalase passes through the solid line circle of  
279 SOC, which shows that there is a positive correlation between SOC and CAT. (Fig. 4B) shows  
280 that four soil enzymes pass through the solid line circle of DOC, which indicates that there are a

281 positive correlation between DOC and the activities of the four soil enzymes. (Fig. 4C) shows  
282 that most of the four soil enzymes fall outside the solid line circle and dotted line circle of  
283  $\text{NH}_4^+\text{-N}$ , indicating that there was no significant relationship between  $\text{NH}_4^+\text{-N}$  and the activities  
284 of the four soil enzymes.

285 Different environmental factors have different effects on soil enzyme activity (Table 6). The  
286 effects of different environmental factors on soil enzyme activities were  $\text{SOC} > \text{DOC} > \text{TP} >$   
287  $\text{MBC} > \text{NH}_4^+\text{-N} > \text{EOC}$ . Among them, the effects of SOC, DOC, TP and MBC were significant  
288 on soil enzyme activity, especially the effect of SOC on soil enzyme activity was extremely  
289 significant. And SOC had the greatest effect on soil enzyme activity, accounting for 45% of the  
290 total explanations ( $F = 27.82, p < 0.01$ ). The effects of  $\text{NH}_4^+\text{-N}$  and EOC were not significant on  
291 soil enzyme activity ( $p > 0.05$ ).

292

293 **Figure.3**

294

295 **Table 6**

296

297 **Figure.4**

298

## 299 **Discussion**

### 300 **Effects of different flooding conditions on soil enzyme activities**

301 Soil enzyme is a kind of proteins with special catalytic ability, which mainly comes from  
302 the decomposition of soil microorganisms, animal and plant secretions and residues (Guan  
303 Songyin, 1986). Soil moisture has a significant correlation with soil microbial activity and type,  
304 and different water conditions will directly affect the existence and activity of soil enzyme  
305 activity (Wan Zhongmei, 2005). This indicates that the degree of flooding did significantly affect  
306 the activity of soil enzymes. The results showed that the activities of sucrase and amylase were  
307 closely related to the degree of flooding, and decreased gradually with the increase of flooding  
308 degree (Wan Zhongmei, 2008; Zhou Xiaoming, 2018), which was consistent with the effect of  
309 flooding degree on sucrase and amylase activities, and the activities of sucrase and amylase in  
310 the rarely flooded and seasonal flooded wetlands were significantly higher than those in the long-  
311 term flooded wetlands. This phenomenon may be due to the increase of soil moisture, which  
312 leads to the decrease of soil permeability, restricts the growth of soil microorganisms, greatly  
313 slows down the decomposition of soil humus, and thus reduces the activity of soil enzymes. It is  
314 also possible that increasing soil water will affect the availability of oxygen in an environment  
315 with abundant substrates and wet soils, then affecting the growth of soil microorganisms and  
316 plant roots, resulting in a decrease in enzyme activity (Guenet et al., 2012).

317 In this study, although the correlation between sucrase and urease activity and flooding  
318 conditions was not significant, the urease activity of rarely flooded wetlands was much higher  
319 than that of long-term flooded wetlands as a whole, which was similar to the results of other  
320 researchers (Xu Jingjing, 2017). However, except that the sucrase activity of 30~40 soil layer

321 decreased significantly with the increase of water content, the sucrose activity in other soil layers  
322 did not change significantly with the increase of water content, which was consistent with that  
323 the catalase activity did not change significantly with the water gradient in non-rhizosphere  
324 soil (Tian Youhua, 2012). This phenomenon may be that the deeper the soil layer and the less  
325 water it contains, the more beneficial it is to increase the content of soil organic matter, the  
326 survival of soil microorganisms and the activity of enzymes.

