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# Distribution patterns of typical enzyme activities in tundra soils on the Fildes Peninsula of maritime Antarctica

DING Wei, WANG Qing, ZHU Renbin\* & MA Dawei

Institute of Polar Environment, School of Earth and Space Sciences, University of Science and Technology of China, Hefei 230026, China

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Abstract Soil enzyme activities can be used as indicators of microbial activity and soil fertility. In this paper, the activities of invertase (IA), phosphatase (PA) and urease (UA) were investigated in tundra soils collected from marine animal colonies, areas of human activity and background areas on Fildes Peninsula, maritime Antarctica. Soil enzyme activities were in the range of  $1.0-82.7 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  for IA,  $0.2-8.2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  for PA and  $0.2-39.8 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  for UA. The spatial distribution patterns for soil enzyme activities corresponded strongly with marine animal activity and human activity. Significantly higher soil IA and PA activities occurred in penguin colony soils, whereas seal colony soils showed higher UA activity. Statistical analysis indicated that soil IA activity was controlled by the levels of soil nutrients (TOC, TN and TP), PA activity was closely related with TP, and UA activity was affected by the soil pH. Overall, the deposition amount of penguin guano or seal excreta could impact the distribution of enzyme activities and heavy metals Cu and Zn ([IA]=0.7[TP]-0.2[Cu]+22.3[TN]+15.1, [PA]=0.3[TP]+0.03[Mc]+0.2, [UA]=16.7[pH]-0.5[Cu]+0.4[Zn]-72.6). These models could be used to predict enzyme activities in the tundra soils, which could be helpful to study the effects of marine animal activity and environmental change on tundra ecosystems in maritime Antarctica.

Keywords enzyme activity, penguin and seal colonies, environmental variables, tundra soils, Antarctica

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## **1** Introduction

Soil enzymes are a generic term for a class of polymerases including endoenzymes in living cells and extracellular enzymes in soil solution or on the surface of soil particles. Generally, there are three enzyme sources in soil systems: secretion from plant roots, microbial activity in soil and release of animal residues during decomposition processes<sup>[11]</sup>. Soil enzymes exist in solid and liquid phase and participate in the decomposition and synthesis of soil organic matter. Their activities in soils are affected by physical properties (temperature, moisture, ventilation conditions and particle compositions, etc.) and chemical properties (pH, organic matter, nutrient contents of nitrogen and phosphorus, etc.), and they are often regarded as an indirect indicator of microbial activity and soil fertility<sup>[2-3]</sup>.

The majority of the biochemical reactions in soils are driven by enzymes<sup>[4]</sup>. Invertase is an important enzyme in the regulation of carbon cycles as it catalyzes the hydrolysis of sucrose<sup>[5]</sup>. Phosphatase plays an important role in the biological liberation of phosphorus in soil systems, and directly affects the decomposition and transformation of soil organic phosphorus and its bioavailability<sup>[6-7]</sup>. Urease catalyzes the hydrolysis of urea into carbon dioxide and ammonia, and its activity tends to increase the soil pH values because of the production of ammonia from the biochemical reactions<sup>[8]</sup>. Soil enzyme activity has been extensively studied in different types of soils<sup>[9-11]</sup>. However, their activity and influencing factors have received little attention in the soils of polar regions. In Antarctica, some ice-free coastal

<sup>\*</sup> Corresponding author(email: zhurb@ustc.edu.cn)

zones are identified as "sea animal colonies" because of the colonization by a large number of sea animals like penguins and seals<sup>[12]</sup>. Soil physical and chemical properties in the colonies are strongly impacted by animal excreta through the effects of microbes. The special soil within seabird colonies is described as ornithogenic soil because of the presence of organic materials including guano, feathers and eggshells<sup>[13]</sup>. The ornithogenic soil is particularly rich in organic carbon, nitrogen, and phosphorus, although the background soil can be barren because of the weak weathering and the absence of vegetation<sup>[14-15]</sup>.

