RESEARCH ARTICLE

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Effects of lanthanum and silicon stress on bio-sequestration of lanthanum in phytoliths in rice seedlings

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Abstract

Plant phytoliths are important for silicon (Si) cycling in natural ecosystems; however, their role in lanthanum (La) sequestration in plants is still unclear. In the present study, we elucidated the mechanism of La-induced damage to the growth of rice (*Oryza sativa* L.) seedling from the viewpoint of the La sequestration by phytoliths (PhytLa). The phytoliths were extracted by using the microwave digestion method. La concentrations within the plants and phytoliths were determined by a modified lithium metaborate fusion method. Analysis showed that pretreatment with low La concentration not only promoted photosynthesis and transpiration in rice but also enhanced the sequestration ability of phytoliths on La. Conversely, high La concentration inhibited photosynthesis and transpiration in rice and the ability of phytoliths to sequester La. Moreover, high Si concentrations promoted the sequestration ability of phytoliths during these processes. Promotion of combined stress of La and Si on the ability of rice seedling was stronger than that of the single La stress. The sequestration ability of phytoliths in different parts of rice varied significantly, following the order: stem > leaf > root. This pattern could be attributed to factors such as the production of various phytolith morphotypes (such as tubes) and PhytLa, PhytLa efficiency, La accumulation, and the rate of photosynthesis and transpiration in different parts of rice seedlings. This study demonstrated that La uptake in rice seedlings was affected by the presence of Si in the medium, and phytolith played a crucial role in the bio-sequestration of La and assuaged the damage caused by La in rice seedlings.

Keywords Lanthanum · Phytolith · Rice · Silicon · Photosynthesis · Transpiration

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Abbreviations

PhytLa	La sequestrated by phytoliths
REE	Rare earth element
Ca + b/Ca/b	Chlorophyll a + b/chlorophyll a/b
EDTA	Ethylenediaminetetraacetic acid
ICP-AES	Inductively coupled plasma-atomic emission spectroscopy
PLCO	PhytLa content of organs
PLSA	Sequestration ability of phytoliths on La
Pn	Net photosynthetic rate
Gs	Stomatal conductance
Tr	Transpiration rate
DMSO	Dimethyl sulfoxide
BSi	Biogenic silicon

Introduction

Phytoliths, or plant opals, are amorphous silica deposits formed in the cell walls, intercellular spaces, cell lumen of various plant

tissues (Song et al. 2012a, b). Phytoliths are ubiquitously found in the plant kingdom and are especially abundant in the members of family Gramineae such as bamboo (*Phyllostachys praecox* f. Prevernalis Chen et Yao) (Huang et al. 2014), millets (*Panicum miliaceum* L.) (Zuo and Lü 2011), sugarcane (*Saccharum officinarum* L.) (Parr et al. 2009), wheat (*Triticum aestivum* L.) (Parr and Sullivan 2011; Zuo et al. 2014), and rice (*Oryza sativa* L.) (Li et al. 2013a). Phytoliths are well-known for their interesting function of encapsulating living organelles into the body, such as the chloroplast, mitochondria, plasmids, and other organelles (Carter 2009). Thus, phytoliths have generated widespread interest, and several methods for effectively managing future climate change have been proposed by using phytolith-occluded carbon (PhytC) as a "safe" carbon sink (Zuo et al. 2014; Li et al. 2013a). Our previous study showed that La^{3+} could enter the cells in the form of nanoscale particles by endocytosis of plant cells (Wang et al. 2014). Kameník et al. (2013) reported that plants could internalize various rare earth elements (REEs) as part of phytoliths. Plant cells respond to the external environment changes by transmitting external signal from the extracellular to the intracellular cell (Aragay et al. 2011). Thus, phytoliths have close correlations with the growth and development of plants and may regulate the toxic effects of REEs in plants, animals, and humans (Hodson and Sangster 1999).

Lanthanum (La) is a metallic element belonging to the REE group, which consists of 17 elements due to their physical and chemical similarity. This group is abundant, making up 0.015% of the Earth's crust (Taylor and McLennan 1985). The REEs have wide-scale application in many branches of the industry, agriculture, medicine, and other areas (EPA 2012). The increasing demand for these elements triggered their release into the environment (Hu et al. 2006). The quantity of La in the environment and microfertilizers was higher compared with other REEs (Hu et al. 2004). In contrast to the other REEs, most toxicological studies have been centered on the uptake of La by living organisms. Optimal concentrations of La were beneficial for plants such as maize (*Zea mays* L.), in which La was found to improve the salt tolerance of maize seedlings by enhancing the functions of the photosynthetic apparatus and antioxidant capacity (Liu et al. 2016). Moreover, in bell pepper (*Capsicum annuum* L.), La improved the seedling quality by modifying the plant morphological features such as plant height and stem diameter, and physiological features such as concentration of chlorophyll, amino acids, proteins, and sugars (García-Jiménez et al. 2017). However, excessively high La concentrations applied to plants may induce negative responses, such as the inhibition of chlorophyll a/b, peroxidase activity, and cell membrane permeability in rice (Zeng et al. 2006), and nutrient imbalance in the root tips of faba bean (*Vicia faba* L.) (Wang et al. 2012a). Notably, individual parts of species contained different concentrations of REE, such as the average REE