327

### 328 **Effects of microbial biomass carbon and soil physical and chemical properties on soil** 329 **enzyme activities**

330 Soil microbial biomass is an essential indicator for detecting soil quality. On the one hand,  
331 it can indicate the number of microorganisms involved in energy flow, the material cycle and  
332 organic matter transformation in soil (Powlson D S, Brookes P C Christensen B T., 1987). On  
333 the other hand, it reflects the process of nutrient transfer and transport and the energy cycle in  
334 soil (Doran JW., 1994). Previous studies have shown that the carbon contents of soil microbial  
335 biomass and soil enzyme activities in the treatment of underwater are higher than those in the  
336 treatment of natural bareness (Liu Yajun et al., 2017). (Table 6) shows that soil microbial  
337 biomass carbon is significantly correlated with soil enzyme activity, which is closely related to  
338 soil microbial biomass carbon and soil enzyme activity obtained by the predecessors. With the  
339 change of water gradient, soil enzyme activity is positively correlated with microbial biomass  
340 carbon (Wan Zhongmei et al., 2008).

341 There was a certain relationship between soil organic carbon and soil enzyme activity (Wan  
342 Zhongmei et al., 2009). It can be seen from Figure. 3 that soil organic carbon was significantly  
343 positively correlated with sucrose, urease and amylase activities, and the correlation was  
344 strongest with the sucrose activity. There was a positive correlation between soil easily oxidized  
345 organic carbon and the activities of sucrose, urease, catalase and amylase, which was consistent  
346 with previous research results (Xiao Ye et al., 2015). In addition, total phosphorus had  
347 significant positive effects on four soil enzymes, and had the strongest correlation with the  
348 catalase activity, which could better explain the change of catalase activity, indicating that total  
349 phosphorus was the main factor affecting urease, catalase and sucrose activity through direct or  
350 indirect effects (Liu Guangshen et al., 2003).

351

### 352 **Conclusions**

353 The results of the experiment showed that most of the wetland soil enzymes in Yili Valley  
354 decreased with the increase of flooding degree under different flooding conditions. The activities  
355 of sucrose and urease in the three flooded wetlands were as follows : rarely flooded wetlands >  
356 seasonal flooded wetlands > long-term flooded wetlands; the activities of sucrose and amylase in  
357 rarely flooded wetlands and seasonal flooded wetlands were significantly higher than those in  
358 long-term flooded wetlands; while the activities of catalase were the highest in seasonal flooded  
359 wetlands. It can be seen that flooded condition is indeed one of the important factors affecting  
360 the enzyme activity.

361 Soil organic carbon (SOC), dissolved organic carbon (DOC) and total phosphorus (TP) had  
362 significant effects on soil enzyme activity in wetland, while ammonium nitrogen (NH<sub>4</sub><sup>+</sup>-N),  
363 easily oxidized organic carbon (EOC) and microbial biomass carbon (MBC) had no significant  
364 effects on soil enzyme activity. Among them, the correlation between soil organic carbon and the  
365 sucrase activity, total phosphorus and the catalase activity were the strongest; soil organic carbon  
366 had a significant positive correlation with sucrase, urease and amylase activity, but had a slight  
367 influence on catalase activity; dissolved organic carbon had a positive correlation with four soil  
368 enzyme activities. It can be seen that the activity of soil enzyme in wetland is closely related to  
369 soil organic carbon and dissolved organic carbon. Appropriate increase of soil organic carbon  
370 content can enhance the activity of soil enzyme.

371

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## **Figure Interpretation**

### **Figure.1**

#### **Diagram of wetland sampling point in Yili 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 soil physical and chemical indexes, microbial biomass carbon and 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.

**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 <sub>4</sub> <sup>+</sup> -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.

5

**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 <sub>4</sub> <sup>+</sup> -N
Factor		(g•kg <sup>-1</sup> )	(g•kg <sup>-1</sup> )	(mg • kg <sup>-1</sup> )	(mg • kg <sup>-1</sup> )	(mg • kg <sup>-1</sup> )
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

4

**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.**

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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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4

**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) <sup>-1</sup> )	Catalase (mg/g)	Amylase (mgC <sub>6</sub> H <sub>12</sub> O <sub>6</sub> /(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)

**Atwo-way ANOVA for the effects of different flooding conditions and soil layerson soil enzyme activities.**

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

Influence Factor		Sucrase	Catalase	Amylase	Urease
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 Conditions	<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.**