In this study, soil samples were collected from marine animal colonies, areas of human activity and background areas on the Fildes Peninsula of maritime Antarctica, and three kinds of soil typical enzymes (invertase (IA), phosphatase (PA) and urease (UA)) and soil chemical properties (organic carbon, total nitrogen, phosphorus fractions and other environmental variables) were analyzed. The objectives of this paper were: (1) to detect the distribution pattern of soil enzyme activity in maritime Antarctica; (2) to investigate the factors affecting soil enzyme activity; (3) to establish the relationship models between soil enzyme activities and environmental variables.

### 2 Materials and methods

#### 2.1 Study area

The study area was on the Fildes Peninsula ( $61^{\circ}51'S-62^{\circ}15'S$ ,  $57^{\circ}30'W-59^{\circ}00'W$ ), in the southwest of King George Island, covering an area of about 30 km<sup>2</sup>, which belongs

to the so-called maritime Antarctica<sup>[16]</sup>(Figure 1). This area is characterized by oceanic climate. According to the meteorological data from the Chinese Antarctic Great Wall Station, mean annual temperature was about -2.51°C, ranging from -26.6°C to 11.71°C, and mean annual precipitation was 630 mm mainly in the form of snow<sup>[17]</sup>. This peninsula is an important sea animal colony. According to annual statistical data, a total of over 10 700 sea animals colonize this peninsula every summer. On the western coast are some established colonies of marine mammals, including five pinnipeds of Weddell seal (Leptonychotes weddellii), elephant seal (Mirounga leonina), leopard seal (Hydrurga leptonyx), fur seal (Arctocephalus gazella) and crabeater seal (Lobodon carcinophagus). Of these seals, the elephant seal is the most abundant (71% of the total seal population), followed by the fur seal with a population of 1 590 (14%). The Great Wall Station is located on the eastern coast. Ardley Island is connected with Fildes Peninsula by a sand dam, covering an area of about 2.0 km<sup>2</sup>. It is one of the most important penguin colonies in maritime Antarctica, and encompasses more than 90% of all penguins on the Fildes Peninsula<sup>[17]</sup>. It is of particular importance for the breeding colonies of Gentoo penguins (Pygoscelis papua), Adélie penguins (Pygoscelis adeliae) and Chinstrap penguins (Pygoscelis antarctica). During the molting and breeding period each summer, penguin guano and feathers are deposited into tundra soils or catchment sediments by snow-melt water<sup>[17]</sup>. Mosses and lichens are the dominant vegetation on this island. However, there is limited vegetation in these colonies because of overmanuring and penguin or seal trampling, and only some coprophilic algae grow there.



**Figure 1** The sampling sites for the tundra soils on Fildes Peninsula and Ardley Island, maritime Antarctica. Note: One penguin colony (PC1-PC5), one seal colony (SC1-SC5 and SH1), human-activity areas (WD, AR, OS and WS) and the background tundra areas (MR, BP, NR2 and SW1) were investigated on Fildes Peninsula and its adjacent area.

#### 2.2 Sampling description

In the summer of 2011/2012, one penguin colony (PC), one seal colony (SC), the human activity areas (AA) and background tundra areas (BA) were investigated on Ardley Island and Fildes Peninsula in maritime Antarctica. The human activity areas included the refuse dump (WD) and sewage farm (WS) near the Great Wall Station, the oilcans near the Russian Antarctic Bellingshausen Station(OS), and the Chilean airport (AR). The background areas were located in the tundra on the top of the hill (MR), the valley tundra (BP), the tundra on Nelson Island (NR), and the slope tundra in Biological Bay (SW). The sampling sites were illustrated in Figure 1. In total, five penguin colony soil samples (PC1-PC5), six seal colony soil samples (SC1-SC5 and SH1), four human activity area soil samples (WD, AR, OS and WS) and four background tundra soil samples (MR, BP, NR2 and SW1) were collected to study the effects of marine animal activity and human activity on soil enzyme activities in maritime Antarctica (Table 1). The 0–10-cm surface soil samples were collected using a clean bamboo scoop, and stored in clean plastic bags. The sediment cores were maintained intact. Immediately after collection, all samples were completely sealed and stored in the dark at  $-20^{\circ}$ C until laboratory analysis. All the samples were mixed homogeneously and divided into two portions in sequence. One portion was used to analyze enzyme activities and the other portion was used to determine other physicochemical properties of the soils after freeze-drying.

