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Original Research

Changes in Land Use and their Effects on Soil Properties in Huixian Karst Wetland System

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Abstract

To gain a better understanding of the impact from the land-use change in the Huixian karst wetland system, we analyzed soil microflora, enzyme activities, and physicochemical properties from three land-use types (natural wetland, paddy field, and dry farmland). The results showed that soil pH, soil organic carbon, total nitrogen, cation exchange capacity, exchangeable Ca and Mg, and the cellulase and alkaline phosphatase activities in the dry farmland were significantly lower than those in the paddy field and natural wetland ($p<0.05$). However, soil pH, soil organic carbon, total nitrogen, cation exchange capacity, exchangeable Ca and Mg, and the cellulase and alkaline phosphatase activities made no significant difference to the paddy field and the natural wetland ($p>0.05$). Moreover, the soil microbial biomass carbon and nitrogen in the dry farmland were also lower than those in the paddy field and natural wetland, although no significant differences were observed ($p>0.05$). This suggests that, in the alkali condition, natural wetland with high biomass and weak microbial activity may be an important carbon sink. In the CCA biplot, it can be seen that the natural wetland cluster intersects with the paddy field cluster and the dry farmland cluster in the same quadrant (although the paddy field and the dry farmland clusters are separate). Therefore, we concluded that the natural wetland usually was reclaimed as paddy field or dry farmland directly for agricultural output in the Huixian karst wetland system. The paddy field has a waterlogged condition and shows the similar results to natural wetland, which can be regarded as artificial wetland. In view of the similar ecosystem services by paddy fields as substitutes of natural wetland, if the degradation trend of natural karst wetland can't be reversed, the paddy field should be preserved in the Huixian karst wetland system for its ecosystem service.

Keywords: alkali condition, carbon sink, artificial wetland, ecosystem service

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Introduction

A wetland has important functions and values in the reduction of flooding and maintaining carbon storage [1]. However, due to anthropogenic factors, some wetlands have been reclaimed as agricultural or urban fields [1].

Wetland conversion involves soil changes, such as soil physical change [2-4], and biogeochemical change, which include changes in decomposition rates and oxidation states and the mobility of nutrient species [5]. Moreover, the land change/conversion of natural wetland can change carbon release to the atmosphere [6-7]. Although organic materials, soil microflora, and enzyme activities are important components of the soil matrix and their transformation plays a significant role in soil fertility and biogeochemical cycles from the local to the global level, little is known about the changes in land use and their effects on soil properties in the karst wetland system [5]. The reason is that their inner relationship relating to land change is extremely complex, especially in the aerobic/anaerobic transient conditions. Therefore, the monitoring and analysis of wetland conversion usually was based on remotely sensed images [8]. To gain insights into the land change/conversion of natural wetland through analysis of soil quality, canonical correspondence analysis (CCA) plus other statistical analysis was adopted to describe their inner relationship.

Canonical correspondence analysis as a useful method can simplify large sets of environmental samples or abundance data, and identify and quantify the internal relationships of environmental factors [9-10]. Although greater data always contains some unusual samples and redundant environmental variables, their intimate relationship can be diagnosed using the CCA method [10]. Therefore, our paper aimed to provide new insights for the quantitative relationships among the soil factors at the Huixian karst wetland system relating to the conversion trend of three land-use types (natural wetland, paddy field, and dry farmland) and the influence on their ecosystem services.

Materials and Methods

Study Site

Huixian karst wetland is located at karst depressions of the town of Huixian in Lingui County, which is the largest karst wetland in China, with a mean annual temperature of 16.520.5 ºC and average annual rainfall of 1,890.4 mm (1951-2008). Since the 1950s, due to the increase of human activities and the lack of effective management and protection, the natural wetland has been continuously undermined. The water area of the wetland has gradually been shrinking and its ecosystems were seriously damaged. Now, the total wetland has been dismembered as a star-studded pond with an area of no more than 1 km^2 [11].

Sample Collection

We collected 11 soil sample plots from the Huixian karst wetland system. Four sample plots were extracted from paddy fields, four sample plots were extracted from dry farmland, and three sample plots were extracted from natural karst wetland. At each sample plot (20×20 m), three soil cores (5 cm diameter) were collected at 0-20 cm depths and mixed. The soil samples were packaged on ice and shipped to the laboratory overnight, where the soil samples were sieved (2 mm), separated from rocks and roots, homogenized, and stored at 4 ºC until analysis.