concentrations in leaves, stems, and roots of a natural fern *Dicranopteris dicbotoma* Bernh. were respectively found to be in the range 3065–3358, 41.0–45.7, and 30.8–38.6 mg/kg (ppm, wt) REE (Wei et al. 2005). The leaves of a perennial fern *Dicropteris dicbotoma* Bernh. were shown to contain 2148 mg/kg (ppm, wt) La (Shan et al. 2003). Nonetheless, the biochemical processes and mechanisms underlying REE uptake and release in plants still remain unknown.

This study was conducted to explore the possibility of La sequestration by phytoliths (PhytLa) in rice seedlings, which typically accumulated Si. Our objectives were to determine the factors that assist the sequestration of La by phytolith and the role of phytolith in the growth of rice seedling. This study will help understand the behavior pattern of REEs in the soil-plant system and explore the role of REEs in natural ecosystem and agricultural systems. Moreover, our data will allow the assessment of REEs environment safety.

Materials and methods

Plant material and growing conditions

Hydroponic experiments were carried out in a growth chamber, under controlled conditions, from June to August in 2015, using the seeds of rice cultivar (cv. Huai No. 8). The seeds were disinfected with 3% (*v/v*) hydrogen peroxide for 30 min, washed several times with deionized water, soaked for 24 h, and germinated on moist gauze at 28 °C for 48 h in a constant temperature incubator. After germination, the seeds were sown in a plastic container with quartz sand and irrigated with 1/2-strength modified Hoagland nutrient solution with the following composition: $(NH_4)_2SO_4$ 1.25 mM, K_2SO_4 0.35 mM, $CaCl_2$ 1.00 mM, $MgSO_4$ 1 mM, $MnCl_2$ 9 μM, Na_2MoO_4 0.39 μM, H_3BO_3 20 μM, $ZnSO_4$ 0.77 μM, $CuSO_4$ 0.32 μM, NaEDTAFe 20 μM, and $C_2H_4N_4$ 23.79 μM in deionized water. The growth chamber was maintained at a temperature of 35 °C for 13 h (light) and 25 °C for 11 h (dark) with a light intensity of 400 μmol/m^2/s, and the relative humidity of 70–80%. At the onset of the third, the seedlings were transplanted into 5-L plastic pots with 96 plants in each of which 4 L nutrient solution was added and renewed completely every 2 days. The nutrient solution was supplemented with available Si ($NaSiO_3 \cdot 9H_2O$) and available La ($LaCl_3$) after 3 days.

Based on the current La level in the global soil (Biasioli et al. 2012; Hu et al. 2006) and average levels of monosilicic acid in the global soil (Sommer et al. 2006; Epstein 2001), we set up 16 treatments as shown in Table 1:

In Table 1, T1 represents the control (CK). The pH of each solution was adjusted to 5.5 with NaOH and a solution of concentrated H_2SO_4 and HNO_3 at a ratio of 3:1 (*v/v*). A solution of 1 mM KH_2PO_4 was used to spray the plants every 2 days; the nutrient solution was renewed completely every 3 days. Three

replicates were maintained for each treatment, and there were five pots per treatment replicate. All treatments were maintained for 22 days under the combined stress of Si and La.

Phytolith analysis

Preparation of plant materials

Both control and treated rice seedlings, under stress for 22 days, were cleaned using an ultrasonic bath for 15 min first and then rinsed with ultrapure water. These samples were dried at 105 °C for 20 min and then at 75 °C to constant weight. All samples were milled in preparation for measurements.

Determination of the phytolith content in rice samples subject to various La and Si treatments

A microwave digestion method as described by Parr et al. (2001) was used to extract phytoliths from plant samples using the Walkley-Black type digest (Walkley and Black 1934). The extracted phytoliths were dried to a constant weight in a drying oven at 75 °C, then sealed in a pre-weighed centrifuge tube and weighed to calculate the phytolith content.

Analytical method for phytolith morphology

To observe phytolith morphology, we used the heating digestion method, as described by Lu et al. (2009). The final uniform liquid samples of 5 μL obtained from plant digestion were mounted on a fixed slide with neutral resins, and the representative phytolith morphology and microstructure on each slide were observed using optical microscopy (Meunier et al. 1999). The phytolith morphotypes on the fixed slide were counted and expressed as the content of phytolith morphology (grains/g).