#### 2.3 Measurement of soil enzyme activity

The activities of the various soil enzymes were based on the release and quantitative determination of the product in the reaction mixture when soil samples were incubated with substrate and buffer solution. Invertase (IA) activity:

						51					
Sites	Latitude	Longitude	Mc/ %	pН	TN / %	TOC / %	$TP / (g \cdot kg^{-1})$	Cu/ (mg·kg <sup>-1</sup> )	$Zn/(mg\cdot kg^{-1})$		
Penguin colony (PC)											
PC1	62°12′49.5″S	58°56′02.5″W	62.0	5.2	0.70	5.33	20.72	150.29	50.07		
PC2	62°12′56.5″S	58°55′59.0″W	49.5	5.4	0.50	3.83	8.13	59.87	88.26		
PC3	62°12′57.0″S	58°55′23.4″W	60.8	6.1	1.85	11.41	20.70	138.67	142.14		
PC4	62°12′57.1″S	58°55′22.1″W	70.4	6.4	3.43	18.60	19.36	466.43	512.26		
PC5	62°12′39.9″S	58°55′37.6″W	72.0	5.9	2.93	17.88	34.78	251.11	266.64		
Mean			62.9a	5.8a	1.88a	11.41a	20.74a	213.27a	211.87a		
Seal colony (SC)											
SC1	62°12′36.1″S	59°00′43.3″W	22.1	7.1	0.13	0.30	0.86	110.96	67.41		
SC2	62°12′36.8″S	59°00′44.3″W	26.9	6.7	0.51	1.93	1.67	90.83	109.80		
SC3	62°12′37.3″S	59°00′44.4″W	31.9	6.4	0.44	1.65	2.36	79.54	74.01		
SC4	62°12′37.7″S	59°00′44.3″W	34.5	7.5	1.91	9.08	7.37	97.24	148.97		
SC5	62°12′37.7″S	59°00′44.6″W	40.2	7.5	2.41	12.35	8.21	112.46	152.49		
SH1	62°12′21″S	58°55′58.2″W	20.2	6.6	0.41	1.18	2.18	66.13	52.98		
Mean			29.3b	7.0a	0.97b	4.42b	3.78b	92.86b	100.94b		
Anthropogenic area (AA)											
WD	62°12′59.4″S	58°57′39.4″W	6.3	7.6	0.09	0.16	1.07	83.62	69.34		
AR	62°12′37.8″S	58°59′39.2″W	60.9	6.7	0.15	0.49	2.93	96.22	115.04		
OS	62°11′59.4″S	58°56′18.3″W	17.5	6.6	0.12	0.33	1.26	96.47	101.02		
WS	62°12′53.1″S	58°57′37.9″W	54.2	7.0	1.51	6.54	5.93	148.01	141.62		
Mean			34.7b	7.0a	0.47c	1.88c	2.80c	106.08b	106.76b		
Background tundra area (BA)											
MR	62°13′5.04″S	58°58′50.1″W	17.6	7.2	0.26	1.53	1.52	57.67	94.36		
BP	62°12′10.3″S	59°00′46.1″W	15.6	7.0	0.19	0.54	0.58	122.54	59.55		
NR2	62°15′1.55″S	58°58′11.7″W	22.8	5.9	0.16	0.59	2.00	51.09	72.39		
SW1	62°12′55.0″S	59°00′4″W	14.3	7.5	0.13	0.20	0.34	134.57	51.91		
Mean			17.6b	6.9a	0.19d	0.72d	1.11d	91.47b	69.55c		

 Table 1 Physicochemical properties for the different types of tundra soils

Notes: Mc: soil gravimetric moisture; TOC: total organic carbon; TN: total nitrogen; TP: total phosphorus. The different lowercase letters indicate statistically significant differences between means within these areas.

