



## Response of phytoliths in *Phragmites australis* to environmental factors in northeast China



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### ABSTRACT

Accuracy of paleovegetation reconstruction and understanding of phytolith formation would both be improved by further study of phytolith size in the *Phragmites australis* under different environmental conditions. Leaves of *P. australis* were collected from 11 sampling sites in northeast China with differences in temperature, precipitation and habitat. Principal component analysis of environmental factors (climatic and edaphic) indicated that the annual averages of temperature and precipitation were the main factors influencing phytolith size. Moreover, three-way analyses of variance (ANOVAs) further showed that phytolith size differed significantly under conditions of different temperature or precipitation gradients, whereas habitat differences had little effect. The changes in phytolith size with temperature differed in the humid, semi-humid and semi-arid areas of northeast China. In the humid and semi-humid areas, moving from the temperate to the warm temperate zone, increasing temperature reduced phytolith size; whereas in the semi-arid area, phytolith became larger with increasing temperature. In the warm temperate and temperate zones, the changes of phytolith size with precipitation showed the same trend—moving from the semi-arid to semi-humid to humid areas, as precipitation increased, phytolith grew larger. Finally, ANOVA revealed that phytoliths were also sensitive to habitat. These findings demonstrated that the size of *P. australis* phytoliths was sensitive to environmental factors: for regional research, the annual averages of temperature and precipitation were the major factors influencing size, but in the same climate district, habitat differences seemed to also have a significant impact on phytolith size. Consequently, phytolith analysis has potential utility in the study of global climate change, palaeoenvironment reconstruction, and environmental conservation and restoration.

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### 1. Introduction

Phytoliths are specific forms of silicon dioxide minerals that precipitate in or between the cells of living plant tissues (Wang and Lu, 1992). In recent years, phytolith analysis has been widely used in archaeology and Quaternary geology as a potential indicator of the paleoenvironment, and has become an important method of reconstructing paleoclimate, paleogeography and paleovegetation (Lu et al., 1996, 2007; Prebble et al., 2002; Iriarte and Paz, 2009). Because phytoliths precipitate in or among the cells of living plant tissues, their morphology is controlled by the plant

tissues themselves, and the plant tissues are influenced, in turn, by the environment (Madella et al., 2009; Jie et al., 2010a; Liu et al., 2013a,b). Plants take up silicon predominantly in the form of monosilicic acid. However, their capacities for silicon absorption vary across species, and are controlled by plant physiological activities, including photosynthesis, respiration and transpiration. Phytoliths are the products of plant physiological activities, and changes in phytoliths can directly reflect variations in plant physiological activities and environment (Li, 2010a). Therefore, the research on the relationship between plant physiological activities and phytoliths could offer new ways to study the phytolith formation.

Research into the relationship between plant physiological activities and environment has become extremely important with the changes in global climate. Previous studies that focused on

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*Phragmites australis* indicated that temperature and precipitation affected the photosynthetic rate, transpiration rate and water use efficiency, and therefore plant biomass (Peter and Kaj, 2006; Wu et al., 2008; Suman et al., 2011; Gong et al., 2011; Xu et al., 2012, 2013). Sinclair et al. (2005) showed in simulations of sorghum growth in four locations in Australia that imposition of a maximum transpiration rate at high precipitation resulted in yield increases of about 75.0% during the growth period of plant. Similarly, Sinclair et al. (2010) found for soybean that grain yield was increased in over 70.0% of the plant growth period for most locations in the US. It has also been reported (Chen and Zhang, 1991; Zhang and Chen, 1991; Davies et al., 1994) that habitat differences can markedly influence photosynthetic and transpiration rates. Consequently, changeable growth conditions affect plant cell shape.

Several studies have examined the relationship between phytoliths and the environment – including temperature, humidity, soil pH and atmospheric carbon dioxide (CO<sub>2</sub>) concentration – and found that phytoliths were sensitive to environment (Ge et al., 2010; Jie et al., 2010a,b; Liu et al., 2013b). Lu et al. (2006) investigated 243 surface soil phytolith samples from China and used canonical correspondence and detrended correspondence analyses to determine the main environmental variables influencing phytolith distribution; they suggested that mean annual precipitation, relative humidity, mean annual temperature and annual evaporation were the four dominant variables, accounting for 60.0% of the total variance. Liu et al. (2013b) analyzed the quantity and size of *P. australis* phytoliths from five sampling sites distributed across three climatic zones in northeastern China, each zone representing a different level of humidity, and showed that the quantity and size of phytoliths differed markedly across the five sampling sites. Studies by Ge et al. (2010) revealed that the elevation of CO<sub>2</sub> concentration significantly changed the contents and sizes of *Leymus chinensis* phytoliths. However, there is no consensus on the phytolith formation in response to different environmental factors.

Aquatic macrophytes are widely distributed in various wet environments, from fresh to salt water. *P. australis* (Cavanilles) Trinius ex Steudel., belonging to the Poaceae family, is one of the most common aquatic plants living in wetland ecosystems. This plant, which can grow up to 6 m high, can withstand extreme environmental conditions, such as the presence of toxic contaminants. Given its capacity of absorption and tolerance to contaminated environments, *P. australis* has thus been used for years for water treatment through phytoremediation and eco-environment restoration (Topal, 2015). Simultaneously, *P. australis* phytoliths are specific forms of silicon dioxide minerals that precipitate in or between the cells of living *P. australis* tissues, to some extent, which would reflect changes in the environment. Due to the widespread distribution of *P. australis* and the close relationship between phytolith and the environment (Wang and Lu, 1992), this study investigated the relationship between *P. australis* phytolith size and environment in northeast China. This relationship was used to offer new ways to understand the environmental implications of phytolith and increase understanding of phytolith formation. This study should provide a reference point for reconstruction of palaeoenvironment and palaeoclimate, and a new way of addressing research into global climate change and environmental conservation and restoration.

## 2. Study area

The study site in northeast China is located at 39°40'N–53°30'N, 115°05'E–135°02'E (Fig. 1). The region can be divided into a cold temperate zone, a temperate zone, and a warm temperate zone from north to south, and a humid area, a semi-humid area, and a semi-arid area from east to west. Northeast China has four distinct

seasons, with a long winter and a short summer. The annual average temperature ranges from –4.0 to 11.0 °C. The average annual precipitation, which is concentrated in the period from July to September, and represents 70.0% of the yearly total, ranges from 1000.0 mm in the east to 350.0 mm in the west (Zhao et al., 2011). *Larix gmelinii* predominates in the cold temperate zone; needled and broad-leaved mixed forest covers the eastern part of the temperate zone; and *Pinus tabulaeformis* Carr is found in the Liaoning Hills in the warm temperate zone. The influence of the monsoon is obvious at 115–135°E by the presence of needled and broad-leaved mixed forest, meadow steppe, and steppe. The main soil types in northeast China are brown coniferous forest soils in the cold temperate zone, dark brown forest soil in the temperate zone, and forest steppe chernozem and meadow steppe chernozem in the temperate zone (Sun et al., 2006).

## 3. Materials and methods

### 3.1. Experimental design

Eleven sampling sites were selected along the precipitation gradient between 115° and 135°E and the temperature gradient between 39° and 53°N (Fig. 1). According to the comprehensive physical regionalization of China (Huang, 1989; Zhao, 1983), the 11 sampling sites could be divided into two sections from south to north along the temperature gradient. These two sections are the warm temperature zone (Dandong–Panjin–Tongliao) and the temperate zone (Longwan–Changchun–Changling, Mudanjiang–Harbin–Daqing, and Tongjiang–Beian–Nehe) (Fig. 1, Table 1). In each section, there are obvious precipitation differences among the sampling sites (Fig. 1). The sampling sites represent three levels of precipitation and two levels of temperature. Five *P. australis* samples were collected from aquatic habitat (W) and from xerophytic habitat (T) respectively (Fig. 2) at each sampling site in September, 2012. And the experimental warming treatment was performed at the experimental station of the Northeast Normal University in Changling County in the Songnen Grassland, China (Jie et al., 2010a).