Extraction of La using lithium metaborate fusion

Phytoliths and plant samples were extracted with a modified lithium metaborate fusion method (Blecker et al. 2006; Ingamels 1964). The samples were placed in a high-purity graphite crucible, stirred homogeneously with lithium metaborate, and then combusted in a muffle furnace. During the ashing process, the temperature was gradually increased to 200 °C and sustained for 10 min, then further increased to 500 °C and sustained for 5 min, and finally heated up to 950 °C and sustained for 15 min. After cooling, the ash was dissolved in dilute nitric acid, and dissolution was accelerated using a magnetic stirrer. The phytolith-sequestrated La (PhytLa) and plant total La were determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES). The Si content was measured by molybdenum blue spectrophotometric method (Mortlock and Froelich 1989), and the

spectrophotometer was used to do colorimetric analysis at 700 nm. All measurements mentioned above were monitored with controls consisting of a standard soil sample (GBW07405, GSS-5) and a standard plant sample (GBW 07603, GSV-2). The precision of better than 5% was achieved through the analysis of the standard sample. PhytLa content of organs (PLCO) (%, wt) was calculated by the following formula:

$$PLCO = Phytolith\ content \times PhytLa\ content/100 \qquad (1)$$

where the phytolith content represented the weight percentage of phytolith to the dry weight of the plant (%, wt), and PhytLa content represented the percentage of La in phytolith by weight (%, wt). Moreover, the sequestration ability of phytoliths on La (PLSA, wt) was estimated by the ratio of the content of PhytLa of organs to La and was calculated by the following formula:

$$PLSA = PLCO/La\ content \qquad (2)$$

where PLCO was calculated by Eq. (1). The greater the PLSA, the more La sequestrated by phytoliths in rice seedlings.

Photosynthesis parameters

The rice leaves from all treatments stressed with La and Si for 22 days were used for measuring the net photosynthetic rate (Pn), stomatal conductance (Gs), and transpiration rate (Tr) using a portable gas exchange fluorescence system (GFS-3000, Heinz WalzGmbH). The measurements were performed using three replicates. The light intensity during the measurements was 1100 mmol/m^2/s, the air temperature was 25–30 °C, and the test time was 9–12 am.

Assay of contents of chlorophyll a and b

Chlorophyll was extracted from 0.02 g of rice leaf of all treatments. Dimethyl sulfoxide (DMSO) was used for extraction as described previously (Lichtenthaler 1987). The absorbance values were measured as A_{663} and A_{645} at 663 and 645 nm respectively, using a spectrophotometer and then chlorophyll a and b were calculated according to the following formulas:

$$Chlorophyll\ a = 12.7A_{663} - 2.69A_{645} \qquad (3)$$
$$Chlorophyll\ b = 22.9A_{645} - 4.68A_{663} \qquad (4)$$

Statistical analysis

The data used in this paper were analyzed by one-way ANOVA, and statistical analysis was performed by Duncan's multiple range test to check for significant differences. Data analysis and mapping were done with the Excel and SPSS software.

Table 1 Various La and Si treatments in the experimental design

| Treatments	SiO$_2$ (mg/L)	LaCl$_3$ (mg/L)	Treatments	SiO$_2$ (mg/L)	LaCl$_3$ (mg/L)
T1 (CK)	0	0	T9	90	0
T2	0	20	T10	90	20
T3	0	100	T11	90	100
T4	0	300	T12	90	300
T5	15	0	T13	210	0
T6	15	20	T14	210	20
T7	15	100	T15	210	100
T8	15	300	T16	210	300

Results

Phytolith morphology in rice seedlings in response to various La and Si treatments

We recorded 10,935 grains of phytoliths with nine morphotypes in our 16 experimental treatments (Table S1). The various morphotypes included concave dumbbell, panicoid dumbbell, complex rondels, bulliform, crenate, tube, fan, elongate smooth, and tabular psilate (Fig. 1). Concave dumbbells and panicoid dumbbells and tubes were the predominant phytolith shapes and accounted for more than 70% of the total phytoliths. Thus, we named these three phytolith morphotypes as the "most common morphotypes" in rice. The content of complex rondels, bulliforms, crenates, and tubes was correlated with the PhytLa content, phytolith content, and Si content (Table S2). We speculated that these morphotypes depended on the La sequestration by phytoliths