Soils (2.5 g) were added into 25-mL Erlenmeyer flasks and then treated with 0.1 mL of toluene, 5 mL of pH 6 modified universal buffer, and 5 mL of 5% sucrose solution. IA activity was measured using the 3,5-dinitrosalicylic acid method<sup>[19]</sup>. Phosphatase (PA) activity: Soils (2.5 g) were weighed into 100-mL volumetric flasks. The reaction mixture consisted of 2.5 mL of benzene disodium (25 mg·mL) and 2.5 mL borate buffer. PA activity was determined by the release of *p*-nitrophenol from *p*-nitrophenyl phosphate (only neutral phosphatase activity was determined because of pH ranges from 6 to 8)<sup>[18]</sup>. Urease (UA) activity: UA activity was qualitatively determined by the amounts of ammonia in the solution based on the indophenol reaction. The reaction mixture contained 0.18 M potassium phosphate buffer (pH 7.0), 50 µmol of urea, and a suitable amount of the enzyme solution in a final volume of 1.0 mL<sup>[20]</sup>. All the samples were incubated for 24 h at 37°C, then filtered and measured spectrophotometrically at 578 nm. Soil enzyme activity was expressed as mg·kg<sup>-1</sup>·h<sup>-1</sup>.

#### 2.4 Analyses of soil properties

Soil pH was determined with an ion selective electrode using a soil-to-water ratio of 1:3 (W/V). Soil gravimetric moisture content (Mc) was determined by drying the soil at 105°C for 12 h, and calculated as: Mc=(mass before drying–mass after drying)/(mass after drying×100%). Total organic carbon (TOC) and total nitrogen (TN) in the soils were measured with the CNS Elemental Analyzer (Vario EL III, Elementar Analysen Syetem GmbH, Germany) with a relative error of 0.1%. Total phosphorus (TP) was measured by the ammonium molybdate spectrophotometric method. For the analyses of other chemical elements including Cu and Zn, samples digested by multi-acids (HNO<sub>3</sub>–HF–HCIO<sub>4</sub>) were analyzed by an Inductively Coupled Plasma Optical Emission Spectrometer (Optima 2100 DV), where the relative error was less than  $1\%^{[18]}$ .

#### 2.5 Statistical analysis

The mean values and standard deviation (mean±sd) were calculated to facilitate comparisons of the data between different samples. The relationships between enzyme activities and primary properties (Mc and pH), biogenic elements (TOC, TN and TP) and heavy metals (Cu and Zn) were analyzed using linear regression analysis, particularly multiple regression analysis to predict the enzyme activities. The factors tested and the relationships were considered statistically significant where p<0.05. Differences in mean enzyme activities and mean concentrations of environmental parameters between different types of soils were tested with Student's *t*-test at p=0.05. All statistical analyses were performed using Microsoft Excel 2007, SigmaPlot 12.0 (Systat Software International, USA) and SPSS 16.0 (IBM, USA) for Windows XP.

## **3** Results and discussion

#### 3.1 Soil physicochemical properties

As summarized in Table 1, overall tundra soil moisture (Mc, expressed on a weight basis) ranged from 6.3% to 72.0% in maritime Antarctica, and the highest Mc occurred in the soils of the penguin colony tundra. The pH ranged between 5.2 and 7.6 with an average of 6.6. Compared with seal colony soils and other types of tundra soils, penguin colony soil pH was slightly acidic, which could be attributed to the production of nitric and sulfuric acid during the mineralization processes of penguin guano<sup>[21-23]</sup>. The concentrations of TN (0.09%–3.4%), TOC (0.2%–18.6%) and TP (0.3–34.8 g·kg<sup>-1</sup>) showed the same distribution patterns in tundra soils, and they were significantly correlated with each other (Table 2). The mean contents of soil nutrients decreased in the order of penguin colony soils>background tundra soils in maritime Antarctica. Our results