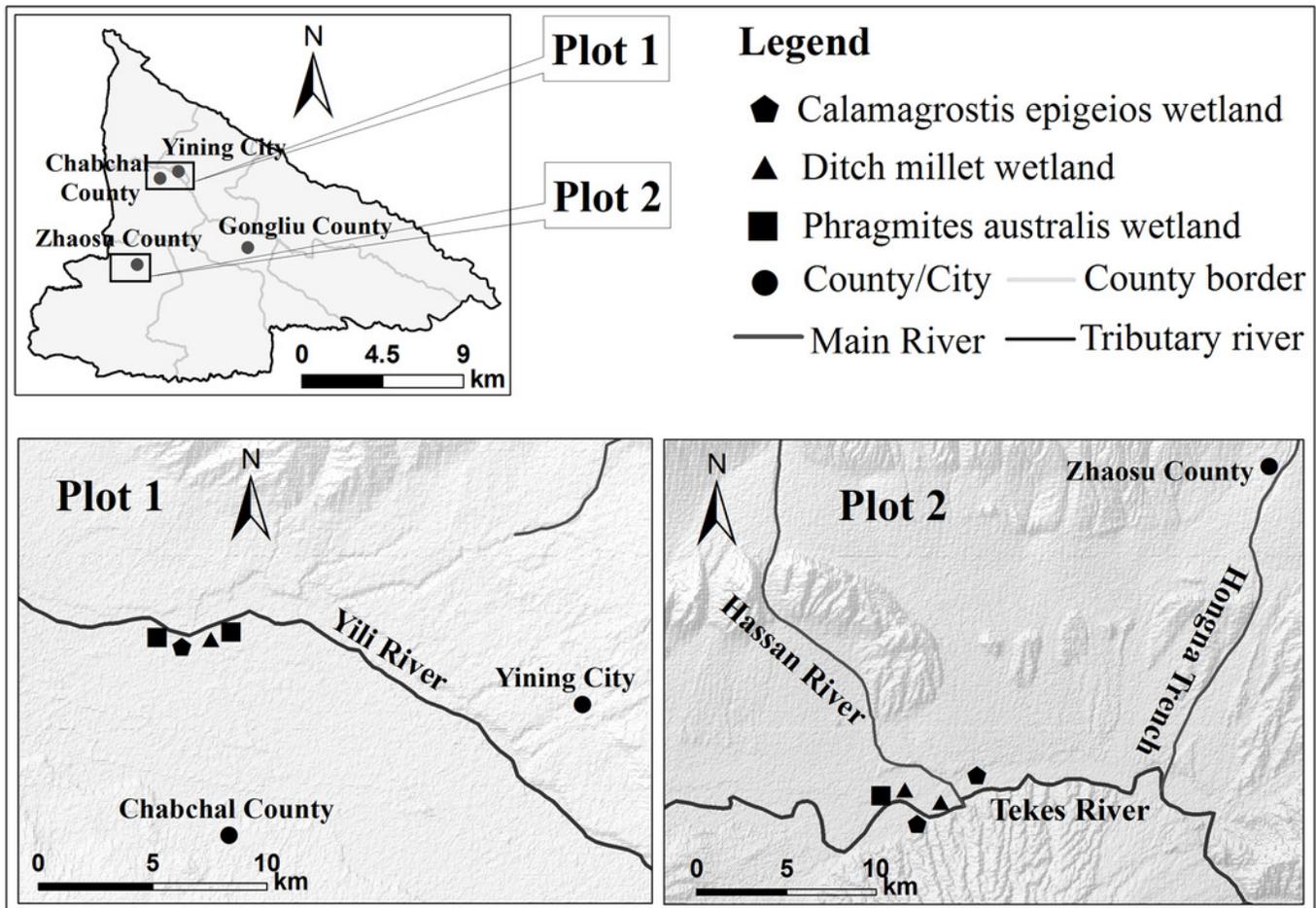
environmental Factor	Sorting of Importance	Degree of Interpretation (%)	Importance ( <i>F</i> value)	Significance ( <i>P</i> value)
SOC	1	45	27.824	0.002
DOC	2	13.3	5.209	0.012
TP	3	8.9	3.337	0.032
MBC	4	5.1	2.728	0.050
NH <sub>4</sub> <sup>+</sup> -N	5	8.5	3.139	0.114
EOC	6	4.7	1.284	0.296

