

Responses of Phytolith in Guinea Grass (*Leymus chinensis*) Leaves to Simulated Warming, Nitrogen Deposition and Elevated CO₂ Concentration in Songnen Grassland, Northeast China

LI Bo^{1,2}, FENG Yingying^{1,2,3}, GUO Jixun^{1,2}, JIE Dongmei^{1,2,3}, SHI Lianxuan^{1,2}

(1. Key Laboratory for Vegetation Ecology, Ministry of Education Grassland Research Institute, College of Life Science, Northeast Normal University, Changchun 130024, China; 2. Key Laboratory for Wetland Ecology and Vegetation Restoration, Ministry of Environmental Protection, Changchun 130024, China; 3. College of Geographical Science, Northeast Normal University, Changchun 130024, China)

Abstract: Deposited in plant cells and their intercellular space, phytoliths, a special form of silica, could be used to determine information on plant structure and physiology especially their size and content. With the hypothesis that phytolith in plant would change under variable climate and environment, the dominant plant species in Songnen grassland, guinea grass (*Leymus chinensis*), was treated by an open-top chamber (OTC) to elevate CO₂ concentration, infrared heaters, and artificial nitrogen (N) addition for three years from 2006–2008. Phytoliths were extracted by wet-ashing method and analyzed by variance analysis and so on. We found that the responses to elevated CO₂ are complicated, and warming is positive while N addition is negative to the deposition of phytoliths in *L. chinensis* leaves. Especially, warming could reduce the negative impact of N addition on phytolith in *L. chinensis*. The short cell's taxonomic in graminea is significant because of no disappearance with simulated environmental changes. The phytolith originated in the long cell and plant intercellular space are more sensitive to elevated CO₂ concentration, warming, and N addition, and could become some new indicators for environmental changes. In conclusion, different phytolith types have various responses to simulated warming, N addition and elevated CO₂ concentration.

Keywords: phytolith; elevated CO₂; warming; N addition; response

Citation: Li Bo, Feng Yingying, Guo Jixun, Jie Dongmei, Shi Lianxuan, 2015. Responses of phytolith in Guinea Grass (*Leymus chinensis*) leaves to simulated warming, nitrogen deposition and elevated CO₂ concentration in Songnen grassland, Northeast China. *Chinese Geographical Science*, 25(4): 404–413. doi: 10.1007/s11769-015-0766-x

1 Introduction

Phytoliths are special form of silica developed in higher plant cells and intercellular space (Wang and Lv, 1993; Rudall *et al.*, 2014). They are abundant in the topsoil. At different climatic conditions, the amounts of different type phytoliths are under statistical law (Jie *et al.*, 2010a). Thus, it is widely used in paleo-climate's reconstruction (Inoue and Sase, 1996; Carter, 2002; Dawson

et al., 2004; Trombold and Israde-Alcantara, 2005; Fan *et al.*, 2006; Li *et al.*, 2011; Li *et al.*, 2013). Silicon not only reduces the toxicity of salt in crops, but also promotes plant growth and protects them from pests and diseases (Lewin and Reimann, 1969; Lanning and Eleuterius, 1985; McNaughton *et al.*, 1985; Zhu *et al.*, 2004). It is proposed that the content and size of phytoliths might play an important role in plant growth. Environmental factors can influence the formation of phy-

Received date: 2014-09-17; accepted date: 2014-12-02

Foundation item: Under the auspices of National Natural Science Foundation of China (No. 40971116, 41471164, 31170303, 31270366), Ministry of Environmental Protection Foundation for Public Welfare Project (No. 201109067), National Undergraduate Training Programs for Innovation and Entrepreneurship (No. 201410200074)

Corresponding author: GUO Jixun. E-mail: gjixun@nenu.edu.cn

© Science Press, Northeast Institute of Geography and Agroecology, CAS and Springer-Verlag Berlin Heidelberg 2015

tolith (Prebble *et al.*, 2002; Lu *et al.*, 2006).

The tendency of warming, elevated CO₂ concentration and nitrogen (N) addition is ongoing nowadays (Phoenix *et al.*, 2006; Sabine, 2014). Elevated CO₂ or temperatures could thicken leaves and increase biomass production (Lin and Hu, 1996; Pritchard *et al.*, 1999; Zeng *et al.*, 2002; Li *et al.*, 2014). Optimal N addition can enhance the growth of plants (Feng Dalan L *et al.*, 2009; Reddy and Matcha, 2010), while excessive nitrogen can reduce the biological diversity of terrestrial ecosystems (Dirnböck *et al.*, 2014). However, no information is available now regarding the responses of phytolith in plants to climate change.

We hypothesized that content and size of phytolith in plants would change under climate and environment changes. Guinea grass (*Leymus chinensis*) is a major grass species in Songnen grassland. There were lots of studies on its physiology previously, therefore, comparative analysis between plant physiology and phytolith was convenient for this species. The purpose of this study was to identify the responses of different types of phytolith in *L. chinensis* to simulated warming, elevated

CO₂ and nitrogen deposition conditions, which could provide guidelines for paleo-environmental rebuilding and more understanding of future ecosystems evolution.