Soil Physicochemical Properties Analysis

Soil samples were air-dried, sieved (0.2 mm), and analyzed for pH, soil organic carbon (SOC), total nitrogen (TN), and cation exchange capacity (CEC) [10-11]. Exchangeable Ca (E-Ca) and Mg (E-Mg) in soil samples were extracted in 1 M KCl and determined by AAS-Zeenit700 [12].

Extraction and Enumeration of Culturable Microbes

Bacteria, actinomycetes, and fungi were extracted from wet soil using the soil-plate method [10]. Three plates of each culture medium were inoculated per dilution [10].

Soil Microbial Biomass Carbon and Nitrogen

Soil microbial biomass carbon (SMBC) was determined by the fumigation-extraction method [13]. In brief, wet soil fumigated with ethanol-free $CHCl_3$ and non-fumigated wet soils (25 g dry weight equivalent) were extracted with 50 mL of 0.5 mol L^{-1} K_2SO_4 (soil:extractant ratio = 1:2) for 30 min by oscillating shaking at 180 rpm, and filtered organic carbon in the extracts was determined after oxidation with 0.2 mol L^{-1} $K_2Cr_2O_7$ at 180 ºC for 5 min [14]. The soil microbial biomass carbon was calculated as follows:

$$\mathrm{SMBC = EC/kEC}$$

…where EC is the difference between organic carbon extracted from fumigated wet soils and non-fumigated wet soils, and kEC = 0.38.

Soil microbial biomass nitrogen (SMBN) was determined by analyzing total nitrogen in the 0.5 mol L^{-1} K_2SO_4 extracts obtained from the wet soil fumigated with ethanol-free $CHCl_3$ and non-fumigated wet soils (25 g dry weight equivalent) [13]. 20 ml K_2SO_4 was transferred into digesting tubes for achieving digesting solution, and 5 ml digesting solution (sulfuric acid-mercuric sulfate potassium sulfate solution) was added to each tube. The mixture in the tubes was digested on a hot block at 160 ºC for 1 h and then 380 ºC for 2.5 h. After cooling, the sample was diluted to 25 ml with ammonia-free water and the concentration of NH^+_4-N in the digester was determined using a discrete auto-analyzer. The soil microbial biomass nitrogen was calculated using the equation:

$$SMBN = EN/kEN, EN = Nf - Nnf$$

…where Nf is the total nitrogen in fumigated wet soil, Nnf is the total nitrogen in non-fumigated wet soil, and kEN = 0.54.

Soil Enzyme Assays

All enzyme activities were determined from wet samples in triplicate [10]. The cellulase activity was expressed on a soil dry weight as mg glucose released g^{-1} h^{-1}. The urease activity was determined using urea as substrate, and the soil mixture was incubated at 37 ºC for 24 h. The produced NH_4^+-N was determined by a colorimetric method, and urease activity was expressed on a soil dry weight as mg NH_4^+-N g^{-1} h^{-1}. The alkaline phosphatase (APA) activity was expressed on a soil dry weight basis by correcting for water content in the soil at the time the sample was removed from the incubation bottle and is given in units of mg p-nitrophenol-produced g^{-1} soil h^{-1}.

Data Analysis

The descriptive statistical analysis in mean and correlation analysis was performed using SPSS 13.0 software for Windows XP. Correlation analysis with the source data was done using the Pearson correlation method with significance defined at $p<0.05$ [10-11].

The significance of differences among means in the same column was calculated using JMP version 5.0, SAS Institute Inc., Cary, NC, USA. Different letters in the same column indicate significant differences in activity at $p<0.05$ [10-11].

The CANOCO for Windows 4.5 program was used to compute CCA for explaining the contribution of each influencing factor [10]. Moreover, Pearson correlation analysis and CCA can prove each other [10].

Results and Discussion

Soil Physicochemical Character

Table 1 shows soil physicochemical properties. Soil pH, SOC, TN, and CEC in the dry farmland were significantly lower than those in the paddy field and natural wetland ($p<0.05$), though the above results have no significant difference in the paddy field and the natural wetland.