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# Figure 1

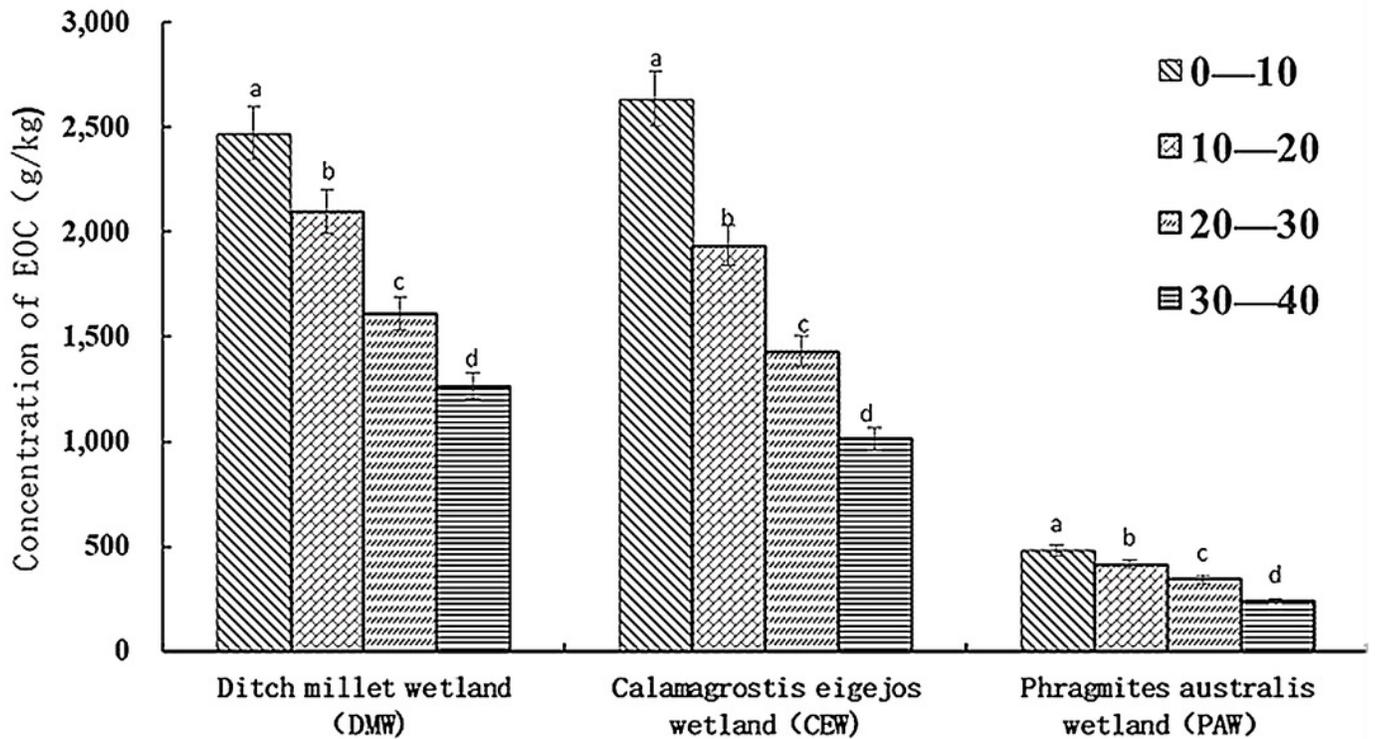
Diagram of wetland samplingpoint in Yili Valley.