Variables	IA	PA	UA	Mc	pН	TN	TOC	TP	Cu	Zn
IA	1	0.76**		0.66**		0.37*	0.34*	0.70**	$0.50^{*}$	0.63**
PA		1	0.51**	$0.80^{**}$	$-0.50^{*}$			$0.90^{**}$	$0.58^{**}$	$0.52^{*}$
UA			1							
Mc				1		0.73**	0.76**	0.82**	0.71**	0.56**
pН					1			$-0.54^{*}$		
TN						1	0.98**	0.76**	$0.78^{**}$	0.83**
TOC							1	0.84**	$0.79^{**}$	0.82**
TP								1	0.62**	0.58**
Cu									1	0.84**
Zn										1

Table 2 Correlation between soil enzyme activities (IA, PA and UA) and environmental variables at the sampling sites

Notes: Only statistically significant correlation coefficients were shown in this table. \* and \*\* indicate correlation significant at the 0.05 and 0.01 level, respectively. IA: invertase activity; PA: phosphatase activity; UA: ureases activity; Mc: soil gravimetric moisture content; TOC: total organic carbon; TN: total nitrogen; TP: total phosphorus.

indicated that penguin activity might have a stronger effect on soil chemical properties than seal activity and human activity in our study area.

In all the soil samples, the concentrations of Cu and Zn ranged from 51.1 to 466.4 mg·kg<sup>-1</sup> and 50.1 to 512.3 mg·kg<sup>-1</sup>, respectively, and the highest levels occurred in penguin colony soils. Additionally, the concentrations of Cu and Zn showed significant positive correlations with Mc, TN, TOC and TP in these soil samples (Table 2). In our study area, the biological and chemical weathering processes are weak because of severe climatic conditions and exposure to the bedrock, and soils are generally devoid of nutrients<sup>[24]</sup>. Our results further confirmed that penguin guano, seal excreta and the soils impacted by sea animal excreta were generally rich in nutrients, and they were important sources for soil nutrients in the local terrestrial ecosystems, similar to previous reports<sup>[22-23]</sup>.

#### 3.2 Distribution of soil enzyme activities

Soil enzyme activities (IA, PA and UA) showed various distribution patterns in the four different soil types (Figure 2). PA showed significant positive correlations (p < 0.01) with IA and UA when all the data were combined (Table 2). Soil IA, PA and UA ranged from 1.0 to 82.7 mg·kg<sup>-1</sup>·h<sup>-1</sup>, from 0.2 to 8.2 mg·kg<sup>-1</sup>·h<sup>-1</sup> and from 0.2 to 39.8 mg·kg<sup>-1</sup>·h<sup>-1</sup>, respectively, in maritime Antarctica. IA and PA activities decreased in the order of penguin colony soils  $(33.48 \pm 11.48 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}, 6.16 \pm$ 1.32 mg·kg<sup>-1</sup>·h<sup>-1</sup>, respectively)>seal colony soils  $(8.91\pm$ 4.92 mg·kg<sup>-1</sup>·h<sup>-1</sup>, 2.08±0.56 mg·kg<sup>-1</sup>·h<sup>-1</sup>, respectively)  $\approx$ anthropogenic area soils (11.28±5.55 mg·kg<sup>-1</sup>·h<sup>-1</sup>, 1.22± 0.49 mg·kg<sup>-1</sup>·h<sup>-1</sup>, respectively)>background tundra soils  $(3.49\pm1.87 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}, 0.80\pm0.28 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}, \text{respectively}).$ IA and PA activities were significantly higher in penguin colony soils than in seal colony soils. However, UA activity was much higher in seal colony soils (22.50±5.62 mg·kg<sup>-1</sup>·h<sup>-1</sup>) compared with penguin colony soils, indicating the existence of different biochemical processes from IA and PA in the soil systems. Specifically, much higher enzyme activities occurred in sites PC3, PC4, PC5, SC4 and SC5 because these sampling sites were closer to the penguin or seal nests than any other sites (Figure 1). This indicated that the deposition amount of penguin guano or seal excreta could impact the distribution of enzyme activity in Antarctic tundra soils. It was found that the activities of proteases, phosphatase, urease and xylanase were also high in the soils of penguin colonies<sup>[25-26]</sup>. These enzymes might be secreted disproportionately from microbes, such as algae and bacteria<sup>[27]</sup>, or could have originated from penguin or seal gut material and fecal organisms<sup>[25]</sup>. Further research is needed to elucidate the sources of these enzymes in penguin or seal colony soils in the future.