Fig. 1 The majority of phytolith morphotypes in rice seedlings are as follows: **a** concave dumbbell; **b**, **c** panicoid dumbbell; **d**, **e** complex rondels; **h**, **j**, **k** bulliform; **f**, **i** crenate; **l** tube; **g** fan; **m** elongate smooth; **n** tabular psilate
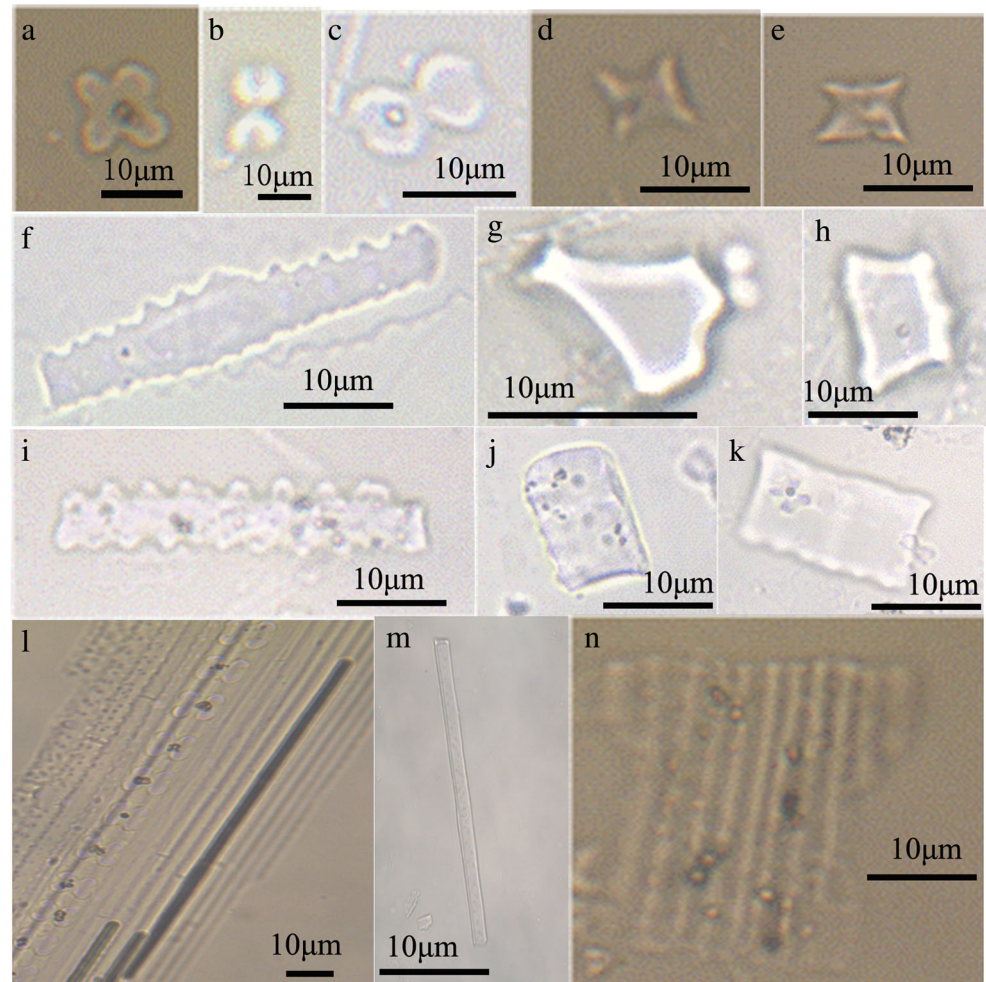
and phytolith accumulation; thus, we named them "sensitive morphotypes" in rice.

This study confirmed that the content of various phytolith morphotypes derived from cells of different shapes had an enormous discrepancy in different organs (Table S1). Unlike other organs, the content of various phytolith morphotypes in the leaves was higher, because it was affected by the important life functions of leaves such as transpiration and photosynthesis, where the transpiration stream ends. For example, the tube content in leaves increased along with the increase of Si level, which was a sharp contrast to that in roots and stems (Table 2). Similar variations were exhibited in complex rondels, bulliforms, and crenates. Thus, the available Si promoted the Si accumulation ability in leaves, where various epidermal cells such as bulliform, dumbbell, and long-short cells were siliconized to enhance transpiration and photosynthetic efficiency of leaves. Moreover, the available La also affected the content of various phytolith morphotypes in leaves. For example, the content of tubes in leaves was maximum at La level of 20 mg/L.

On the other hand, under combined treatments with La and Si, the content of tubes was significantly different from the single treatment with La or Si. Firstly, compared with the control (CK) and the single treatment with Si (15 or 90 mg/L), the content of tubes increased remarkably in the combined treatments with low La (20 mg/L) and appropriate Si (15 or 90 mg/L). While compared with the single treatment with low La, the content of tubes was unchanged in the combined treatment with low La and low Si, but it increased significantly in the combined treatment with low La and 90 mg/L Si. Compared with the CK and the single treatment with low La, the content of tubes in the combined treatments with low La and 210 mg/L Si increased evidently, while it decreased remarkably compared with the single treatment with 210 mg/L La. Secondly, compared with the CK, the content of tubes in the combined treatment with low Si and 100 mg/L La was unchanged, while it decreased significantly compared with the single treatment with 100 mg/L La or 15 mg/L Si. Compared with the CK, the single treatment with 100 mg/L La or 90 mg/L Si, the content of tubes increased remarkably in the combined treatments with 100 mg/L La and 90 mg/L Si. Compared with the CK, the single treatment with 100 mg/L La, the content of tubes increased evidently in the combined treatments with 100 mg/L La and 210 mg/L Si, while it