2 Materials and Methods

2.1 Study site

The study was conducted at Grassland Ecosystem Experimental Station of Northeast Normal University, which is located in the southwestern part of Songnen grassland region (44°40′–44°44′N, 123°44′–123°47′E) (Fig. 1). The region is in temperate zone with semi-arid monsoon climate, hot in the summer and cold in the winter. The average annual temperature is 4.9°C with the warmest month of 22°C–25°C and the coldest month of –22°C––16°C. The annual precipitation is about 470 mm, and 70% of the total precipitation occurs from June to September. The dominant grass in this region is *Leymus chinensis*, accompanied by reed (*Phragmites australis*) and other forbs. The soil type is mainly Hapli-Ustic Isohumosols, and others are salinized or alkalized soils. The soil organic matter content is around 30–40 g/kg.

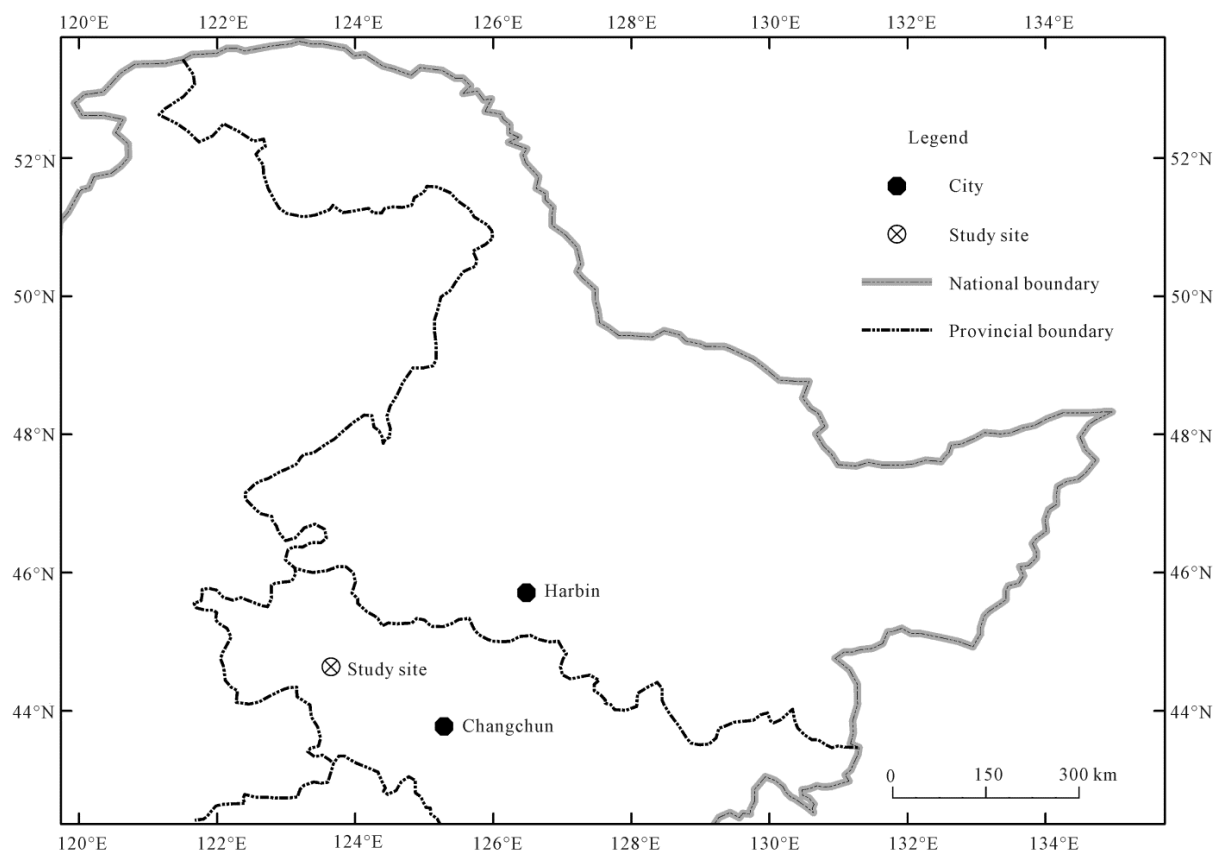


Fig. 1 Location of study site

2.2 Experimental design

There were five treatments in the experiment: elevated CO₂, warming, N addition, warming combined with N, and control. Elevated CO₂ experiment was achieved by a 5 m² open top chamber (OTC). The OTC was continuously supplied CO₂ in four directions by liquid CO₂ cylinder, and a flow meter was used to maintain the CO₂ concentration to 750 μmol/mol. For simulated warming and N addition treatments, 12 rectangular plots were established with each of 4 m × 3 m. Six randomly selected plots were heated by infrared heaters 2.25 m high above the ground throughout the year, while the other six plots were treated as control. Ammonium nitrate (10 g/m²) was applied to 1/2 of each plot on May 30th of each year (Fig. 2). In all, there formed an OTC for elevated CO₂ treatment, six subplots for simulated warming, six subplots for N addition, six subplots for warming combined N addition, and six subplots for control treatment. The community structure was simple in the plot. *L. Chinensis* was the main plants with a contagious distribution. Besides, only a few *P. australis*, Indian kalimeris herb (*Kalimeris integrifolia*), and sedge (*Carex duriuscula*) grew in the plot. The experiments were repeated for three years from 2006 to 2008. At harvest season in 2008, one 1 m × 1 m quadrat from each subplot was chosen, totally 25 quadrats. In each quadrat, leaves of *L. Chinensis* were sampled using five point sampling mode. Samples were then taken back to laboratory and dried.