A wetland is a land area saturated with water, either permanently or seasonally [1]. Although wetlands occupy only 4-6% of the earth's land area (~530-570 mha), they store a substantial amount of carbon [15]. Yu et al. reported that the highest mean SOC density (209.9 t C ha^{-1}) was recorded from a wetland ecosystem and the lowest (29.0 t C ha^{-1}) from a desert ecosystem in China [16]. Due to the greatly reduced supply of O_2 to soil and the decreased organic carbon mineralization rates in the natural wetland, the natural wetland shows a high content of soil organic carbon in the anaerobic environment [17]. In the anaerobic environment, anaerobic microorganisms as well as photosynthesis by submerged macrophytes can create high pH values in the aquatic environment [18]. Moreover, the study site is located at the karst area with calcium-rich and high alkaline character. So the pH value in the natural wetland is higher than that in the dry farmland.

In the waterlogged condition, increased water residence times in wetlands are due, in part, to the dense stands of aquatic plants that characterize these ecosystems [15, 19]. Aquatic plants increase nitrogen retention through vegetative uptake and provide favorable conditions for sedimentation and denitrification [19]. In our study, the natural wetland retains the highest proportion of total nitrogen loading (Table 1) and the importance of water residence time to nitrogen retention was supported by Jacobs and Harrison [19].

CEC is calculated as the sum of exchangeable cations (Ca, Mg, K, Na, Al, Fe, and Mn), which drives nitrification and oxygen transfer in flood and drains wetland treatment systems [20]. Ammonium cations are adsorbed to negatively charge surfaces when wetland cells are flooded. Babadi et al. reported that adsorption of nitrogen products is known to be stronger on soils with high cation-exchange capacity [21]. Moreover, accumulated organic materials in wetland form humic substances that have significant CEC [22]. So, in the natural wetland, CEC is greater than that in the dry farmland. In the wetland of Brazilian Pantanal, surface (0-10 cm) soil also had significantly higher soil organic matter and cation exchange capacity [23]. Moreover, greater content of exchangeable Ca or Mg was relating to higher cation exchange capacity as observed in the natural wetland (Table 1).

As for the paddy field, it also has a waterlogged condition that is similar to the wetland in many ways and is regarded as an artificial wetland [24]. Then it was found that soil pH, SOC, TN, CEC, and exchangeable Ca and Mg in the paddy field were similar to those in the natural wetland, which are higher than those in the dry farmland ($p>0.05$).

Soil Microbial Population and Biomass

Table 2 lists soil microbial population and biomass. The microbial category in the natural wetland, paddy field, and dry farmland was performed in the order of bacteria > actinomycetes > fungi. The bacteria number was $0.61\times10^7 \sim 4.36\times10^7$ cfu g^{-1}, with the highest proportion of 67.30 ~ 95.61% in the total soil microbial population; followed by actinomycetes, whose number and proportion were $1.44\times10^6 \sim 2.65\times10^6$ cfu g^{-1} and 5.81~18.95%, respectively. However, the fungi number was

Table 1. Soil physicochemical properties in karst wetland system (mean values).

Land-use type	pH	SOC ($g\ kg^{-1}$)	TN ($g\ kg^{-1}$)	CEC ($mol\ kg^{-1}$)	Exchangeable Ca ($mol\ kg^{-1}$)	Exchangeable Mg ($mol\ kg^{-1}$)
Paddy field	7.91 a	22.33 a	2.33 a	24.09 a	1.22 ab	1.23 ab
Natural wetland	7.87 a	21.77 a	2.28 a	19.46 a	1.44 a	1.34 a
Dry farmland	6.64 b	12.78 b	1.38 b	9.56 b	1.01 b	0.99 b

Notes: Different letters in the same column are significantly different at the 0.05 probability level.

2.92×10^4~6.52×10^4 cfu g^{-1} and the proportion was less than 1%, just only 0.14~0.38%. However, the numbers of living bacteria, actinomycetes, and fungi in the natural wetland are lower than those in the paddy field and dry farmland. The reason is that the microbial population was counted with the aerobic plate count method using a serial dilution [10]. The microorganisms from the natural wetland are adapted to anaerobic soils. Under aerobic stress, the growth and activity of anaerobic microbial communities will be inhibited. So the soil microbial population from the natural wetland sample only reflects the aerobic microbial communities and the anaerobic microbial communities were ignored. The paddy field has high microbial communities as a consequence of drought and flooding conversion from aerobic to anaerobic conditions [25-26]. The frequent changes of hydrological conditions lead to continuous stress for microorganisms in soil and thus to increased microbial populations from the paddy field using the plate count method. So the soil microbial population in the paddy field is significantly higher than that in the dry farmland ($p<0.05$).