showed a decreasing trend compared with the single treatment 210 mg/L Si. Thirdly, compared with the CK, the single treatment with La (300 mg/L) or Si (15 or 90 mg/L), the content of tubes increased significantly in the combined treatments with 300 mg/L La and appropriate Si (15 or 90 mg/L), and the increase was more obvious in the combined treatment with 300 mg/L La and 90 mg/L Si. Compared with the CK and the single treatment with 300 mg/L La, the content of tubes increased remarkably in the combined treatments with 300 mg/L La and 210 mg/L Si, while it decreased evidently compared with the single treatment with 210 mg/L Si. In addition, by calculating the mean of the tube content in the treatments with different Si, we found that the content of tubes showed an increasing trend along with the increasing Si level, and it was similar with the variation trend of the single treatment with different Si (T1, T5, T9, and T13). Hence, these confirmed that Si and La affected the formation of tubes, and the tubes were important morphotype for the accumulation of phytoliths in rice.

Phytolith accumulation in various tissues of rice seedlings subject to different Si and La concentrations

Figure 2 shows the phytolith content in various rice organs affected by different Si and La concentration, and we found the following four phenomena: firstly, in the single treatment with Si, the phytolith content in various rice organs all showed an increasing trend along with the increasing Si level. While in the single treatment with La, along with the increasing La level, the phytolith content in rice leaves and stems first increased then decreased, 20 mg/L La was its threshold, and the phytolith content reached maximum, while the phytolith content in rice roots reached maximum when La was 100 mg/L, and it was higher in the single treatments with 100 or 300 mg/L La compared with that in the single treatment with low La and CK. Secondly, compared with the CK and the single treatment with low La, the phytolith content in rice leaves and stems increased gradually in the combined treatment with low La and increasing Si, while variation of phytolith content in rice roots was not obvious, and it decreased first then increased. Thirdly, compared with the CK and the single treatment with 100 mg/L La, the phytolith content in rice leaves increased gradually in the combined treatment with 100 mg/L

Table 2 Tube content in leaves under different treatments

Treatments	Grains/g	Treatments	Grains/g	Treatments	Grains/g	Treatments	Grains/g
T1 (CK)	15 (2.15)	T5	20 (2.13)	T9	55 (5.33)	T13	175 (7.55)
T2	45 (3.23)	T6	45 (4.26)	T10	65 (6.43)	T14	85 (8.12)
T3	30 (5.22)	T7	15 (3.12)	T11	130 (8.75)	T15	140 (9.45)
T4	15 (2.11)	T8	50 (5.25)	T12	90 (6.55)	T16	50 (5.13)
Means	26.25	Means	32.5	Means	85	Means	112.5

The data in the table represent the average of three repetitions; the data in the bracket mean its standard deviation
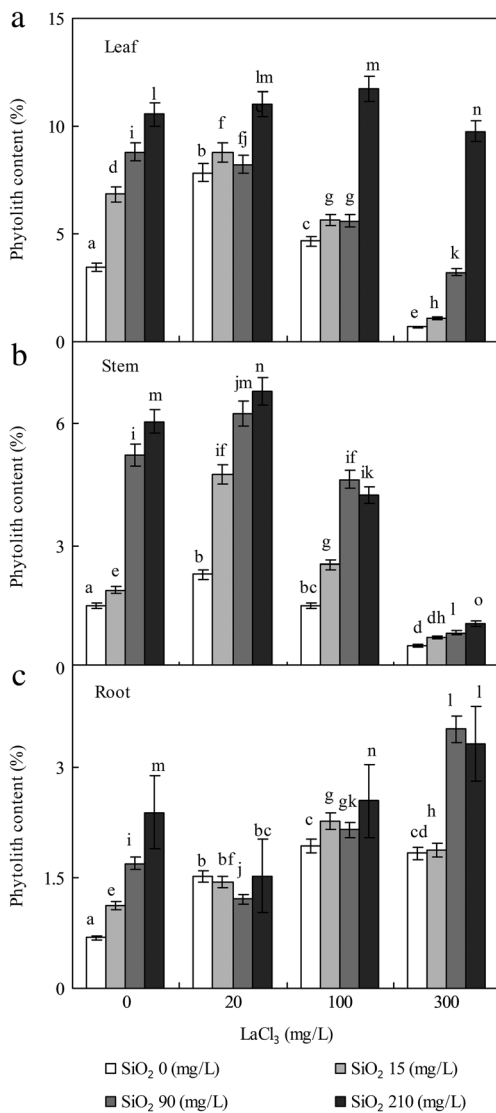
Fig. 2 a–c Variation of phytolith content in the combined treatments with different Si and La levels in rice leaf, stem, and root tissues. Means with various letters are significantly different at the $P < 0.05$ level of confidence according to Duncan's multiple range test. Error bars represent the standard deviations of the mean. $n = 3$