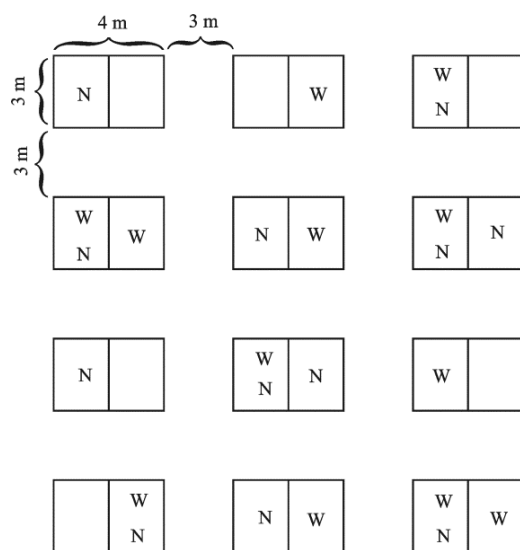


Fig. 2 Experimental design of simulated warming and nitrogen deposition treatments

2.3 Extraction method

Wet-ashing method was used to isolate phytolith (Jie *et al.*, 2010b). For each sample, we randomly selected 5 g dry leaves, cleaned them by microwave, and then put them into numbered tubes. The tubes were added with 20 mL concentrated nitric acid, heated by water bath. After complete oxidization of leaves, the concentrated nitric acid was removed, and distilled water was added. Centrifugation was done at rotation speed of 2000 r/min for 10 min, which was repeated three times. The extracted phytoliths were dried, weighed, and then made into permanent slide. Phytoliths were observed and the size of phytolith was measured under a biological microscope (BA210 Digital, MOTIC, USA), magnified 900 times. For each phytolith slide, the number of every phytolith type was counted, and relative content of each type was calculated.

2.4 Analysis method

One-way ANOVA was used to determine the size differences of phytolith between the five treatments. The significant level was at $P < 0.05$.

3 Results

3.1 Phytolith type

According to the International Code for Phytolith Nomenclature 1.0 (ICPN1.0), the phytoliths observed in *L. chinensis* mainly included rondel, elongate, hair cell, laminate, favose, and others (Table 1). Among them, rondel, a primary phytolith, accounted for 70% of total phytolith amount. The sum of rondel, elongate and hair cell accounted for more than 90% of total phytolith amount. Based on the subtle differences in morphology, a more detailed classification for rondels, elongates, and hair cells was set. Rondels were divided into three subtypes: ridged rondel, flat rondel, and echinate rondel. Elongates were divided into four subtypes: smooth elongate, echinate elongate, horn-ends elongate, and hollow elongate. Hair cells were divided into two subtypes: unciform haircell and lanceolate. Hollow elongate was only observed in elevated CO₂ and N fertilization treatments, while it was not observed in other treatments.

3.2 Relative content of phytolith between treatments

For elevated CO₂ treatment, the relative content of rondel increased compared to the control. Specific to the

Table 1 Relative contents of all types of phytolith in *Leymus chinensis* leaves

Phytolith type		Percentage (%)				
		CK	CO ₂	W	N	WN
Rondel	Total rondel	64.0	70.0	69.0	73.0	76.0
	Ridged rondel	27.0	46.0	31.0	33.0	50.0
	Flat rondel	29.0	28.0	31.0	36.0	34.0
	Echinate rondel	44.0	26.0	38.0	31.0	16.0
Elongate	Total elongate	7.0	13.0	5.0	7.0	9.0
	Smooth elongate	29.0	15.0	14.0	21.0	41.0
	Echinate elongate	59.0	56.0	76.0	29.0	45.0
	Horn-ends elongate	12.0	23.0	10.0	4.0	14.0
	Hollow elongate	—	6.0	—	46.0	—
Hair cell	Total hair cell	25.0	12.0	24.0	10.0	12.0
	Unciform haircell	2.0	10.0	2.0	23.0	21.0
	Lanceolate	98.0	90.0	98.0	77.0	79.0
Other types	Total other types	4.0	5.0	2.0	10.0	3.0
	Laminate	13.0	20.0	40.0	29.0	29.0
	Favose	33.0	7.0	30.0	56.0	28.0
	Homo-dentate	37.0	13.0	10.0	3.0	29.0
	Rare type	17.0	60.0	20.0	12.0	14.0

Notes: CK, control group; CO₂, elevated CO₂ group; W, warming group; N, excessive N group; WN, warming combined excessive N group. '—' represents no phytolith of such type was observed

three kinds of rondels, the relative content of ridged rondel increased, while the content of flat rondel and echinate rondel descended. There were more elongates in the treatment than the control group, and the relative content of hair cells decreased.

In warming treatment, rondels, elongates and hair cells took up more percentage from total phytolith amount, reaching up to 98%. It was hardly to find laminates, favoses, tooth shapes, and other minority phytolith types. The relative content of rondels increased from 64% (the control group) to 69%, while that of elongates and hair cells changed slightly.

The three type of rondel responded differently to N addition. In all, the relative content of rondels increased from 64% (control group) to 73%. For the three types of rondel, the relative content in ridge rondel and echinate rondel increased but flat rondel declined. As for hair cells, the relative content decreased, and the scale between unciform hair cell and lanceolate also changed. The content of unciform hair cells increased obviously, which was almost 10 times than the warming treatment. As for elongates and hair cells, their contents decreased. Besides, the content of laminates, favoses, tooth shapes,

and broken-shape increased sharply, reaching to 10%. Specially, we observed some hollow elongates which were not fully silicified.