### 3.2. Extraction methods

The phytoliths are isolated using wet ashing (Wang and Lu, 1992), as follows. (1) Cleaning: from top to bottom, in turn, the third or fourth leaf of each plant sample is selected to reduce the experimental error. Each leaf is added to a test tube, to which distilled water is added. The leaves are then cleaned in an ultrasonic shaking instrument to remove any soil contamination. (2) Oxidization: the five dry clean leaves from each site are cut into small pieces and mixed. A 0.2 g sample of the dry clean leaves is added to a test tube and mixed with concentrated nitric acid until the organic matter is fully oxidized. (3) Centrifugation and cleaning: to dilute the acid, distilled water is added and the mixture is centrifuged three times at 2000 rpm for 20 min. Absolute ethanol is then added to the test tube and the mixture is centrifuged at 2000 rpm for 20 min. (4) Slide preparation: the liquid is shaken well and 1–3 drops of it is placed on a glass microscope slide, which is heated over a spirit lamp until all the ethanol has evaporated. Canada balsam oil (1–2 drops) is added and a cover slip placed on top. (5) Identification: the samples are examined and measured under a MOTIC biomicroscope (DMBA 300, MOTIC China Group Co., Ltd., China) at a magnification of 900 ×. More than 300 phytolith particles are counted for each sampling slide. Only phytoliths with a diameter >10.0 μm and that could be taxonomically identified are counted.

The physicochemical properties of soil (pH, anions, cations, etc.) were all determined by following the methods described in the

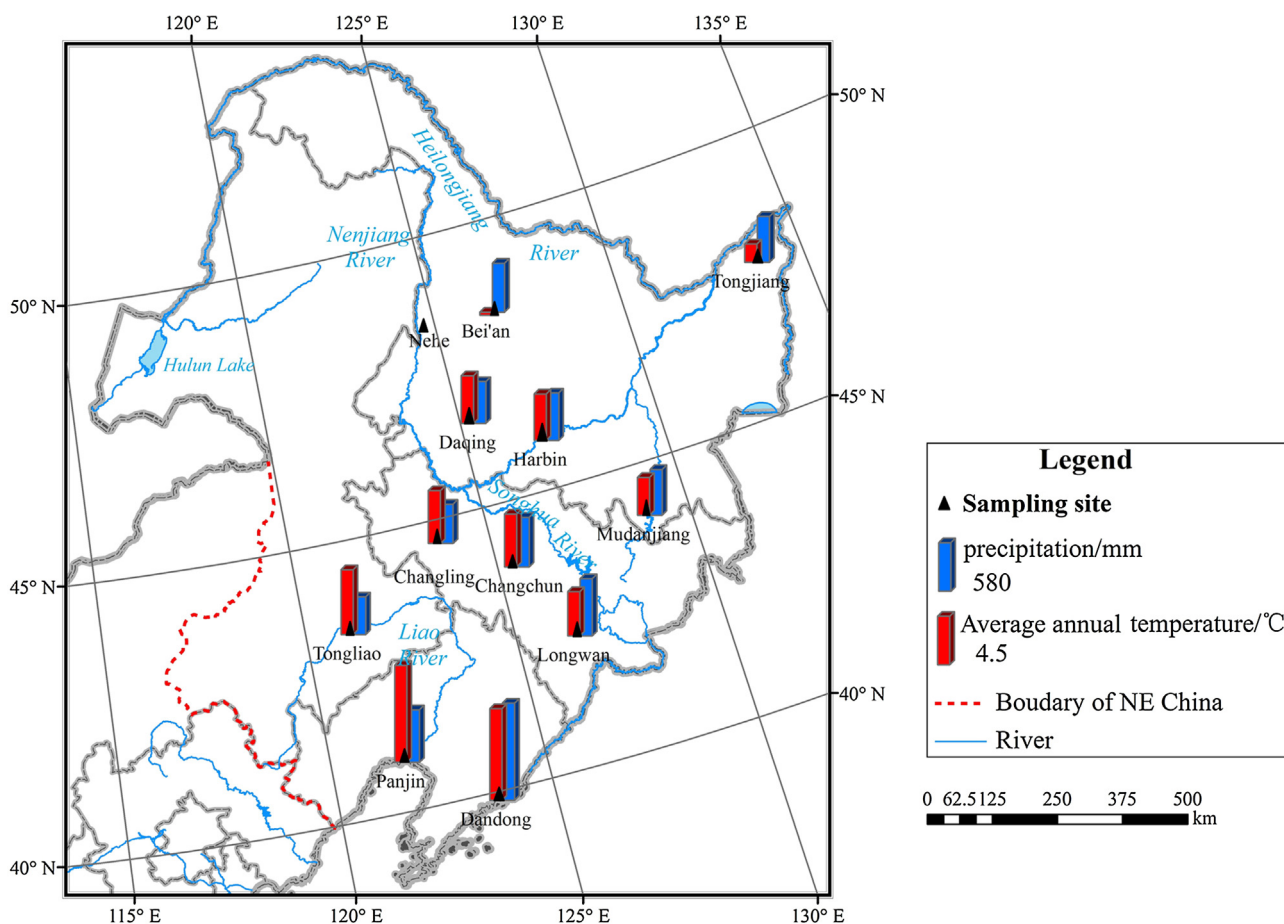


Fig. 1. Geographical location and environment description of sampling sites.

book *Soil Agricultural Chemistry Analysis* (Bao, 2010). The physical composition was tested using a laser diffraction particle size analyzer (Microtrac S3500, Montgomeryville, Pennsylvania, USA) which can measure particle sizes from 0.02  $\mu\text{m}$  to 2800.00  $\mu\text{m}$ . More detailed experimental procedures for determining the physico-chemical properties of soil can be found in the literature (Ordóñez et al., 2016).

### 3.3. Selection of phytolith types

To exactly determine the relationship between phytolith and the environment, the short cell phytoliths, hair cell phytoliths and silicified stomata were respectively studied. As the proportion of saddle phytoliths in *P. australis* was greatest among all phytolith types, which are a characteristic phytolith of *P. australis*, and the proportion of rondel phytoliths was also high, these types were selected as representative of short cell phytoliths. Hair cell phytoliths are sensitive to environmental change, and lanceolate phytoliths were selected as representative of this type. Plant stomata play an important role in plant photosynthesis, respiration and

water use efficiency, and the size of the pore opening is adjusted directly in response to the internal and external environment of the plant. Silicified stomata are discrete bodies of solid silicon dioxide deposited in plant guard cells and were analyzed as the guard cell phytoliths.

### 3.4. Selection of research method

The northeast China region can be divided north–south into cold temperate, temperate and warm temperate zones; and east–west into humid, semi-humid and semi-arid areas. We examined the changes in *P. australis* phytoliths along the temperature gradient in the following two ways. (1) The 11 sampling sites could be divided south–north along the temperature gradient into two sections: warm temperate zone (including Dandong–Panjin–Tongliao), and temperate zone (including Longwan–Changchun–Changling, Mudanjiang–Harbin–Daqing and Tongjiang–Beian) (Table 1). There are obvious temperature differences among these two zones and we used these to investigate the effect of temperature on *P. australis* phytoliths. The phytolith size of each temperature zone is the

Table 1  
Climate data of sampling sites in northeast China.