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



## Figure 2

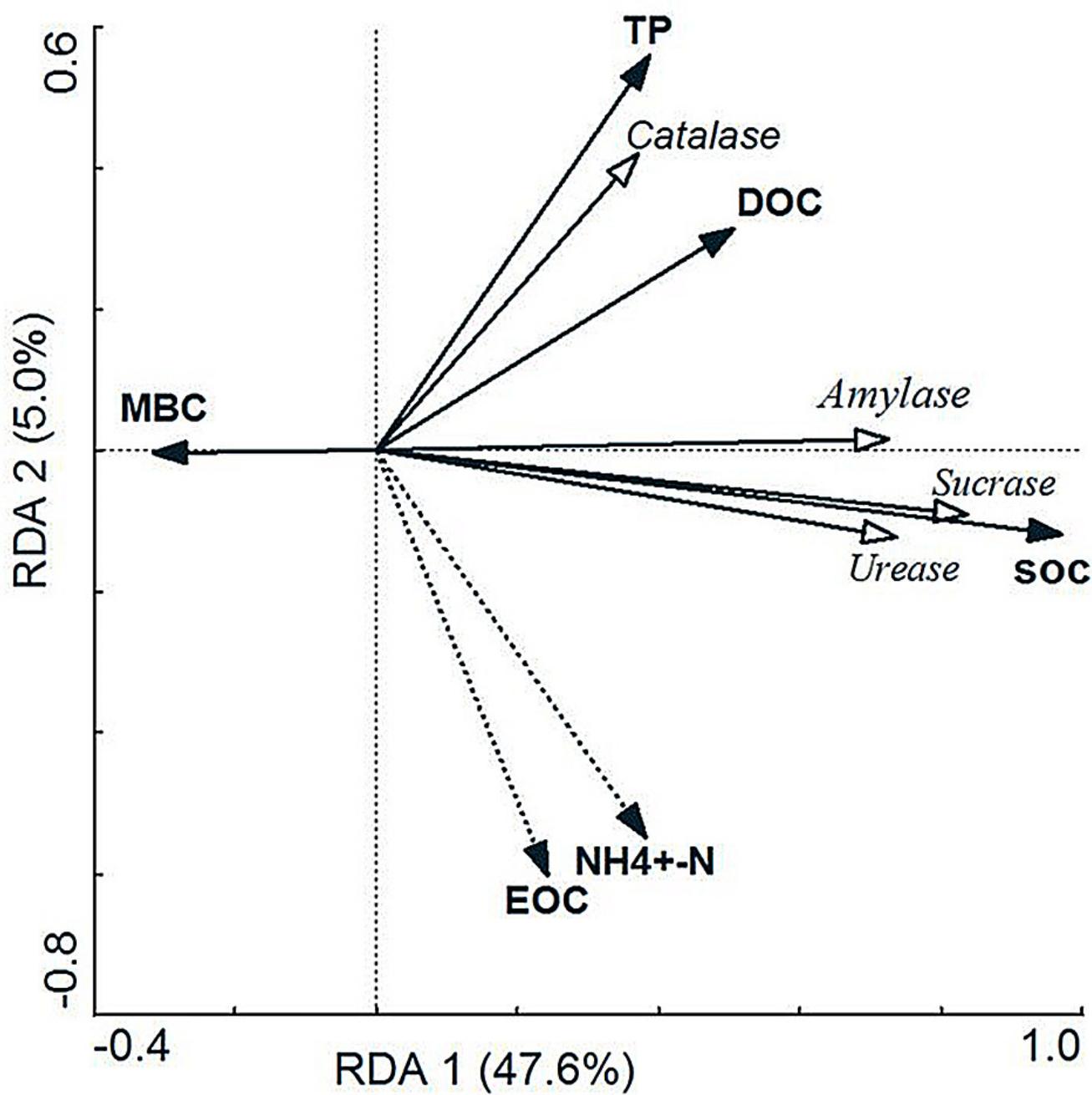
Soil microbial biomass carbon content in wetland soil of different layers under different flooding conditions.