In warming combined with N addition treatment, the content of laminates, favoses and tooth-shapes fell down, although they were higher in single N treatment. The relative content of rondels and elongates increased compared to the other treatments except elevated CO₂. The hair cells descended compared to the control and single warming, although the percentage increased comparing with the single N addition.

3.3 Length and width of phytolith between treatments

For elevated CO₂ treatment, the size of rondels tended to become smaller, although some sub-types did not change significantly (Table 2, Table 3). Specific to the three kinds of rondels, the length of ridged rondel increased whereas its width decreased. As for flat rondel and echinate rondel, both length and width became smaller (Fig. 3). Elongates tended to stretch and increased in length (Fig. 4a). The size of hair cells became larger (Fig. 4b).

Table 2 Significant coefficients of phytolith length in experimental treatments in *Leymus chinensis*

Phytolith type	Experimental treatment	CK	CO ₂	W	N	WN	
Rondel	Ridged rondel	CK	—	0.120	0.007	0.007	0.059
		CO ₂	0.120	—	0.252	0.252	0.738
		W	0.007	0.252	—	0.857	0.416
		N	0.012	0.333	0.857	—	0.526
		WN	0.059	0.738	0.416	0.526	—
	Flat rondel	CK	—	0.186	0.826	0.826	0.000
		CO ₂	0.186	—	0.124	0.124	0.000
		W	0.826	0.124	—	0.609	0.000
		N	0.770	0.302	0.609	—	0.000
		WN	0.000	0.000	0.000	0.000	—
	Echinate rondel	CK	—	0.190	0.221	0.604	0.041
		CO ₂	0.190	—	0.012	0.068	0.001
		W	0.221	0.012	—	0.479	0.408
		N	0.604	0.068	0.479	—	0.126
		WM	0.041	0.001	0.408	0.126	—
Elongate	Echinate elongate	CK	—	0.342	0.010	0.556	0.043
		CO ₂	0.342	—	0.102	0.125	0.279
		W	0.010	0.102	—	0.002	0.578
		N	0.556	0.125	0.002	—	0.009
		WN	0.043	0.279	0.578	0.009	—
Hair cell	Lanceolate	CK	—	0.113	0.604	0.946	0.043
		CO ₂	0.113	—	0.036	0.129	0.000
		W	0.604	0.036	—	0.557	0.131
		N	0.946	0.129	0.557	—	0.037
		WN	0.043	0.000	0.131	0.037	—

Notes: Results were from one-way ANOVA. CK, control group; CO₂, elevated CO₂ group; W, warming group; N, excessive N group; WN, warming combined excessive N group

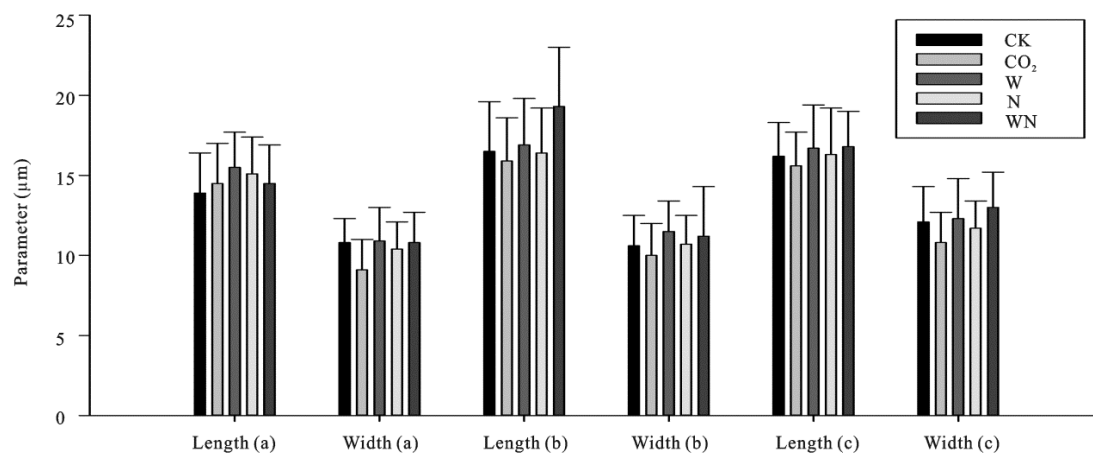


Fig. 3 Length and width of three types of rondels. a, ridged rondel; b, flat rondel; c, echinate rondel. CK, control group; CO₂, elevated CO₂ group; W, warming group; N, excessive N group; WN, warming combined excessive N group. Error bars represent the standard error of the mean

Table 3 Significant coefficients of phytolith width in experimental treatments in *Leymus chinensis*

Phytolith type		Experimental treatment	CK	CO ₂	W	N	WN
Rondel	Ridged rondel	CK	—	0.000	0.000	0.704	0.796
		CO ₂	0.000	—	0.005	0.000	0.000
		W	0.000	0.005	—	0.000	0.000
		N	0.704	0.000	0.000	—	0.903
		WN	0.796	0.000	0.000	0.903	—
	Flat rondel	CK	—	0.985	0.010	0.181	0.159
		CO ₂	0.985	—	0.011	0.187	0.165
		W	0.010	0.011	—	0.208	0.234
		N	0.181	0.187	0.208	—	0.944
		WN	0.159	0.165	0.234	0.944	—
	Echinate rondel	CK	—	0.113	0.604	0.946	0.043
		CO ₂	0.113	—	0.036	0.129	0.000
		W	0.604	0.036	—	0.557	0.131
		N	0.946	0.129	0.557	—	0.037
		WM	0.043	0.000	0.131	0.037	—
Elongate	Echinate elongate	CK	—	0.000	0.022	0.000	0.000
		CO ₂	0.000	—	0.066	0.578	0.717
		W	0.022	0.066	—	0.196	0.138
		N	0.000	0.578	0.196	—	0.846
		WN	0.000	0.717	0.138	0.846	—
Hair cell	Lanceolate	CK	—	0.015	0.106	0.106	0.012
		CO ₂	0.015	—	0.409	0.409	0.927
		W	0.106	0.409	—	0.154	0.359
		N	0.106	0.409	0.154	—	0.020
		WN	0.012	0.927	0.359	0.020	—