Sections		The annual average temperature ( $^{\circ}\text{C}$ )	The annual average precipitation (mm)
Temperature sections	The warm temperature zone	7.83	748.33
	The temperature zone	3.51	560.37
Precipitation sections	The humid area	4.45	737.03
	The semi-humid area	4.48	594.53
	The semi-arid area	5.10	474.30

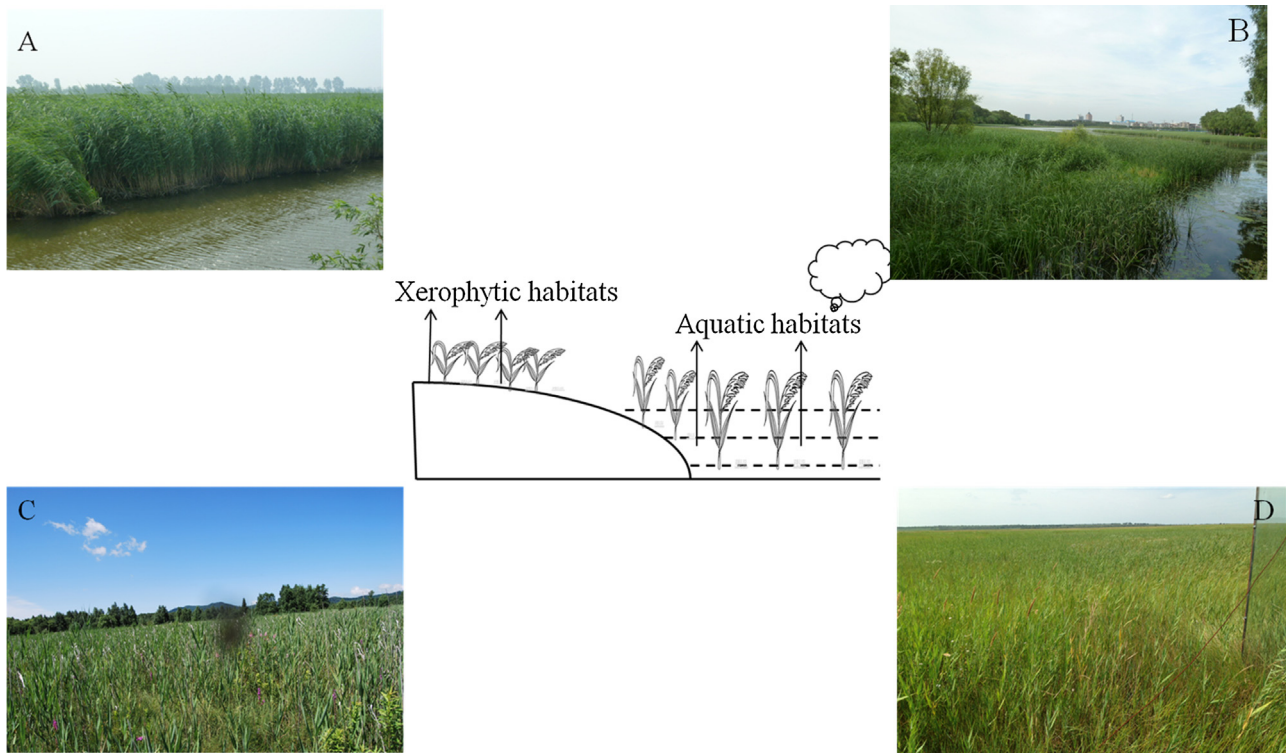


Fig. 2. The aquatic and xerophytic habitats of sampling sites. (A, B) the aquatic habitats, (C, D) the xerophytic habitats.

average of phytolith sizes of the all sampling sites in each zone. (2) We also examined the changes in saddle, rondel and lanceolate phytoliths and silicified stomata, along the temperature gradient (from the warm temperate to temperate zones) moving east–west for humid, semi-humid and semi-arid areas in northeast China. The phytolith size of each temperature zone is the average of phytolith sizes of the all sampling sites from each zone in the same humidity area.

Additionally, the 11 sampling sites could be also divided east–west into three sections along the precipitation gradient. Among the sections, there are slight temperature differences but larger precipitation differences. To further analyze the changes in *P. australis* phytoliths with precipitation, we investigated the changes in the following two ways. (1) The 11 sampling sites could be divided along the precipitation gradient east–west into three sections: humid (including Dandong, Longwan, Mudanjiang and Tongjiang), semi-humid (including Panjin, Changchun and Harbin) and semi-arid (including Tongliao, Changling, Daqing and Beian) areas (Table 1). We used the precipitation differences among the three humidity areas to analyze the changes in *P. australis* phytoliths with precipitation. The phytolith size for each humidity area is the average of phytolith sizes of the four sampling sites in each area. (2) We also examined the changes in saddle, rondel, lanceolate phytoliths and silicified stomata along the precipitation gradient in the warm temperate and temperate zones. And the phytolith size for each humidity area is the average of phytolith sizes of the all sampling sites from each area in the same temperature zone.

### 3.5. Statistical analysis

Principal components analysis (PCA) was conducted to investigate the main environmental factors influencing phytolith size of *P. australis*. Three-way and one-way analyses of variance (ANOVAs) were used to examine the effects of temperature, precipitation and

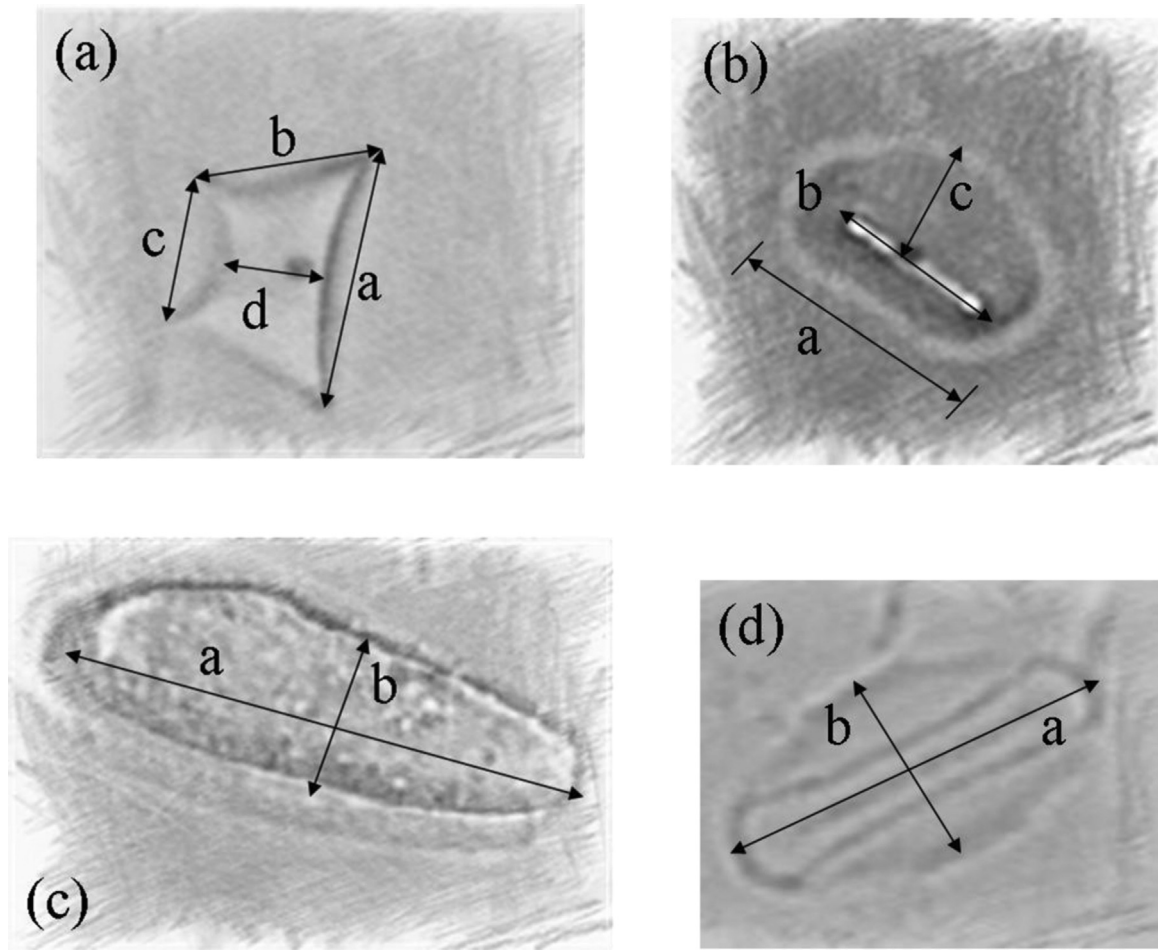
habitat on phytolith size of *P. australis*. All statistical analyses of the data were performed using SPSS 18.0 software.