La and increasing Si level, while the phytolith content in rice stems reached maximum when Si was 90 mg/L and it decreased slightly when Si was 210 mg/L, but it showed an increasing trend in general, but the phytolith content in rice roots showed a fluctuant increase trend. Compared with the single treatment with 15 or 90 mg/L Si, the phytolith content in rice leaves decreased significantly in the combined treatment with 100 mg/L La and 15 mg/L or 90 mg/L Si, while compared with the single treatment with 210 mg/L Si, it increased obviously in the combined treatment with 100 mg/L La and 210 mg/L Si. Compared with the single treatment with 15 mg/L Si, the phytolith content in stems increased significantly in the combined treatment with 100 mg/L La and 15 mg/L Si. Compared with the single treatment with 90 or

210 mg/L Si, the phytolith content in stems decreased remarkably in the combined treatment with 100 mg/L La and 90 or 210 mg/L Si. Compared with the single treatments with various Si concentration (15, 90, or 210 mg/L), the phytolith content in roots all increased significantly in the combined treatments with 100 mg/L La and various Si concentration (15, 90, or 210 mg/L). Fourthly, compared with the CK, the phytolith content in leaves and roots were higher and all showed an increasing trend in the combined treatment with 300 mg/L La and increasing Si level, while the phytolith content in stems was lower but it also showed an increasing trend. Compared with the single treatment with Si (15, 90, or 210 mg/L), in the combined treatments with 300 mg/L La and corresponding Si (15, 90, or 210 mg/L), we found that the phytolith content in leaves and stems showed a decreasing trend, while the phytolith content in roots showed an obvious increasing trend.

The order of the mean of phytolith content in different rice organs was: leaves > stems > roots (Table 3). Moreover, a significant correlation existed between Si content and phytolith content in leaves ($P < 0.0005$), but Si content and phytolith content in stems and roots were not significant ($P > 0.05$ in both stems and roots) (Fig. 7a). Thus, the accumulation of phytoliths in different parts was significantly different, and available Si in culture solutions promoted the production of phytoliths in rice seedlings.

The accumulation of PhytLa and La in various rice tissues in response to different Si and La concentrations

The PhytLa content in various rice organs means the efficiency of La sequestration by phytoliths in different rice organs. Figure 3 shows the variation of PhytLa content in the roots, stems and leaves of rice seedlings affected by La and Si. The results exhibited three phenomena: firstly, compared with the CK, in the single treatments with increasing Si level, the leaf PhytLa content in rice seedlings increased first then decreased, and it reached maximum when Si level was 90 mg/L, while the stem PhytLa content showed adverse variation trend, and it reached minimum when Si level was 90 mg/L; the root PhytLa content first increased then decreased, and it reached maximum when Si level was 15 mg/L. In the single treatments with increasing La level, the PhytLa content in various rice organs showed similar variation trend, and they all increased gradually. Secondly, in the combined treatments with low La and different Si level, the variation of PhytLa content in various rice organs was different. Compared with the CK, the PhytLa content in leaves was higher in the combined treatments with low La and different Si (15, 90 or 210 mg/L), but it was lower compared with that of the single treatment with low La, and it also increased compared with that of the single treatment with corresponding Si concentration (15, 90, or 210 mg/L). Compared with the CK and the single treatment

Table 3 Comparison of phytolith content, PhytLa content, total La content, and the PLSA in different rice seedlings' tissues (roots, leaves, stem) under various Si concentrations regardless of the La concentration

SiO$_2$ (mg/L)	Phytolith content (%)			PhytLa content (%)		
	Root	Stem	Leaf	Root	Stem	Leaf
AT0	1.4934 ± 0.5657	1.4713 ± 0.7170	4.1663 ± 2.9633	0.0756 ± 0.0613	0.0303 ± 0.0222	0.0175 ± 0.0115
AT15	1.6796 ± 0.5043	2.4856 ± 1.6899	5.5916 ± 3.2514	0.0864 ± 0.0704	0.0396 ± 0.0317	0.0150 ± 0.0099
AT90	2.1482 ± 0.9957	4.2361 ± 2.3454	6.4628 ± 2.5746	0.0881 ± 0.0910	0.0270 ± 0.0240	0.0146 ± 0.0064
AT210	2.4500 ± 0.7386	4.5376 ± 2.5380	10.7712 ± 0.8371	0.1156 ± 0.1269	0.0282 ± 0.0206	0.0074 ± 0.0011
Means	1.9428 ± 0.4361	3.1827 ± 1.4561	6.7480 ± 2.8443	0.0914 ± 0.0171	0.0313 ± 0.0057	0.0136 ± 0.0044
SiO$_2$ (mg/L)	La content (%)			PLSA		
	Root	Stem	Leaf	Root	Stem	Leaf
AT0	0.2227 ± 0.2889	0.0428 ± 0.0570	0.0500 ± 0.0530	0.0189 ± 0.0263	0.0201 ± 0.0158	0.0226 ± 0.0198
AT15	0.1827 ± 0.1958	0.0235 ± 0.0197	0.0347 ± 0.0324	0.0103 ± 0.0025	0.0434 ± 0.0309	0.0306 ± 0.0197
AT90	0.1739 ± 0.1860	0.0227 ± 0.0184	0.0224 ± 0.0168	0.0114 ± 0.0048	0.0468 ± 0.0242	0.0496 ± 0.0233
AT210	0.1639 ± 0.1834	0.0225 ± 0.0164	0.0213 ± 0.0115	0.0172 ± 0.0061	0.0586 ± 0.0360	0.0436 ± 0.0157
Means	0.1858 ± 0.0258	0.0278 ± 0.0099	0.0321 ± 0.0133	0.0144 ± 0.0042	0.0422 ± 0.0161	0.0366 ± 0.0122

