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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_2) 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_2 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, representes 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 temperature zone (Longwan–Changchun–Changling, Mudanjing–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

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