## 4. Results

Samples of *P. australis* leaves from 11 sampling sites were examined. In total, 33,148 phytolith particles were counted and classified into six types. The identification of phytoliths was based on their morphological characteristics and all phytolith morphotypes identified here were described with the classification criteria of Wang and Lu (1992) and the International Code for Phytolith Nomenclature (ICPN) 1.0 protocol. The main phytolith morphotypes were saddle, lanceolate, bulliform (including square), rondel and elongated phytoliths and silicified stomata. Phytolith size was measured under a microscope (DMBA 300, MOTIC China Group Co., Ltd., China) at a magnification of 900 $\times$ . The measured parameters included the length, length of saddle, width, and width of saddle for saddle phytoliths; the upper base, etue and height for rondel phytoliths; and the length and width for lanceolate phytoliths and silicified stomata. The detailed parameters used for the different phytolith types are shown in Fig. 3. For each phytolith type, 30 phytolith particles were measured. In total, 2640 phytolith particles were measured, and 7260 original data values of phytolith size were obtained.

### 4.1. Principal components analysis (PCA) of environmental factors influencing phytolith size

PCA is a method of dimensional reduction useful for reducing the complexity of many variables. As a number of environmental factors, including climatic and edaphic factors, may affect silica uptake and phytolith formation, PCA was used to investigate the main environmental factors influencing phytolith size. The PCA showed that the four main factors were obtained and explained 79.59% of the variation, and the first and second axes explained



**Fig. 3.** sketch of *P. australis* phytoliths.

(a) saddle: a–length, b–width, c–length of saddle, d–width of saddle, (b) rondel: a–etue, b–upper base, c–height, (c) lanceolate: a–length, b–width, (d) silicified stomata: a–length, b–width.

60.45% (Table 2), indicating that phytolith size was mainly affected by the environmental factors represented by these two axes. The factor best represented by the first axis was the most important. To further determine the environmental factors represented by first and second axes, the correlation coefficients of environmental factors in each axis were compared among themselves by pairing them with each other, and the environmental factor with the highest correlation coefficient could be considered as represented by the axis (Xia, 2010), therefore, the first and second axes could be considered as representing the annual averages of temperature and precipita-

tion, respectively. Thus the *P. australis* phytolith sizes were mainly related to the annual averages of temperature and precipitation.

#### 4.2. Three-way and one-way analyses of variance (ANOVAs) for phytolith size

ANOVA is a method of testing whether the differences among the mean values of two populations or multiple populations showed statistical significance or not. The above analysis indicated that the annual averages of temperature and precipitation were the main factors influencing *P. australis* phytolith size. In addition,

**Table 2**  
Results of PCA on the environmental factors related to *P. australis* phytolith sizes.

Environmental factors		The main composition factors			
		1	2	3	4
Climate factors	The annual average temperature	0.758	0.372	−0.091	0.308
	The annual average precipitation	0.104	0.814	−0.123	0.413
Micro-terrain	Habitat differences	−0.024	−0.194	0.415	0.774
Edaphic factors	Soil available silicon	0.222	0.551	0.559	−0.313
	Soil pH	0.468	−0.339	−0.148	−0.033
	Soil electrical conductivity	0.699	0.042	0.524	−0.275
	Soil organic matter	−0.483	−0.227	0.608	0.106
	Soil mechanical composition-sand	0.525	−0.646	0.044	0.161
Accumulative contribution rate (%)		29.84	51.44	66.15	79.59

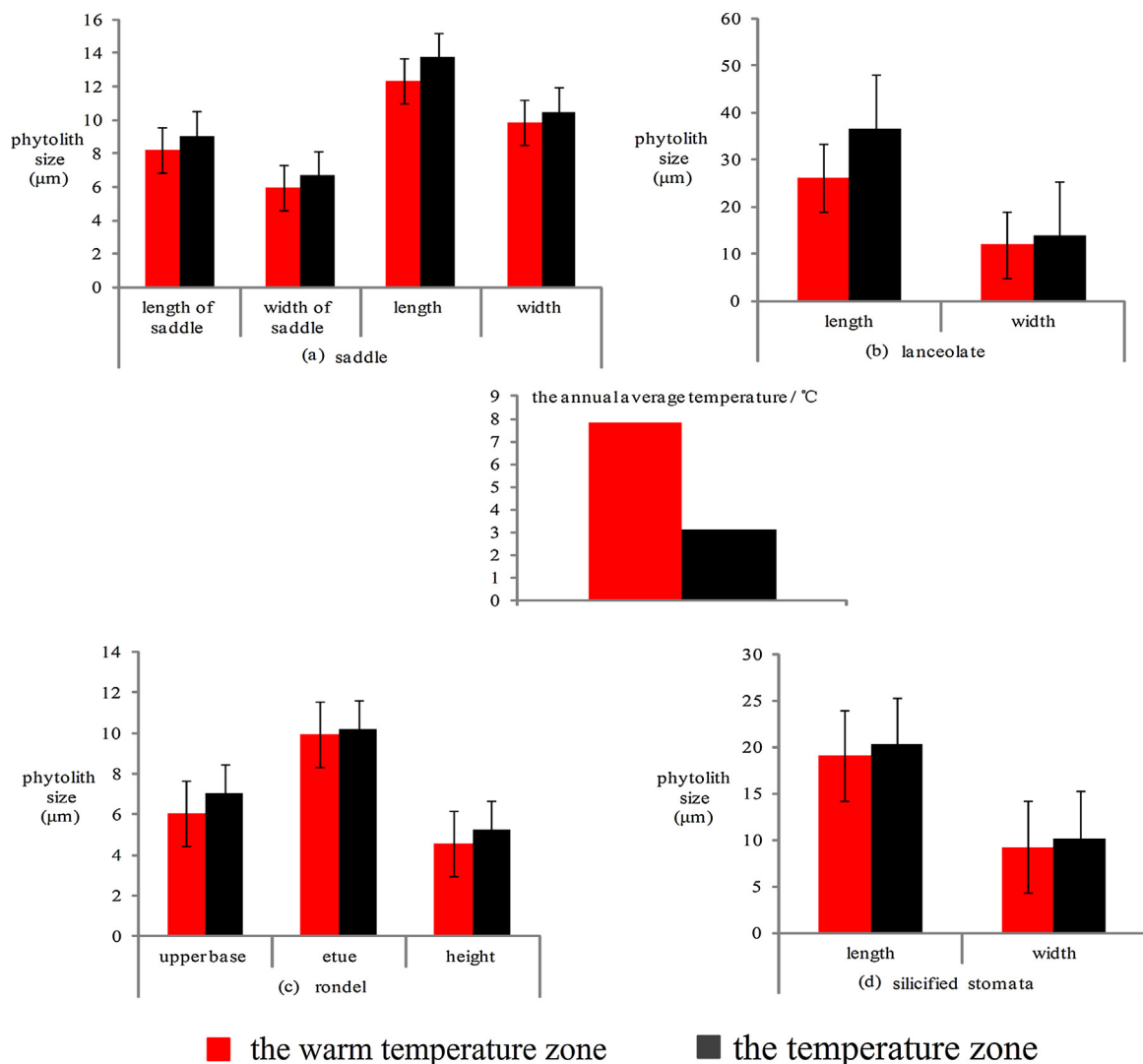
**Table 3**  
Results of the three-way ANOVAs on the *P. australis* phytolith size.