# **3.3** Effects of environmental variables on soil enzyme activities

Tundra soil IA and PA activities showed positive correlations



**Figure 2** Distributions of invertase (IA), phosphatase (PA) and urease (UA) for the different types of soils. Note: PC, SC, AS and BS indicate the soils for penguin colony, seal colony, humanactivity area and background tundra area, respectively, maritime Antarctica.

(p < 0.05, Figure 3 and Table 2) with TN, TOC and TP levels in maritime Antarctica, indicating that soil organic C, N and P levels were predominant factors affecting IA and PA activities, consistent with previous results<sup>[28-29]</sup>. The soil physiochemical properties were strongly influenced by marine animal activity and their excreta in coastal Antarctica. The enrichment in soil OC, TN and TP stimulated microbial population abundance and the activity of IA and PA in the tundra soils, and thus IA and PA could be used as indicators of soil fertility in Antarctica<sup>[11,29]</sup>. The TN, TOC and TP levels were much higher in penguin colony soils than in seal colony soils, suggesting that penguin activity and the deposition of penguin guano had more significant effects on IA and PA than seal activity and seal excreta in our study area. When all the data were combined together, PA activity showed a strong positive correlation (r=0.90, p<0.001) with TP because soil PA was affected by soil phosphorus levels and bioavailability<sup>[30]</sup>. UA activity had no significant correlations



Figure 3 Effects of soil TN, TOC and TP levels on IA, PA and UA in maritime Antarctica. Note: IA, PA and UA indicate that the activities of invertase, phosphatase and urease in tundra soils. The *r* and *p* represent Spearman's rank correlation coefficient and the significant level (2-tailed) between enzyme activity and environmental parameters, respectively. Correlation is significant at the 0.05 level with p < 0.05.

with soil TOC, TN and TP levels, indicating that the nutrient levels in ornithogenic soils might not significantly influence soil UA activity in Antarctica (Figure 3). Ma et al.<sup>[30]</sup> studied ex situ enzyme activity through soil depth profiles in penguin and seal colonies in Vestfold Hills, East Antarctica, and found that the activities of IA and PA at different soil depths showed a significant positive correlation with soil TOC and TN. Soil nutrients were predominantly derived from penguin guano or seal excreta, indicating that the deposition amount of penguin guano or seal excreta could impact the vertical distribution of enzyme activity through soil depth profiles. A 3-year experiment in an Antarctic dry valley showed that soil respiration rates and the activities of soil  $\beta$ -glucosidase, acid and alkaline PA were significantly increased by C and N supplementation, compared with control soils without C and N addition<sup>[31]</sup>, which was in agreement with our results.

The ornithogenic soils are important OC and N reservoirs in Antarctic terrestrial ecosystems<sup>[16-17]</sup>. IA is

an important enzyme for regulating carbon cycling by catalyzing the hydrolysis (breakdown) of sucrose<sup>[4-5]</sup>. In this study, the significant correlation between IA activity and TOC contents indicated that soil IA activity might have an important effect on tundra carbon cycles in maritime Antarctica. PA is involved in regulating P cycling and catalyzes the hydrolysis of organic esters and anhydrides via the following reaction: Phosphate+ $H_2O \rightarrow ROH + H_3PO_4$ . The decomposition and transformation of soil organic phosphorus and its bioavailability are directly affected by PA activity<sup>[6-7]</sup>. Therefore, PA activity plays an important role in the biological liberation of P in Antarctic soil systems. UA, which belongs to the superfamily of amidohydrolases and phosphotriesterases, is an enzyme that catalyzes the hydrolysis of urea into carbon dioxide and ammonia through the reaction:  $(NH_2)_2CO+$  $H_2O \rightarrow CO_2 + 2NH_3$ . UA catalyzes the hydrolysis of urea to produce ammonia and carbonate<sup>[8]</sup>, and its activity tends to increase the pH in the environment because of the ammonia

produced. High UA activity generally exists in neutral soils. In our study area, penguin colony soil pH was acidic because of the production of nitric and sulfuric acid during the mineralization processes of penguin guano<sup>[23-25]</sup>. Penguin colony soils did not show high UA activity in maritime Antarctica.