## Figure 3

### **Redundancy analysis of soil physical and chemical indexes, microbial biomass carbon and soil enzyme activity.**

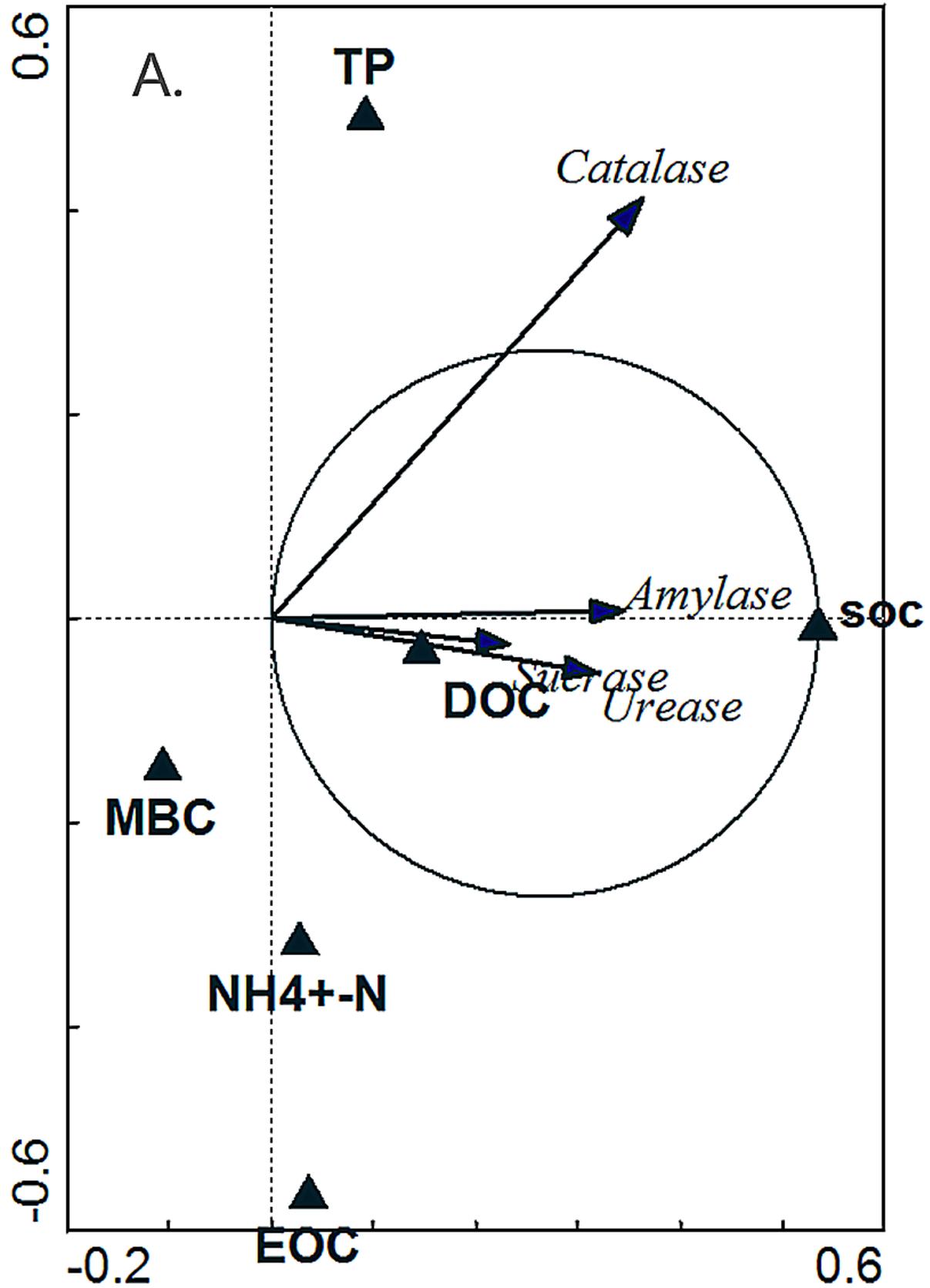
The quadrant of arrow in the figure represents the positive and negative correlation between different factors and the sorting axis, the hollow arrow represents several soil enzymes, the solid arrow represents environmental variables, and the cosine value of corresponding angle represents the correlation 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 singlefactor influencing varied of soil enzyme activities in wetland.