Environmental factors	Saddle		Rondel			Lanceolate		Silicified stomata			
	Length of saddle	Width of saddle	Length	Width	Upper base	Etue	Height	Length	Width	Length	Width
The annual average temperature	0.000	0.065	0.003	0.009	0.109	0.001	0.361	0.001	0.002	0.505	0.357
The annual average precipitation	0.001	0.161	0.013	0.020	0.084	0.001	0.387	0.002	0.014	0.160	0.087
Habitats	0.930	0.512	0.764	0.650	0.988	0.700	0.612	0.225	0.314	0.137	0.359
The annual average temperature × habitats	0.301	0.513	0.416	0.232	0.421	0.230	0.365	0.560	0.612	0.693	0.993
The annual average precipitation × habitats	0.003	0.054	0.020	0.244	0.002	0.538	0.007	0.585	0.669	0.025	0.024
The annual average temperature × the annual average precipitation	0.136	0.451	0.300	0.416	0.076	0.686	0.161	0.773	0.532	0.044	0.191

it had been reported that the height, biomass and spatial distribution of wetland plants were determined mainly by the availability of water in the different habitats of wetland (Webb et al., 2012). Hence, the habitat differences were also considered in the present study. Therefore, three-way ANOVAs were used to examine the effects of temperature, precipitation and habitat on phytolith size. The three-way ANOVAs showed significant differences in the length of saddle, length and width of saddle phytoliths, the etue of rondel phytoliths, and the length and width of lanceolate phytoliths with the temperature or precipitation gradients ( $P < 0.05$ ; Table 3),

whereas habitat differences and any two factors interacted with each other had little effect on all parameters, except the significance of some parameters was below the significance level of 0.05 under the influence of “the annual average precipitation × habitats”. On the whole, this revealed that phytolith size was more sensitive to the annual averages of temperature and precipitation.

To further clarify the changes in phytolith sizes with habitat, One-way ANOVAs were conducted for phytolith sizes among different temperature zones and humidity areas. One-way ANOVAs showed that the changes in *P. australis* phytoliths especially the



**Fig. 4.** Average size of *P. australis* phytoliths along temperature gradient in the NE China.

**Table 4**  
Results of the one-way ANOVA on the *P. australis* phytolith sizes between xerophytic and aquatic habitats.

Phytolith morphotype	Parameter	Sig. P				
		The warm temperature zone	The temperature zone	The humid area	The semi-humid area	The semi-arid area
Saddle	Length of saddle	0.798	0.331	0.845	0.851	0.041
	Width of saddle	0.577	0.913	0.264	0.013	0.333
	Length	0.417	0.140	0.202	0.612	0.538
	Width	0.299	0.615	0.008	0.513	0.416
Rondel	Upper base	0.210	0.010	0.757	0.585	0.902
	Etue	0.002	0.033	0.024	0.251	0.230
	Height	0.980	0.360	0.076	0.004	0.014
Lanceolate	Length	0.000	0.012	0.096	0.031	0.232
	Width	0.002	0.031	0.024	0.035	0.025
Silicified stomata	Length	0.000	0.011	0.153	0.042	0.012
	Width	0.011	0.006	0.012	0.342	0.024

lanceolate and silicified stomata were significantly affected by habitat ( $P < 0.05$ ; Table 4). Thus we speculated that in the same climate district, the phytolith size would change remarkably with the habitat differences.

The above analysis showed that, for regional research, temperature and precipitation were the main factors influencing size, but habitat was not a major influencing factor. However in the same climate district, habitat differences seemed to also have a significant impact on phytolith size.

4.3. Size of phytoliths with environmental changes

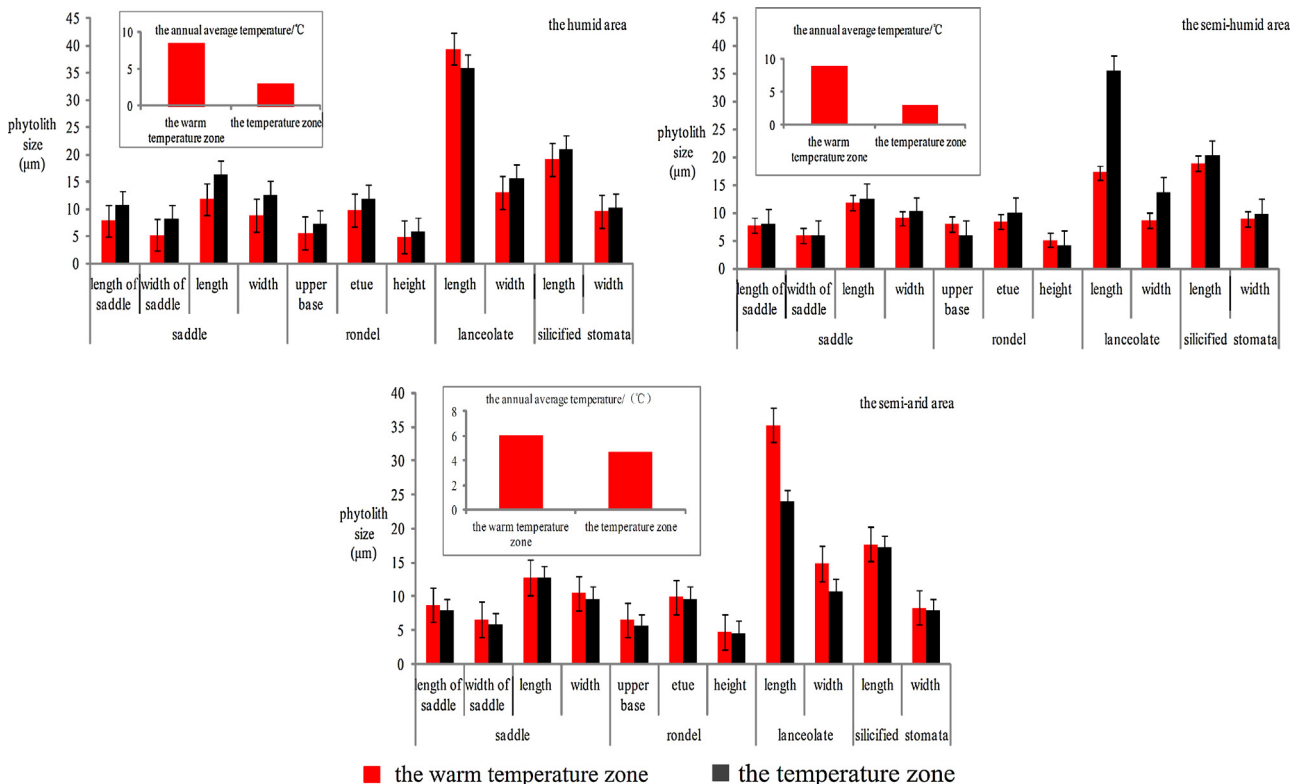
The above research revealed that *P. australis* phytolith size was sensitive to the annual average temperature, the annual averages precipitation and habitat differences. Consequently, we studied the changes in phytolith size with three environmental changes as fol-

lows: along a temperature gradient; along a precipitation gradient; and with habitat differences.

4.3.1. Size of phytoliths along the temperature gradient

The *P. australis* phytolith size for each temperature zone (Fig. 4) was the average of phytolith sizes from the all sampling sites in each zone to reduce the influence of precipitation. Phytolith sizes along the temperature gradient were clearly ranked as follows: the temperate zone > warm temperate zone for saddle (Fig. 4(a)), rondel (Fig. 4(c)), lanceolate (Fig. 4(b)) and silicified stomata (Fig. 4(d)). Overall, low temperature favored phytolith development, and moving from the warm temperate to the temperate zone, as temperature decreased, phytoliths were larger.