Soil microbial biomass is closely related to soil microbial population. Mitsch et al. pointed out that natural wetlands, which are primary carbon sinks, are usually characterized by high biomass, low temperature, high humidity, weak microbial activity, and hence a low carbon dioxide release rate [15]. In our study, SMBC and SMBN in the natural wetland were higher than those in the dry farmland. Due to the paddy field with many characteristics similar to the natural wetland, the values of SMBC and SMBN in the paddy field approximated to those in the natural wetland.

Soil Enzymatic Activities

Soil enzymes play an essential role in catalyzing reactions necessary for organic matter decomposition and nutrient cycling in ecosystems [27]. Cellulase catalyzes an endohydrolysis of 1, 4-ß-D glucosidic linkages in cellulose. In our study, cellulase activity in the natural wetland and paddy field is significantly higher than that in dry farmland. Liu and Toyohara suggested that low cellulose activity in soil could be ascribed to a low level of cellulase supplied by microorganisms [28]. In the dry farmland, farmers were accustomed to removing the aboveground parts of plants, so the limited supply of plant organic matter in the soil as the enzyme substrate will retard the cellulase activity (Table 3). Alkaline phosphatase could catalyze the conversion of organic phosphorus to an inorganic one and is involved in P nutrient cycling [29]. Xia et al. proposed that extracellular phosphatase activity was high in the streambed sediments, which probably contributed significantly to the flux of phosphorus in sediment by hydrolyzing phosphomonoesters, making free phosphate available to the sediment microorganisms [30]. Hence, the alkaline phosphatase activity in the natural wetland and paddy field is significantly higher than that in dry farmland (Table 3). Urease is the enzyme responsible for the hydrolysis of urea to NH_3, which can be assimilated by microbes and plants. The rate of urea hydrolysis depends on several factors such as soil type, organic matter content, soil moisture content, $CaCO_3$ content, temperature, level of salinity, and alkalinity. Some of these factors accelerate and others retard the rate of urea hydrolysis in soils [31]. Reports of the influences of some soil properties on soil urease activities are inconsistent [32]. In our study, the urease activities have no notable variety with land-use change (Table 3).

Statistical and Canonical Correspondence Analysis

Canonical correspondence analysis and Pearson correlation analysis were used to explore the relationships

Table 2. Soil microbial population, biomass, and quotients in karst wetland system (mean values).

Land-use type	Bacteria ($\times10^7$ CFU g^{-1})	Fungi ($\times10^4$ CFU g^{-1})	Actinomycete ($\times10^6$ CFU g^{-1})	Total microbial population ($\times10^7$ CFU g^{-1})	SMBC ($mg\ kg^{-1}$)	SMBN ($mg\ kg^{-1}$)
Paddy field	4.36 a	6.41 a	2.65 a	4.56 a	267.07 a	21.26 a
Natural wetland	0.61 b	2.92 b	1.44 b	0.76 b	273.80 a	20.96 a
Dry farmland	1.42 b	6.52 a	1.93 ab	2.11 b	159.13 a	16.00 a

Notes: Different letters in the same column are significantly different at the 0.05 probability level.

Table 3. Soil enzyme activities in the Huixian karst wetland system (mean values).

Land-use type	Cellulase ($mg\ g^{-1}\ h^{-1}$)	Urease ($mg\ g^{-1}\ h^{-1}$)	APA ($mg\ g^{-1}\ h^{-1}$)
Paddy field	66.87 a	1.57 a	1.61 ab
Natural wetland	51.56 ab	1.35 a	2.07 a
Dry farmland	38.57 b	1.71 a	0.88 b

Notes: Different letters in the same column are significantly different at the 0.05 probability level.

between soil physicochemical properties, soil microflora, and enzyme activities in the karst wetland systems (Fig. 1 and Table 4). From Fig. 1, it can be seen that the CCA biplot is divided into four quadrants. The soil physicochemical properties are clustered near the center of the CCA biplot. The soil microflora and enzyme activity features appear in the left quadrant and the upper right quadrant of the CCA biplot, respectively. The relative importance of soil microflora and enzyme activity features also differed in three land-use types on the CCA biplot and was clustered on the third quadrant. Natural wetland dominates the third quadrant, the paddy field dominates the second quadrant, and dry farmland dominates the right quadrant, which suggests that the natural wetland was degraded and reclaimed as paddy field or dry farmland directly. In other words, the land-use change history did not follow the sequence: natural wetland → paddy field → dry farmland. Moreover, the conclusion was also proved by local farmers.