Notes: Results were from one-way ANOVA. CK, control group; CO₂, elevated CO₂ group; W, warming group; N, excessive N group; WN, warming combined excessive N group

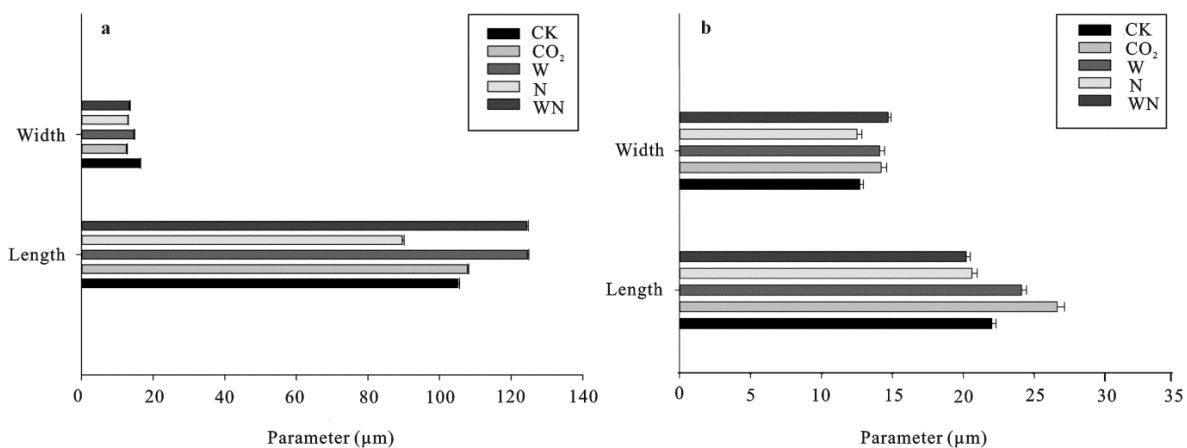


Fig. 4 Parameters of elongate (a) and hair cell (b) in different treatments. CK, control group; CO₂, elevated CO₂ group; W, warming group; N, excessive N group; WN, warming combined excessive N group. Error bars represent the standard error of the mean

In warming treatment, rondels and elongates, as well as hair cells became larger. Among them, the length of ridged rondel, echinate elongate, and lanceolate increased significantly, while the width of flat rondel,

echinate rondel, echinate elongate changed significantly.

The three type of rondel responded differently to N addition. The length of ridged rondel and echinate rondel increased by 8.6%, 0.6%, respectively, while the width decreased by 3.7% and 3.3%. Length in flat rondel decreased by 0.6% while width increased by 0.9%. Except for length of ridged rondel, no variations in coefficients of size were observed among them. The mean length and width of elongates and hair cells were both decreased, along with their decreasing content.

When warming combined with N addition, both length and width of 3 types of the rondels increased significantly compared to the control group, and sizes of echinate rondels became even larger than the single warming group. As for elongates, length and width were larger than single N addition treatment, but smaller than single warming treatment.

3.4 Difference response between phytolith deposit in short cell and long cell

Rondel was the dominant phytolith in *L. chinensis* leaves. For the four treatments, the relative content of rondels all increased. As for sizes, rondels were larger in warming treatments. However, sizes of three types of rondels did not change consistently for elevated CO₂ or N fertilization treatment, and flat rondels and echinate rondels in warming combined with N addition treatment tended to become bigger compared to single treatment.

The relative content of elongates increased under elevated CO₂ treatment, and did not change significantly in warming, N addition, and warming combined with N addition treatment. Length increased while width decreased in all treatments except N addition, which may indicate the elongation of long cells. Excessive nitrogen shortened the length of elongates, what's more, there were a large number of hollow elongates not completely silicified.

For hair cells, the relative content declined in both elevated CO₂, N addition, and N combined with N addition treatment. In warming treatment, the content almost had no change. Microscopic measurements showed that under elevated CO₂ or warming treatment, length and width of hair cells increased significantly, and the hair cells became smaller under N addition treatment. For warming combined with N addition, the length was longer than single warming or single N addition treatment, while width was smaller than single warming or N

addition treatment.

4 Discussion

The content and size of phytolith are associated with plant physiological state and cell morphology (Rudall *et al.*, 2014), while silicon content affects the cell structure, the content of chlorophyll, the absorption of nitrogen, as well as cell transpiration in return (Ahmad *et al.*, 1992; Epstein, 1994; Liang, 1997; Liang, 1999; Liang *et al.*, 2003; Ma, 2004; Romero-Aranda *et al.*, 2006). For gramineae species, phytolith is assembled in leaf epidermal cells. Many researchers proposed that silicon accumulation started from the cell edge (Wang and Lv, 1993), thus, phytoliths in gramineae might record the shape and status of plant cells, and their responses to different conditions could reflect the responses of cells.