The changes of *P. australis* phytolith size with temperature in the humid, semi-humid and semi-arid areas are shown in Fig. 5. The changes of phytolith size with temperature in the semi-arid area (including Tongliao, Changling and Daqing) were opposite to



**Fig. 5.** Variation trend of the phytolith size with temperature in the humid, semi-humid and semi-arid areas.

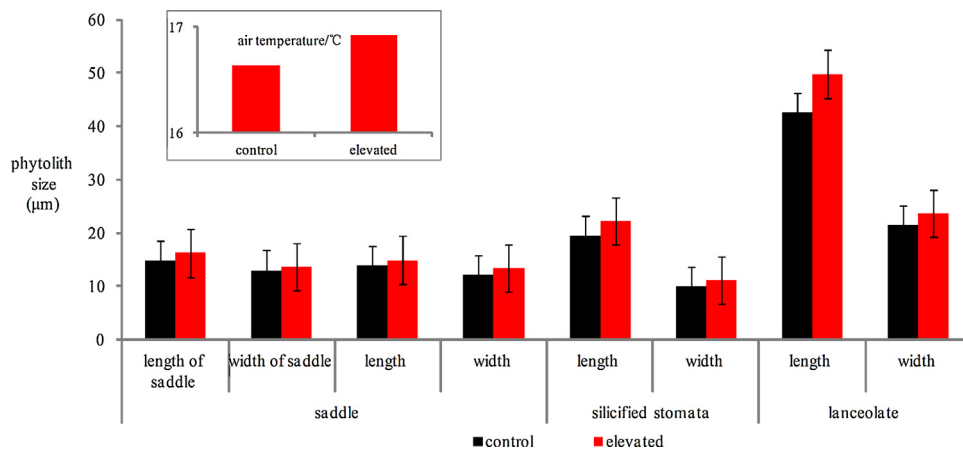


Fig. 6. Variation trend in *P. australis* phytolith size in September in Changling County, China.

those in the humid (including Dandong, Longwan, Mudanjiang and Tongjiang) and semi-humid areas (including Panjin, Changchun, Harbin and Beian) of northeast China. In the humid area, phytoliths from temperate zone (Longwan, Changchun and Changling) were larger than those from the warmer warm temperate zone (Dandong), and similarly in the semi-humid area. However, in the semi-arid area, moving from temperate zone (Changling and Daqing) to warm temperate zone (Tongliao), as the temperature increased, the phytoliths were larger. In the humid, semi-humid and semi-arid areas in northeast China, there were some differences in change trends of phytolith size with temperature due to precipitation differences. Simultaneously, the changes in size of phytoliths with temperature in the semi-arid area were consistent with the results of the warming experiment using the infrared radiators (Kalglo Electronics Inc. Bethlehem, PA, MSR-2420, USA) in Changling (Fig. 6). This illustrated that in the semi-arid area, the phytolith size was mainly affected by temperature. Thus, in the same humidity area, temperature was still the main factor influencing phytolith size.

#### 4.3.2. Size of phytoliths along the precipitation gradient

The phytolith size for each humidity area (Fig. 7) was the average value of the sizes of *P. australis* phytoliths from the four sampling sites in each area to reduce the influence of temperature. The phytolith sizes along the precipitation gradient (Fig. 7) were ranked as follows: the humid area (including Dandong, Longwan, Mudanjiang and Tongjiang) > semi-humid (including Panjin, Changchun, Harbin and Beian) > semi-arid (including Tongliao, Changling and Daqing) for saddle (Fig. 7(a)), rondel (Fig. 7(c)), lanceolate (Fig. 7(b)) and silicified stomata (Fig. 7(d)). Consequently, moving from the semi-arid to semi-humid and to the humid area, phytoliths became larger as precipitation increased.

The changes of size of *P. australis* phytoliths with precipitation in the warm temperate (including Dandong, Panjin and Tongliao) and temperate zones (including Longwan–Changchun–Changling, Mudanjiang–Harbin–Daqing and Tongjiang–Beian) are also shown in Fig. 8. In the warm temperate and temperate zones, moving from the semi-arid to semi-humid to humid areas, as precipitation increased, the phytoliths increased in size. These changes indicated that precipitation actively promoted the growth of phytoliths both in the warm temperate and temperate zones.

#### 4.3.3. Size of phytoliths with habitat differences

The sizes of the *P. australis* phytoliths from the xerophytic and aquatic habitats (Fig. 9) were average values of the size of phytoliths from the respective 11 sampling sites. The phytoliths were larger in

aquatic (W) than in xerophytic habitats (T), leading us to speculate that aquatic habitats greatly favored the development of phytoliths.

## 5. Discussion

### 5.1. the major factors influencing the size of *P. australis* phytolith

The relationship between the physicochemical properties of soil and the environment has been studied extensively, and temperature and precipitation were shown to be the dominant factors controlling the variations in the electrical conductivity of soil (Li, 2010b), pH, available silicon (Si, 2013; Han et al., 2011), physical composition, and soil anions and cations (Liu, 2013). Wang et al. (2009) and Lu et al. (2014) also showed that soil organic carbon and anions in NE China were controlled mainly by the annual average temperature and precipitation. These results suggest that the physicochemical properties of soil are influenced mainly by the annual average temperature and precipitation and thus serve to explain the close relationship between these two parameters and phytolith size as well.

### 5.2. Phytoliths size variations with the environmental changes

#### 5.2.1. Phytolith size variations with temperature

The experimental results showed that in northeast China, moving from the warm temperate to the temperate zone, as temperature decreased, the saddle, rondel and lanceolate phytoliths and silicified stomata of *P. australis* became larger. Generally, temperature is one of the most important environmental factors affecting plant photosynthesis and transpiration. Previous studies showed that high temperature was a factor that positively affected plant functions, increasing the net photosynthesis rate (Hou et al., 2005; Shi et al., 2009), stomatal conductance and stomatal density (Staeher and Sand, 2006), and consequently increasing photosynthesis rate. Thus, as temperature increased, the photosynthesis rate of *P. australis* increased, and more silicon could be absorbed. It has also been reported that the transpiration rate was higher at high temperatures (Zhu et al., 2006), suggesting that as temperature increased, the transpiration rate of *P. australis* increased, and more silicon could be deposited in plant cells. Simultaneously, research on the response of the plant leaf cell morphology to temperature found that plant leaf epidermal cell density (the number of epidermal cells per unit area) and stomatal density (the number of stoma per unit area) were all higher at elevated temperatures relative to controls, but plant leaf epidermal cells and stomata all became smaller (Ferris et al., 1996; An et al., 2011). This suggested that phytolith morphology changed according to the cells in which