Values are means ± standard deviation error, $n = 4$. AT0 represents the average of the treatments of T1, T2, T3, and T4; AT15 represents the average of the treatments of T5, T6, T7, and T8; AT90 represents the average of the treatments of T9, T10, T11, and T12; AT210 represents the average of the treatments of T13, T14, T15, and T16

with low La, the PhytLa content in stems in the combined treatment with low La and 15 mg/L Si increased significantly, while it decreased slightly along with the increasing Si level, but it showed an increasing trend compared with the single treatment with corresponding Si level (15, 90, or 210 mg/L). In roots, compared with the CK, the PhytLa content increased in the combined treatments with low La and different Si (15, 90, or 210 mg/L), while compared with the single treatment with low La, it increased in the combined treatment with low La and 15 mg/L Si, but decreased in the combined treatments with low La and 90 or 210 mg/L Si. Generally, low La prompted the PhytLa content in various organs of rice seedling under different Si levels, while 15 mg/L Si improved the PhytLa content in stems and roots but inhibited the PhytLa content in leaves under low La level, and 90 or 210 mg/L Si caused the decrease of the PhytLa content in various organs, implying that the La sequestration by phytoliths was inhibited. Thirdly, compared with the CK, the PhytLa content in various organs increased significantly in the combined treatments with high La (100 or 300 mg/L) and increasing Si (15, 90, or 210 mg/L). Compared with the single treatment with 100 or 300 mg/L La, the PhytLa content in leaves decreased in the combined treatments with high La (100 or 300 mg/L) and increasing Si (15, 90, or 210 mg/L), while the PhytLa content in stems first increased then decreased, and it reached maximum when Si was 15 mg/L and La was 100 or 300 mg/L; the PhytLa content in roots increased gradually. Consequently, La in the environment can promote the efficiency of La sequestration by phytoliths, while Si inhibited it in leaves but promoted it in roots, and low Si (15 mg/L) promoted it while high Si (90 or 210 mg/L) inhibited it in stems.