Previous studies indicated that the typical phytolith in pooidae were mainly reniform, rondel, conical, stellate, and trapeziform-sinuate, and elongates and hair cells were frequently found while square-shape was hard to find (Wang and Lv, 1993). Huang *et al.* (2004) observed that phytoliths of *L. chinensis* grown in Inner Mongolia were orbicular in shape (we named rondel in this paper), accounting for 69.1% of total phytolith amount, which was consistent with our observation. We not only observed rondels, elongates, hair cells and favoses, but also found new phytolith types including hollow elongates, horn-ends elongates and laminate. We also observed a few broken-shapes and irregular shapes.

4.1 Effect of elevated CO₂ concentration and warming on phytolith

Under the present atmospheric CO₂ level, C₃ plants are CO₂-limited (Zou, 2005). Previous studies showed short-term excessive CO₂ concentration had positive effect on the growth of plants, while long-term excessive CO₂ concentration had more complicated effects (Sage *et al.*, 1989; Gunderson *et al.*, 1993; Poorter, 1993; Makino and Mae, 1999). Masle (2000) discovered that higher CO₂ level enhanced the cell elongation and cell division in wheat (C₃), however, the epidermal cells did not elongate obviously. Other studies also proposed that increasing CO₂ concentration significantly promoted cell division (Lin and Hu, 1996). The increased sum content of rondels and elongates as well as larger but fewer hair cells under elevated CO₂ level in our ob-

servation fully agreed with the findings that high CO₂ concentration promoted cell division.

Positive or negative plant growth in response to warming largely depends on the optimum temperature of plant. If temperature is above the optimum temperature, higher temperature levels will not enhance the plant growth anymore (Berry and Bjorkman, 1980; Yamori *et al.*, 2014). For the 3 types of rondels observed in present study, the content of ridged rondels and flat rondels increased while echinate rondels decreased, which indicated warming had different effect on the three rondels, and the three types may have different formation mechanisms. As for hair cells, the content declined from 25% (control group) to 24%, and the scale between unciform hair cell and lanceolate did not change, which demonstrated that warming had little influence on hair cells. However, larger rondels and hair cells in warming treatment inferred that warming enhanced the phytolith growth. Li *et al.* (2014) also found summer nighttime warming increased the aboveground production of *L. chinensis* significantly.

4.2 Effect of N addition and warming combined with N addition on phytolith

Nitrogen addition could increase foliar nitrogen significantly (Talhelm *et al.*, 2011). Optimal nitrogen addition enhanced the plant growth (Yao and Liu, 2007; Feng L *et al.*, 2009; Reddy and Matcha, 2010), however, high N level had negative effects on plant (Li *et al.*, 2003; Qi *et al.*, 2011). In our experiment, the relative content of rondel increased while the size did not change regularly, similar to warming treatment, which indicated that the three types rondel have different formation mechanism. Besides, both length and width of echinate rondels increased in N addition treatment. As for elongates and hair cells, both content and size decreased sharply, and almost half of elongates in this treatment were hollow, that is, they were not silicified completely. Therefore, excessive N may affect the absorption of silicon, and suppress the formation of elongate. It can be inferred that high N level had a negative impact on phytolith in *L. chinensis*. Because silicification could improve the mechanical strength of cells, enhance the plant ability to resist pests and salt, the suppressed phytolith deposition caused by excessive nitrogen is not beneficial to growth of *L. chinensis* and its saline-alkali tolerance.

The increased relative content in rondels in warming

combined with N addition treatment differed from single warming or single N addition treatment, indicated that warming and N fertilization played cooperative effect on content of rondels in *L. chinensis*. The relative content in rondels and scale of two sub-types in hair cells was similar to single N addition, which further demonstrated that hair cells were more sensitive to N concentration than temperature level. Disappeared hollow elongates in this treatment means warming could reduce the negative effect of excessive N for elongates.

5 Conclusions

(1) Warming is positive while excessive nitrogen is negative to the deposition of phytoliths in *L. chinensis* leaves. Warming could reduce the negative impact of excessive nitrogen on phytolith in *L. chinensis*. The responses to elevated CO₂ were complicated, which needs further investigation.

(2) *L. chinensis* is a high quality pasture in Songnen grassland, as the negative effect of excessive N on phytolith could reduce the mechanical strength as well as the insect-resistance capacity of *L. chinensis*, more attention should be paid to the growth of *L. chinensis* in the future.

(3) The taxonomic of the short cell in graminea is significant because of no disappearance with simulated environmental changes. The phytolith originated in the long cell and plant intercellular space are more sensitive to elevated CO₂ concentration, warming, and excessive nitrogen, and could become some new indicators for environmental changes.

References

- Ahmad R, Zaheer S H, Ismail S, 1992. Role of silicon in salt tolerance of wheat (*Triticum aestivum* L.). *Plant Science*, 85(1): 43–50. doi: 10.1016/0168-9452(92)90092-Z
- Berry J, Bjorkman O, 1980. Photosynthetic response and adaptation to temperature in higher plants. *Annual Review of Plant Physiology*, 31(1): 491–543. doi: 10.1146/annurev.pp.31.060180.002423
- Carter J A, 2002. Phytolith analysis and paleoenvironmental reconstruction from Lake Poukawa Core, Hawkes Bay, New Zealand. *Global and Planetary Change*, 33(3): 257–267. doi: 10.1016/S0921-8181(02)00081-4
- Dawson R, Wei R, Tao S *et al.*, 2004. Analysis of silicon concentration periodicity for the past 2.4 Ma in sediments from Lake Baikal site BDP 96-2. *Climate Research*, 26(3): 193–197. doi:

- 10.1016/S0921-8181(02)00081-4
- Dirnböck T, Grandin U, Bernhardt-Römermann M *et al.*, 2014. Forest floor vegetation response to nitrogen deposition in Europe. *Global Change Biology*, 20(2): 429–440. doi: 10.1111/gcb.12440
- Epstein E, 1994. The anomaly of silicon in plant biology. *Proceedings of the National Academy of Sciences*, 91(1): 11–17. doi: 10.1073/pnas.91.1.11
- Fan Bin, Xu Shiyuan, Yu Lizhong *et al.*, 2006. Phytolith in the sediment of the Lake Chaohu since Middle Holocene and its paleoenvironmental implications. *Journal of Lake Sciences*, 18(3): 273–279. (in Chinese)
- Feng Dalan, Liu Yun, Huang Jianguo, 2009. Changes of biomass and nitrogen content of the reed (*Phragmites communis*) under different soil moisture conditions in the hydro-fluctuation belt of the Three Gorges Reservoir. *Acta Scientiae Circum Stantiae*, 29(9): 2003–2009. (in Chinese)
- Feng L, Li H, Jiao J *et al.*, 2009. Reduction in SBPase activity by antisense RNA in transgenic rice plants: effect on photosynthesis, growth, and biomass allocation at different nitrogen levels. *Journal of Plant Biology*, 52(5): 382–394. doi: 10.1007/s12374-009-9049-3
- Gunderson C A, Norby R J, Wullschlegel S D, 1993. Foliar gas exchange responses of two deciduous hardwoods during 3 years of growth in elevated CO₂: no loss of photosynthetic enhancement. *Plant, Cell and Environment*, 16(7): 797–807. doi: 10.1111/j.1365-3040.1993.tb00501.x
- Huang Fei, Kealhofer Lisa, Huang Fengbao, 2004. Diagnostic phytoliths from Nei Mongol Grassland. *Acta Palaeontologia Sinica*, 43(2): 246–253. (in Chinese)
- Inoue K, Sase T, 1996. Paleoenvironmental history of post-Toya ash tephric deposits and paleosols at Iwate volcano, Japan, using aeolian dust content and phytolith composition. *Quaternary International*, 34: 127–137.
- Jie D M, Liu Z Y, Shi L X *et al.*, 2010a. Characteristics of phytoliths in *Leymus chinensis* from different habitats on the Songnen Plain in Northeast China and their environmental implications. *Science China Earth Sciences*, 53(7): 984–992. doi: 10.1007/s11430-010-0047-6
- Jie Dongmei, Ge Yong, Guo Jixun *et al.*, 2010b. Response of phytolith in *Leymus chinensis* to the simulation of global warming and nitrogen deposition on Songnen grassland, China. *Environmental Science*, 31(8): 1708–1715. (in Chinese)
- Lanning F C, Eleuterius L N, 1985. Silica and ash in tissues of some plants growing in the coastal area of Mississippi, USA. *Annals of Botany*, 56(2): 157–172.
- Lewin J, Reimann B E F, 1969. Silicon and plant growth. *Annual Review of Plant Physiology*, 20(1): 289–304.
- Li Dejun, Mo Jiangming, Fang Yunting *et al.*, 2003. Impact of nitrogen deposition on forest plants. *Acta Ecologica Sinica*, 23(9): 1891–1900. (in Chinese)
- Li Nannan, Jie Dongmei, Wang Liukui *et al.*, 2013. The paleoclimate evolution recorded by phytolith in Gushantun peatland since the Late Pleistocene. *Journal of Northeast Normal University (Natural Science Edition)*, (3): 138–145. (in Chinese)
- Li Ronglin, Jie Dongmei, Liu Yanping *et al.*, 2011. Phytolith as an environmental indicator at the Hushan peat section from the northern Changbai Mountain, NE China. *Acta Micropalaeontologica Sinica*, 28(3): 329–336. (in Chinese)
- Li Z, Lin J, Zhang T *et al.*, 2014. Effects of summer nocturnal warming on biomass production of *Leymus chinensis* in the Songnen grassland of China: from bud bank and photosynthetic compensation. *Journal of Agronomy and Crop Science*, 200(1): 66–76. doi: 10.1111/jac.12041
- Liang Y, 1997. Effect of silicon on leaf ultrastructure, chlorophyll content and photosynthetic activity of barley under salt stress. *Pedosphere*, 8(4): 289–296.
- Liang Y, 1999. Effects of silicon on enzyme activity and sodium, potassium and calcium concentration in barley under salt stress. *Plant and Soil*, 209(2): 217–224. doi: 10.1023/A:1004526604913
- Liang Y, Chen Q, Liu Q *et al.*, 2003. Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (*Hordeum vulgare* L.). *Journal of Plant Physiology*, 160(10): 1157–1164. doi: 10.1078/0176-1617-01065
- Lin J X, Hu Y X, 1996. Structural response of soybean leaf to elevated CO₂ concentration. *Acta Botanica Sinica*, 38(1): 31–34.
- Lu H Y, Wu N Q, Yang X D *et al.*, 2006. Phytoliths as quantitative indicators for the reconstruction of past environmental conditions in China I: phytolith-based transfer functions. *Quaternary Science Reviews*, 25: 945–959. doi: 10.1016/j.quascirev.2006.10.006
- Ma J F, 2004. Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Science and Plant Nutrition*, 50(1): 11–18. doi: 10.1080/00380768.2004.10408447
- Makino A, Mae T, 1999. Photosynthesis and plant growth at elevated levels of CO₂. *Plant and Cell Physiology*, 40(10): 999–1006. doi: 10.1093/oxfordjournals.pcp.a029493
- Masle J, 2000. The effects of elevated CO₂ concentrations on cell division rates, growth patterns, and blade anatomy in young wheat plants are modulated by factors related to leaf position, vernalization, and genotype. *Plant Physiology*, 122(4): 1399–1416. doi: 10.1104/pp.122.4.1399
- McNaughton S J, Tarrants J L, McNaughton M M *et al.*, 1985. Silica as a defense against herbivory and a growth promotor in African grasses. *Ecology*, 66(2): 528–535. doi: 10.2307/1940401
- Phoenix G K, Hicks W K, Cinderby S *et al.*, 2006. Atmospheric nitrogen deposition in world biodiversity hotspots: the need for a greater global perspective in assessing N deposition impacts. *Global Change Biology*, 12(3): 470–476. doi: 10.1111/j.1365-2486.2006.01104.x
- Poorter H, 1993. Interspecific variation in the growth response of plants to an elevated ambient CO₂ concentration. *Vegetation*, 104(1): 77–97. doi: 10.1007/978-94-011-1797-5_6
- Prebble M, Schallenberg M, Carter J *et al.*, 2002. An analysis of phytolith assemblages for the quantitative reconstruction of