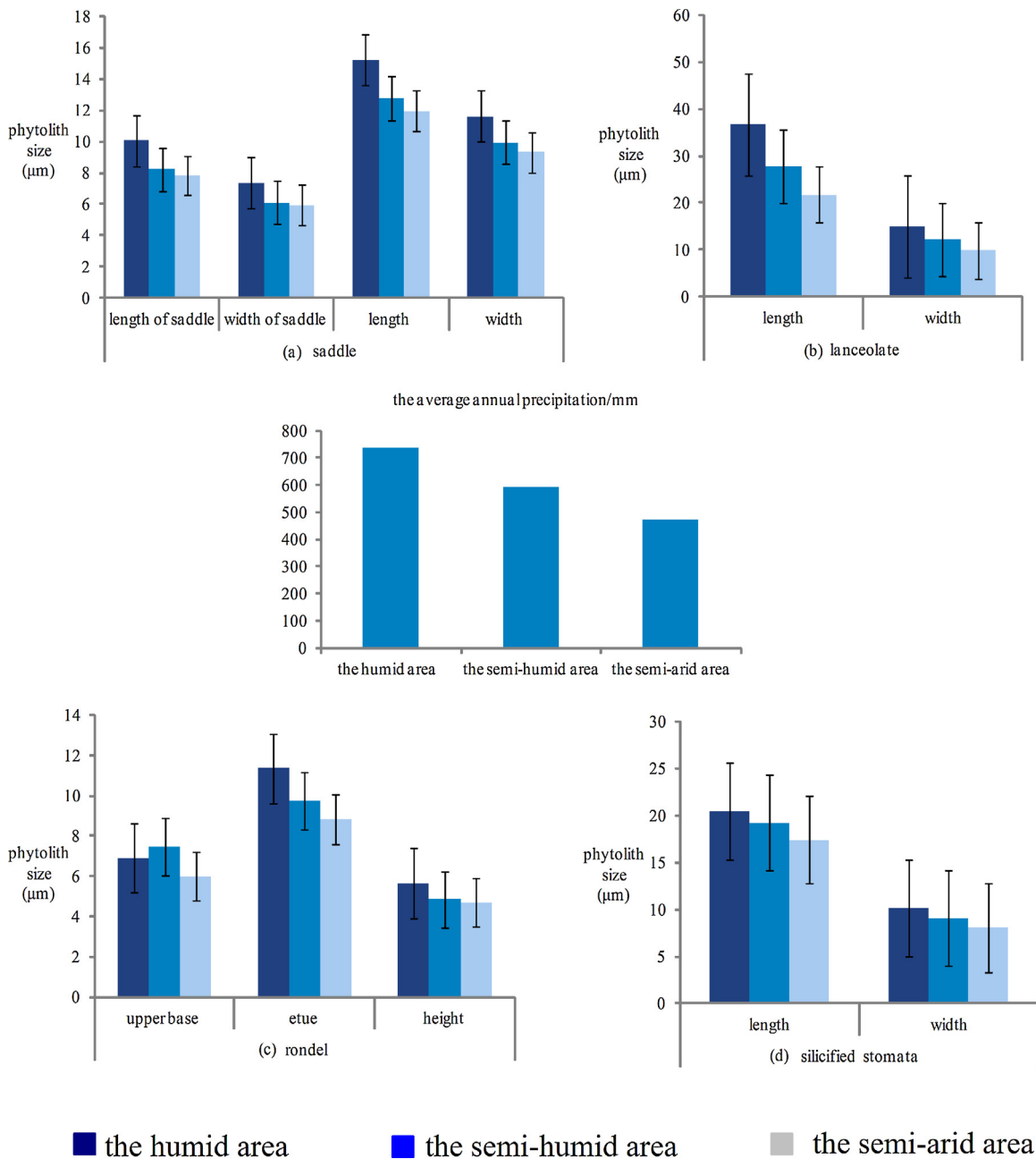


Fig. 7. Average size of *P. australis* phytoliths along precipitation gradient in the NE China.

they were deposited, and that phytolith size was closely related to cell size. Therefore, under high temperature conditions, because of more silicon deposited in or between cells and smaller epidermal cells, more phytoliths appeared, and of lesser size.

The phytoliths in *P. australis* in northeast China responded to temperature with a clearly discernible pattern. In the humid and semi-humid areas, from the warm temperate to the temperate zone, as temperature declined, the phytoliths grew larger; whereas in the semi-arid area, from the temperate to the warm temperate zone, as temperature increased, phytoliths became larger. The semi-arid area has a typical semi-arid monsoon climate characterized by large variation in temperature and precipitation across seasons, and the annual evaporation is 3–4 times the precipitation. As a result, phytoliths were larger, and may be an adaptation enabling *P. australis* to tolerate physiological and environmental drought. At the same time, the *P. australis* from the semi-arid area

had been growing in a semi-arid environment for a long time, and had gradually adapted to this environment. Silicon in these plants would protect them from the influence of environmental factors and stress (Gao and Li, 1995). *P. australis* absorbed large quantities of silicon, which increased their ability to grow under the influence of aridity (Wang et al., 2007). Hence, *P. australis* phytoliths in the semi-arid area became larger, enabling them to adjust their physiological activities to cope with the environment (Jie et al., 2010b). These results revealed that the phytoliths of *P. australis* in the wetland would grow small with global warming, which decreased their supporting ability to further weaken the development and distribution of wetland *P. australis* community. Accordingly, in the *P. australis* wetland ecosystem, relevant government departments should pay attention to cultivating the new plant varieties with high temperature resistance to adapt to global warming, especially in the humid and semi-humid areas. This would be beneficial to

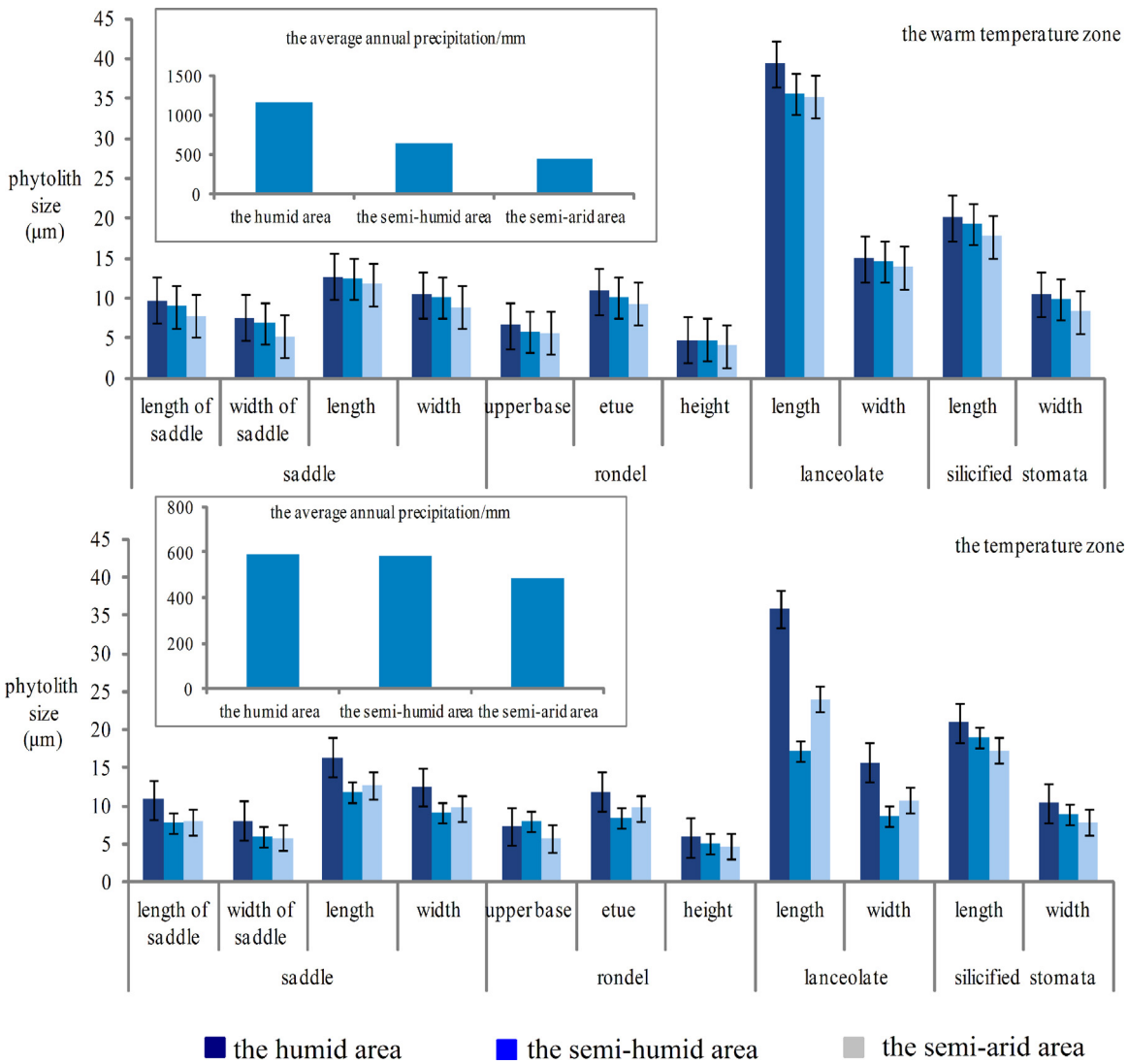


Fig. 8. Variation trend in the phytolith size with precipitation in the warm temperate and temperate zones.

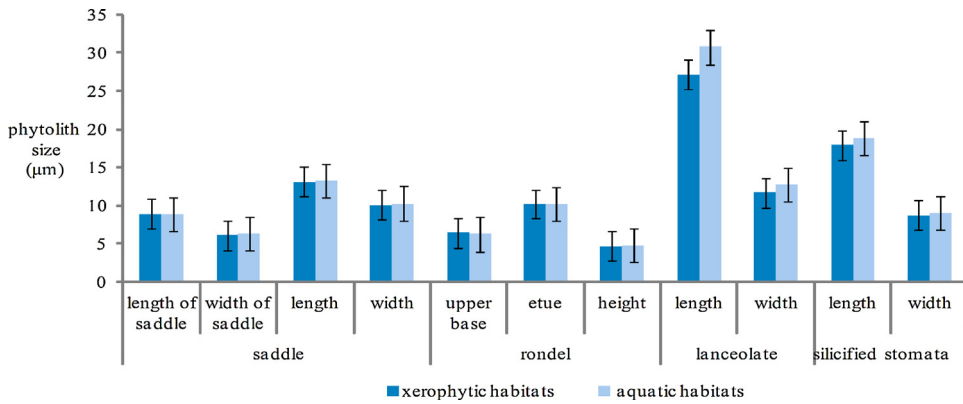


Fig. 9. Average size of *P. australis* phytoliths in xerophytic and aquatic habitats.

the development and diversity of plant community to further promote environmental restoration and researches on global climate change.