The relationships between enzyme activities, Mc and pH are shown in Figure 4. IA and PA activity in soils showed significant positive correlations with Mc (p<0.001), and weak or significant negative correlations with pH. A simulated experiment has confirmed that a moderate increase in moisture content would contribute to soil enzyme activities<sup>[32]</sup>, which was consistent with our results. Therefore the melting of glaciers and permafrost in maritime Antarctica because of global climate warming might enhance soil enzyme activity showed a weak positive correlation with pH and a weak negative correlation with Mc. Every soil enzyme has an optimum pH range (i.e. 6.5–7.0 for UA activity)<sup>[33]</sup>. Soil UA activity increased with the increase of pH in Antarctic soils in this study.

IA and PA activity showed positive correlations with Cu (p=0.036 and p=0.01, respectively) and Zn (p=0.005 and p=0.02, respectively) contents in the soils (Figure 4). UA had no significant correlations with Cu and Zn levels in all the soil samples (Table 2). The monitoring results of soil IA, PA,

UA and dehydrogenase also showed great disparities because of the differences in enzyme types and soil properties<sup>[34]</sup>. Generally Cu and Zn were significantly enriched in penguin guano or seal excreta, their levels in the soils were significantly affected by the input amount of sea animal excreta<sup>[11]</sup>. Stimulation or inhibition effects of heavy metals on soil enzymes were mainly through their enzyme reactions, as a prosthetic group to stimulate the reaction or occupying the active center to inhibit the reaction<sup>[30]</sup>.

We further analyzed the relationships between soil enzyme activities and environmental variables using multiple stepwise regression analysis. The following regression models were obtained between enzyme activities and environmental variables:

[IA]=0.7[TP]+22.3[TN]-0.2[Cu]+15.1 (*r*=0.54, *F*=5.2, *p*=0.005);

[PA]=0.3[TP]+0.03[Mc]+0.2 (*r*=0.91, *F*=36.7, *p*<0.001);

[UA]=16.7[pH]-0.5[Cu]+0.4[Zn]-72.6 (*r*=0.70, *F*=11.3, *p*<0.001).

According to the multiple stepwise regression models above and the simple linear analysis (Figures 3 and 4), IA activity was related to TP and TN levels, indicating it was controlled by TP and TN levels in the Antarctic soil and could be used as an indicator of soil fertility. PA activity was related to TP, indicating that PA activity was controlled by TP levels



**Figure 4** Effects of other environmental variables (Mc, pH, Cu and Zn) on IA, PA and UA in maritime Antarctica. Note: IA, PA and UA indicate that the activities of invertase, phosphatase and urease in tundra soils. The *r* and *p* represent Spearman's rank correlation coefficient and the significant level (2-tailed) between enzyme activity and other parameters, respectively. Correlation is significant at p < 0.05.

in the Antarctic soils, and it could be used as an indicator for soil P levels. UA activity was mainly affected by the pH in the soils. According to the models, the regression values of enzyme activities showed strong positive correlations (p < 0.01) with their measurement values, suggesting that the models above could be used to predict soil enzyme activities in coastal Antarctica.

### 4 Conclusions

The results are summarized as follows:

(1) Penguin activity had more important influences on soil invertase and phosphatase activities, whereas seal activity had more important influences on soil urease activity. Overall higher activities of invertase, phosphatase and urease occurred in the soils closer to the penguin or seal colony sites in maritime Antarctica.

(2) Soil invertase and phosphatase activities were both stimulated by the contents of biogenic elements (carbon, nitrogen and phosphorus), but urease activity was mainly affected by the soil pH.

(3) The multiple stepwise regression analysis confirmed that invertase activity was controlled by TP and TN levels, phosphatase by TP levels, and urease by soil pH, indicating that invertase and phosphatase activities could be used as indicators for soil nutrient levels in maritime Antarctica.

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