- late Quaternary environments of the Lower Taieri Plain, Otago South Island, New Zealand I. Modern assemblages and transferfunctions. *Journal of Paleolimnology*, 27: 393–413. doi: 10.1023/A:1020314719427
- Pritchard S H, Rogers H O, Prior S A *et al.*, 1999. Elevated CO₂ and plant structure: a review. *Global Change Biology*, 5(7): 807–837. doi: 10.1046/j.1365-2486.1999.00268.x
- Qi Y, Huang Y M, Wang Y *et al.*, 2011. Biomass and its allocation of four grassland species under different nitrogen levels. *Acta Ecologica Sinica*, 31(18): 5121–5129. (in Chinese)
- Reddy K R, Matcha S K, 2010. Quantifying nitrogen effects on castor bean (*Ricinus communis* L.) development, growth, and photosynthesis. *Industrial Crops and Products*, 31(1): 185–191. doi: 10.1016/j.indcrop.2009.10.004
- Romero-Aranda M R, Jurado O, Cuartero J, 2006. Silicon alleviates the deleterious salt effect on tomato plant growth by improving plant water status. *Journal of Plant Physiology*, 163(8): 847–855. doi: 10.1016/j.jplph.2005.05.010
- Rudall P J, Prychid C J, Gregory T, 2014. Epidermal patterning and silica phytoliths in grasses: an evolutionary history. *The Botanical Review*, 80(1): 59–71. doi: 10.1007/s12229-014-9133-3
- Sabine C, 2014. The IPCC fifth assessment report. *Carbon*, 5(1): 17–25. doi: 10.4155/cmt.13.80
- Sage R F, Sharkey T D, Seemann J R, 1989. Acclimation of photosynthesis to elevated CO₂ in five C₃ species. *Plant Physiology*, 89(2): 590–596. doi: 10.1104/pp.89.2.590
- Talhelm A F, Pregitzer K S, Burton A J, 2011. No evidence that chronic nitrogen additions increase photosynthesis in mature sugar maple forests. *Ecological Applications*, 21(7): 2413–2424. doi: 10.1890/10-2076.1
- Trombold C D, Israde-Alcantara I, 2005. Paleoenvironment and plant cultivation on terraces at La Quemada, Zacatecas, Mexico: the pollen, phytolith and diatom evidence. *Journal of Archaeological Science*, 32(3): 341–353. doi: 10.1016/j.jas.2004.10.005
- Wang Yongji, Lv Houyuan, 1993. *Study and Application of Phytolith*. Beijing: China Ocean Press. (in Chinese)
- Yamori W, Hikosaka K, Way D A, 2014. Temperature response of photosynthesis in C₃, C₄, and CAM plants: temperature acclimation and temperature adaptation. *Photosynthesis Research*, 119(1–2): 101–117. doi: 10.1007/s11120-013-9874-6
- Yao X, Liu Q, 2007. Changes in photosynthesis and antioxidant defenses of *Picea asperata* seedlings to enhanced ultraviolet-B and to nitrogen supply. *Physiologia Plantarum*, 129(2): 364–374. doi: 10.1111/j.1399-3054.2006.00815.x
- Zeng Qing, Zhu Jianguo, Liu Gang *et al.*, 2002. Effect of FACE on competition between a C₃ crop (rice, *Oryza sativa*) and a C₄ weed (brary ardgrass, *Echinochloa crusgalli*). *Chinese Journal of Applied Ecology*, 13(10): 1231–1234. (in Chinese)
- Zhu Z, Wei G, Li J *et al.*, 2004. Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (*Cucumis sativus* L.). *Plant Science*, 167(3): 527–533. doi: 10.1016/j.plantsci.2004.04.020
- Zou D, 2005. Effects of elevated atmospheric CO₂ on growth, photosynthesis and nitrogen metabolism in the economic brown seaweed, *Hizikia fusiforme* (Sargassaceae, Phaeophyta). *Aquaculture*, 250(3): 726–735. doi: 10.1016/j.aquaculture.2005.05.014