The bacteria population and the total soil microorganisms in topsoil had the significant positive correlation coefficient ($r = 0.999$, $p<0.01$). From the CCA results, it will be found that the angle of vectors between bacteria and total soil microorganisms has a small corner dimension. The results indicate that bacteria are the dominant population in topsoil.

In knowledge of wetland hydrology, soil pH is the key component required to understand the capacity of wetlands to function [18]. According to Table 4, the results show the significantly positive correlation between soil pH and soil organic carbon, TN, extractable Ca, extractable Mg, cellulose activity, APA activity, and SMBC (r = 0.961, 0.922, 0.644, 0.738, 0.641, 0.730, and 0.686, respectively), and the significant negative correlation between soil pH and actinomyces activity (r = -0.673). Hence, pH rise might be a key factor controlling soil property in the anaerobic experiment. Zhang et al. reported that Huixian karst wetland lake is an HCO_3-Ca-Mg type, which was based on in-situ titrating and laboratory analysis [33]. So, under the hydrochemical effect from Huixian karst wetland lake, soil pH has a positive tie with the soil-extractable Ca and Mg and their relationship also is reflected on the CCA biplot when their triangular symbols have the adjacent position.

In the alkali condition, the complex compound of Ca-SOM (soil organic matter) and Mg-SOM will be formed. Nitrogen mineralization is the important process transferred from organic nitrogen to plant-available inorganic forms by soil microorganism. Such a positive relationship between TN and SOC content appeared due to the greater stabilization of humic molecules [34]. Moreover, elevated soil pH can impede soil nitrogen mineralization in a strong alkaline environment [35]. Liu et al. pointed out that the stocks of soil organic carbon in the karst area are higher than those in the no-karst area, and karst ecosystems in southwestern China may play active roles in mitigating the increasing CO_2 concentration in the atmosphere [36]. Then, soil pH has the significantly positive correlation with SOC and SMBC, and SOC and SMBC also have the significantly positive correlation with extractable Ca and extractable Mg. Soil pH can affect microbial activity in soils. Microbial activity would be inhibited in the alkali condition [37]. Whittinghill et al. reported that microbial activity was inhibited by high Ca and Mg concentrations at alkali condition [38]. Under alkali stress, bacteria as the dominant population in topsoil can adopt the environment. When nutritional conditions are not favorable, bacterial size can be reduced and duplication time can be significantly extended in comparison to rich growth conditions, which suggest that essential cellular processes like cell division, morphogenesis, and chromosome dynamics are highly coordinated with central metabolism to ensure the production of fit progeny [39]. Therefore, in the alkali condition, the effect of pH on soil microorganisms is bacteria < fungi < actinomyces, which was reflected on the CCA biplot.

Urease is produced by soil microorganisms and released into the soil for its action. Nayak et al. reported that around 80% urease activity was extracellular and complexed by soil colloids [40]. Bowles et al. (2014) showed that urease activity decreased with increasing application of NH_3- based N fertilizers [41]. It was hypothesized that the addition of the end product of the enzymatic reaction (NH_4^+) suppressed urease synthesis. From Table 4 and Fig. 1 it will be found that urease activities have a negative relationship with soil TN. Therefore, urease and TN appear in a different quadrant. Moreover, our results correspond to Saha et al. that urease activity was decreased in the alkali condition [42]. Cellulases play an important role as a group of enzymes in global recycling of the most abundant polymer and cellulose in nature. Phosphatases play a meaningful role in P cycling, because they provide P for plant uptake by releasing PO_4 from immobile organic P. The significantly greater activity of alkaline phosphatase in manure and straw residues treated soil could be attributed to enhanced microbial activity [43]. Manure into soil also resulted in changes in origin, states, and/or persistence of enzymes in soil [44]. Due to the positive relationship between TN and SOC in topsoil, our results correspond to Saha et al. that cellulose showed a strong positive correlation with alkaline phosphatase in the alkali environment [42].