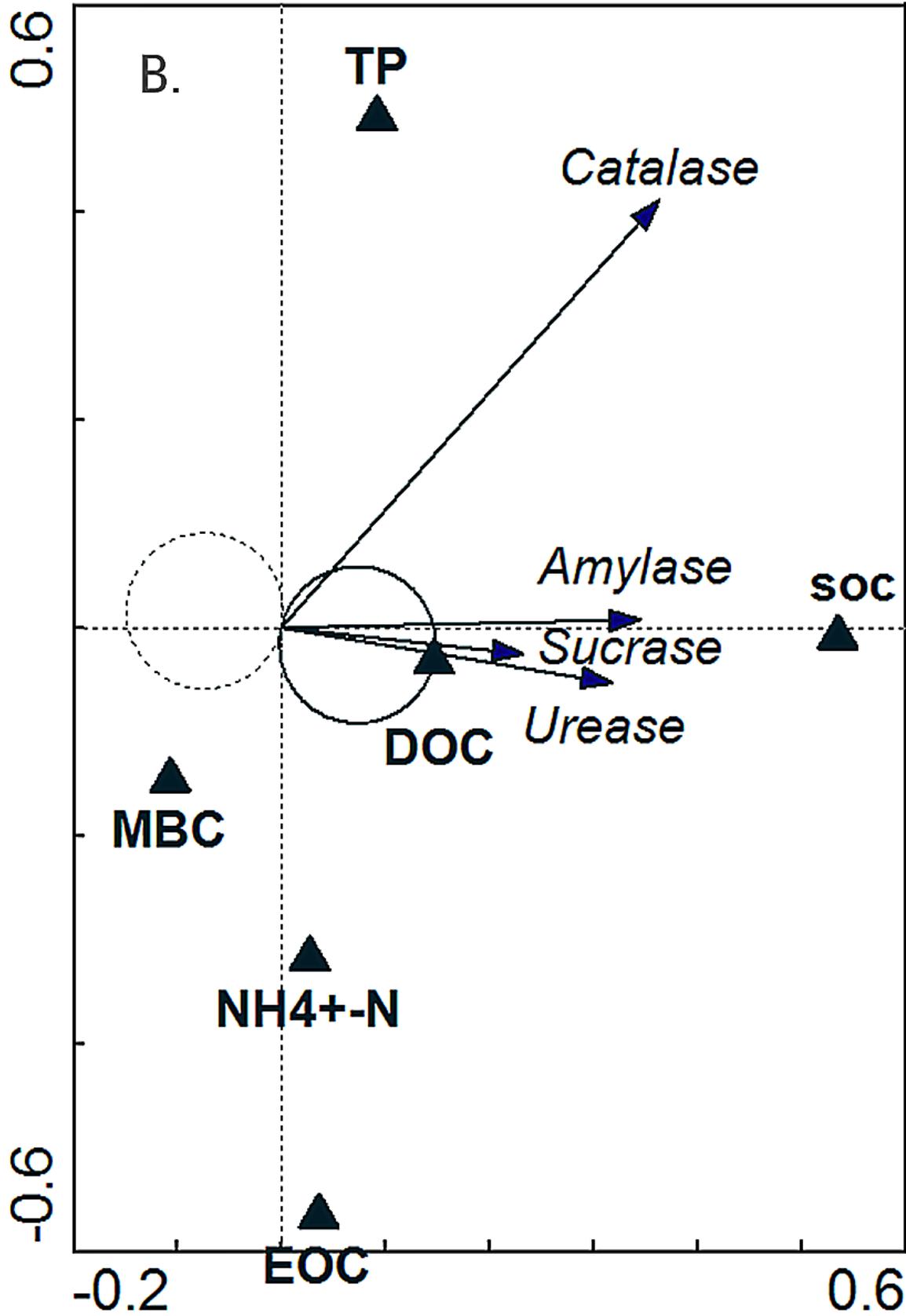
The quadrant of the arrow in the figure represents the positive and negative correlation between different factors and the sorting axis. The arrow represents several soil enzymes, and the solid triangle represents environmental variables.



## Figure 5

**The T-value for a singlefactor 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.



## Figure 6

**The T-value for a singlefactor 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.