5.2.2. Phytolith size variations with precipitation

Precipitation played an important role in the formation of *P. australis* phytoliths. Our analysis of their changes with precipita-

tion revealed the same pattern at all sampling sites. Moving from the semi-arid to semi-humid to the humid areas, as precipitation increased, the phytoliths became larger. Precipitation is very important for plant growth, and keeping the balance between the water absorbed by the root and the water lost via leaf transpiration is necessary to ensure the normal life of plants. Consequently, precipitation exerts specific effects on the physiological processes

of plants. Studies have shown that as precipitation increased, the plant net photosynthesis rate increased (Huber et al., 1984; Liu et al., 2005), as did the chlorophyll a and b contents, leaf area and stomatal conductance, causing the photosynthesis rate of *P. australis* to increase (Horton et al., 2001; Deng et al., 2012). Under conditions of water stress, the transpiration rate decreased to prevent plant leaves wilting from water loss (Gao et al., 2007). In contrast, transpiration rate increased with more precipitation. Consequently, as photosynthesis and transpiration rates of *P. australis* increased with more precipitation, more silicon would be absorbed and deposited in or between cells. It has also been reported that the plant leaf area was larger and the leaves thinner in damp compared to drier environments, making both the cells and their intercellular spaces larger, and controlling phytolith morphology in plant tissues (Decchetti et al., 2008). This explained why *P. australis* phytoliths were sensitive to precipitation, and why they were larger in wetter environments. This was also consistent with a previous suggestion that larger phytoliths reflected higher precipitation (Wang and Lu, 1992).

Our experimental results also demonstrated that changes in *P. australis* phytoliths with precipitation showed the same trends in the warm temperate and temperate zones in NE China, in that phytoliths became larger as precipitation increased. But the trend is not a linear change, such as, the phytoliths from Tongliao, Changling, and Daqing (the semi-arid area) were large. This may be an adaptation enabling *P. australis* to tolerate physiological and environmental drought. Overall, the changes in phytolith size in the warm temperate and temperate zones were all largely influenced by precipitation. This indicated that Hong et al. (2008) found that the anatomical structures of *P. australis* in northeast China were sensitive to precipitation, and along with the increase in precipitation, the diameter of vascular bundles also increased. As the change in diameter of vascular bundles was the result of leaf cell morphological change, we speculated that plant leaf cells were also sensitive to precipitation in northeast China. Guo and Zhu (1994) analyzed the relationship between the *Aneurolepidium chinense* (Trin.) Keng community yield and climatic factors during 1978–1990 in northeast China, using correlation analysis, and showed that precipitation was the main limiting factor affecting the community yield. The height, biomass and spatial distribution of wetland plants were also determined by water availability (Wang et al., 1999), suggesting that phytolith size in *P. australis* in northeast China could also be closely related to precipitation. In conclusion, in the present study, increased precipitation may have strengthened the assimilation of *P. australis*, meaning more silicon was absorbed and deposited, and increasing the size of phytoliths. These data suggested that in the warm temperate and temperate zones, *P. australis* phytoliths would become large with increased precipitation, enabling them to increase their ability to cope with the arid environment. Consequently, under the influence of global warming, measures of water retention and supplement should be applied properly to ensure the normal growth of plants. This provided a reference for growth and development of wetland plants to further improve the surrounding environment.

### 5.2.3. Phytolith size variations with habitat differences

Our experimental results demonstrated that *P. australis* phytoliths were sensitive to habitat differences—with aquatic habitats more favorable to larger phytoliths than xerophytic habitats.

*P. australis* is widely distributed throughout the world, especially in wetlands, and plays an important role in maintaining the ecological balance and species diversity in wetland ecosystems. Water stress in the habitat is a major environmental factor limiting the growth of *P. australis* (Zhao et al., 2008). Previous studies have shown that the physiological activities of plants differed with habitat differences, and that plant net photosynthesis rate was higher in

aquatic habitat (Farquhar and Sharkey, 1982). It was also reported that in xerophytic habitat, plant stomatal conductance, number of chloroplasts in mesophyll cells and leaf area were lower than in aquatic habitat, resulting in low photosynthesis and transpiration rates (Chen and Zhang, 1991; Davies et al., 1994; Liu et al., 2011). The photosynthesis and transpiration rates of *P. australis* differed across different habitats, causing them to absorb and deposit silicon differently. In conclusion, in aquatic habitat, the photosynthetic and transpiration capacity of plants increased and improved the capacities of silicon absorption and deposition, thus making *P. australis* phytoliths larger. Because phytoliths grew in the space between the cell lumen and the intercellular space, the phytolith characteristics changed in response to changes in different habitats (Jie et al., 2010b).

### 5.3. Implication for phytolith size in palaeoenvironment reconstruction

Study of direct phytolith–environmental relationships proved that certain phytolith morphotypes occurred preferentially in certain climates and at certain elevations. Blinnikov (2005) assessed variability of phytoliths in 38 species of plants and 58 modern soil samples from 24 locations in the interior Pacific Northwest, and found that phytolith assemblages were mostly related to elevation, annual average temperature and annual average precipitation. Lu et al. (2006) investigated 243 surface soil phytolith samples from China, and results of canonical correspondence analysis and detrended correspondence analysis suggested that the annual average precipitation was the dominant variable controlling the spatial distribution of the phytolith assemblages. Our results demonstrated that the sizes of *P. australis* phytoliths were all sensitive to annual average temperature, annual average precipitation and habitat differences. Consequently, as similar with different phytolith assemblages, phytolith size is also strongly related to environmental changes. Some studies have also used phytolith size as a supplementary source of paleoenvironment data to study the relationship between phytolith and climate than can be achieved with phytolith assemblages alone. Combined phytolith assemblages and size results from surface soils in wetlands of Changbai mountain region also revealed that the phytolith assemblages and the size were all sensitive to temperature change (Guo et al., 2012). Overall, more even sampling of sites along a regular grid and over a larger geographical area, as was recently done with phytolith assemblages, would better illustrate current phytolith–climate relationships.

## 6. Conclusions

- 1) PCA of environmental factors (climatic and edaphic) and the three-way ANOVAs (annual average temperature, annual average precipitation and habitat differences) all indicated that for regional research, the annual averages of temperature and precipitation were the main factors influencing *P. australis* phytolith size. The results would help increase understanding of the phytolith formation and improve the precision of models for quantitative reconstruction of the palaeoenvironment based on phytolith data. Accordingly, relevant government departments should monitor the conditions of temperature and precipitation constantly to further provide help for growth of wetland plants and environmental conservation or restoration.
- 2) The responses of *P. australis* phytoliths to temperature differed among the humid, semi-humid and semi-arid areas of northeast China. In the humid and semi-humid areas, from the temperate to the warm temperate zone, increasing temperature reduced the size of phytoliths; whereas in the semi-arid area, the phy-

tololiths became larger with increasing temperature. Moreover, in the warm temperate and temperate zones, moving from the semi-arid to semi-humid to humid areas, as precipitation increased, phytoliths grew larger. These data demonstrated that phytolith size was sensitive to environmental factors. Depending on the changes of *P. australis* sizes at different environmental conditions, relevant measures such as water retention and supplement, cultivation of plant varieties with high temperature resistance should be applied accordingly to promote the growth of plant community under the influence of global warming. This would provide a reference and new ways for vegetation restoration and environmental conservation.

3) ANOVA indicated that *P. australis* phytoliths were sensitive to habitat differences—phytoliths were larger in aquatic than xerophytic habitats. It was highly significant in predicting changes in the competitiveness, adaptability and productivity of wetland plants. These data also suggested that in the same climate district, the habitat differences significantly affected size of *P. australis* phytoliths.

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