Table 4. Correlation coefficients for the soil microbial features and the soil physicochemical properties.

	pH	SOC	TN	E-Ca	E-Mg	CEC	Bacteria	Fungi	Actinomyces	T-Micor	Cellulase	Urease	APA	SMBC
SOC	0.961**													
TN	0.923**	0.958**												
E-Ca	0.644*	0.686*	0.795**											
E-Mg	0.738**	0.693*	0.779**	0.720*										
CEC	0.520	0.527	0.478	-0.037	0.220									
Bacteria	0.185	0.217	0.092	-0.293	-0.219	0.722*								
Fungi	-0.318	-0.288	-0.439	-0.680*	-0.675*	-0.018	0.392							
Actinomyces	-0.673*	-0.698*	-0.762**	-0.845**	-0.903**	-0.108	0.201	0.771**						
T-micror	0.159	0.190	0.064	-0.322	-0.250	0.712*	0.999**	0.417	0.236					
Cellulase	0.641*	0.643*	0.631*	0.316	0.293	0.368	0.252	0.321	-0.112	0.246				
Urease	-0.257	-0.336	-0.420	-0.595	-0.377	-0.065	0.100	0.420	0.411	0.114	-0.142			
APA	0.730*	0.675*	0.787**	0.647*	0.872**	0.203	-0.306	-0.474	-0.695*	-0.329	0.528	-0.199		
SMBC	0.686*	0.654*	0.757**	0.792**	0.835**	0.019	-0.222	-0.442	-0.740**	-0.248	0.533	-0.511	0.747**	
SMBN	0.366	0.466	0.584	0.725*	0.348	-0.021	-0.119	-0.267	-0.421	-0.134	0.452	-0.660*	0.290	0.710*

Notes: T-micror is Total microbial population
*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

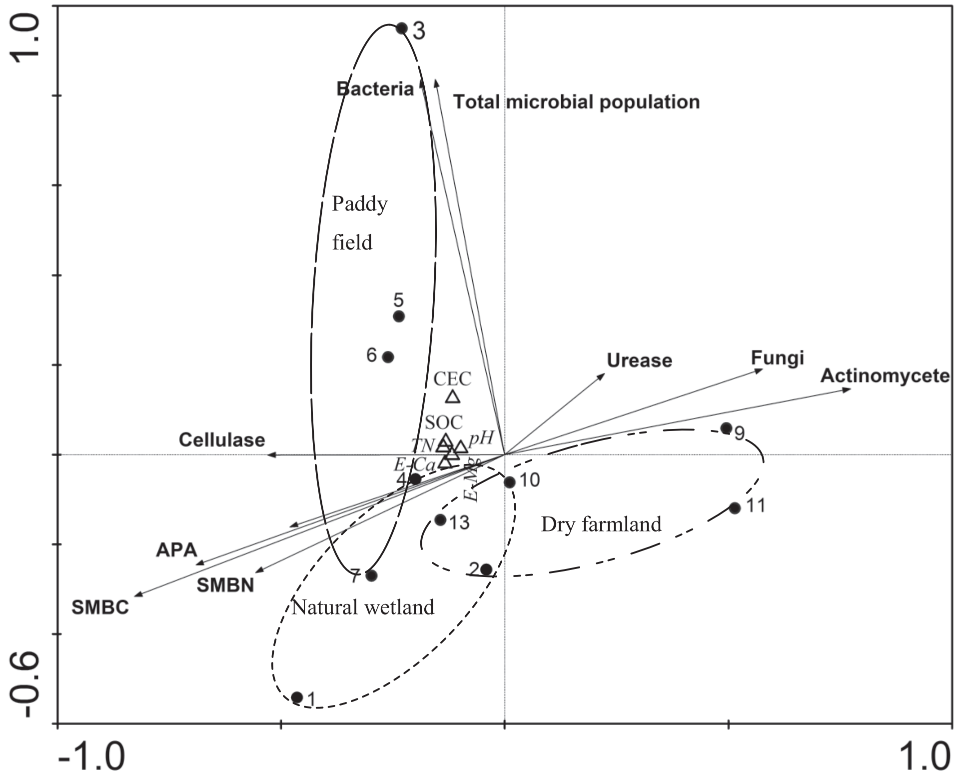

Fig. 1. CCA biplot of soil microbial features and soil physicochemical properties.
● Sample plot number

Conclusions

Wetlands play an active role in stocking soil organic carbon in a karst area. The paddy field has the similar function to natural wetland, which can be regarded as an artificial wetland class. Today, under anthropogenic impact, the natural wetland is gradually shrinking and wetland ecosystems have been damaged due to agricultural management. According to our results, the land degradation pattern was traced from natural wetland to paddy field or dry farmland directly in the Huixian karst wetland system. If a paddy field or dry farmland cannot be reversed to natural wetland due to human activities, effective management and protection in the Huixian karst wetland system is to maintain the paddy field as a substitute for natural wetland due to its similar ecosystem services, which can keep the greater stabilization of humic molecules in the alkali environment.

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