The La content in various organs means the total content of the accumulation of La, including the content of La sequestrated by phytoliths, namely PLCO. Figure 4 shows the variation trend of La content in the roots, stems, and leaves of rice seedlings affected by different La and Si concentrations. We observed the following three phenomena: firstly, in the single treatments with Si, the La content in rice leaves, stems, and roots changed slightly compared with other treatments with different Si level, to be specific, the La content in leaves and stems decreased slightly along with the increase of Si level, while the La content in roots increased slightly. Moreover, in the single treatments with La, the La content in leaves, stems, and roots all increased gradually along with the increase of La level. Secondly, in the combined treatments with low La and different Si concentration, the La content in rice leaves and stems decreased gradually along with the increase of Si level, while the La content in roots first increased then decreased, and 15 mg/L Si concentration caused it to reach maximum under the condition of low La concentration. Besides, the La content in all combined treatments with low La and various Si concentration (15, 90, or 210 mg/L) was higher than that of the single treatment with low La concentration. Compared with the single treatments with different Si concentration (15, 90, or 210 mg/L), the La content in leaves, stems and roots all showed an increasing trend in the combined treatments with low La and different Si level, while they all increased compared with the CK, Thirdly, compared with single treatment with high La (100 or 300 mg/L), the La content in leaves, stems and roots all decreased gradually in the combined treatments with high La (100 or 300 mg/L) and increasing Si level, except for the La content in stems in the combined treatments with 100 mg/L La and increasing Si, which increased gradually compared with
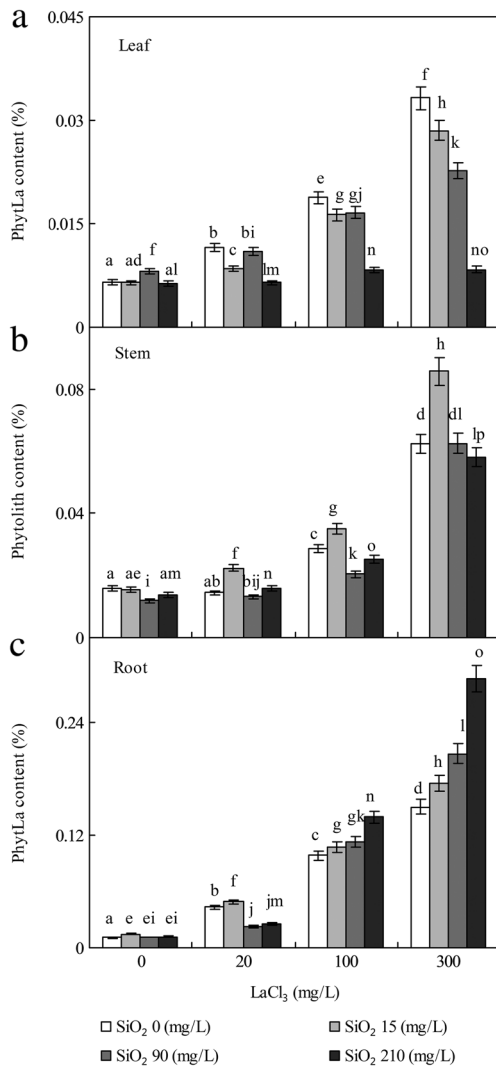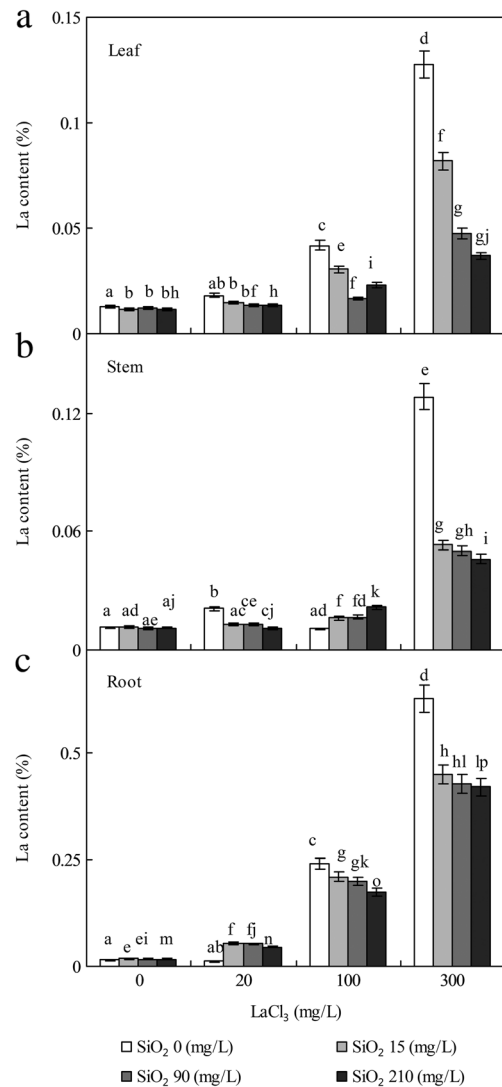
Fig. 3 a–c Variation of PhytLa content in the combined treatments with different Si and La levels in rice leaf, stem, and root tissues. Means with various letters are significantly different at the $P < 0.05$ level of confidence according to Duncan's multiple range test. Error bars represent the standard deviations of the mean. $n = 3$

Fig. 4 a–c Variation of the La content in the combined treatments with different Si and La levels in rice leaf, stem, and root tissues. Means with various letters are significantly different at the $P < 0.05$ level of confidence according to Duncan's multiple range test. Error bars represent the standard deviations of the mean. $n = 3$

the single treatment with 100 mg/L La. Compared with the CK and the single treatment with different Si level, the La content in leaves, stems, and roots all increased significantly in the combined treatments with 100 or 300 mg/L La and different Si level. All these proved that La promoted the accumulation of La in leaves, stems, and roots, while adding Si broke this process and inhibited the La accumulation.

Moreover, in roots with increased Si levels, the content of PhytLa increased, but the content of La decreased (Table 3). However, in leaves and stems with increased Si, the content of PhytLa and La both tended to decrease (Table 3). The average PhytLa content was the highest in roots followed by the stem, and the lowest in leaves (Table 3). Moreover, La was significantly positively correlated with PhytLa content of the roots, stems and leaves of rice seedlings ($P < 0.0005$ in all parts). Similarly, there was a significant positive correlation between the content of

PhytLa and the phytoliths in roots ($P < 0.0005$); however, there was a significant negative correlation between the content of PhytLa and phytolith in both leaves and stems ($P < 0.0005$ in leaves, and $P < 0.001$ in stems) (Fig. 7b, c). These results showed that the accumulation patterns of PhytLa and La were significantly different but showed a change with external La and Si among various organs within rice seedlings.

Sequestration ability of phytoliths on La in various tissues of rice seedlings subject to different Si and La concentrations

To find out the variation of La sequestration ability by phytolith of rice seedling in different pretreatments, we investigated the PLSA value of rice leaves in different treatments. Figure 5b
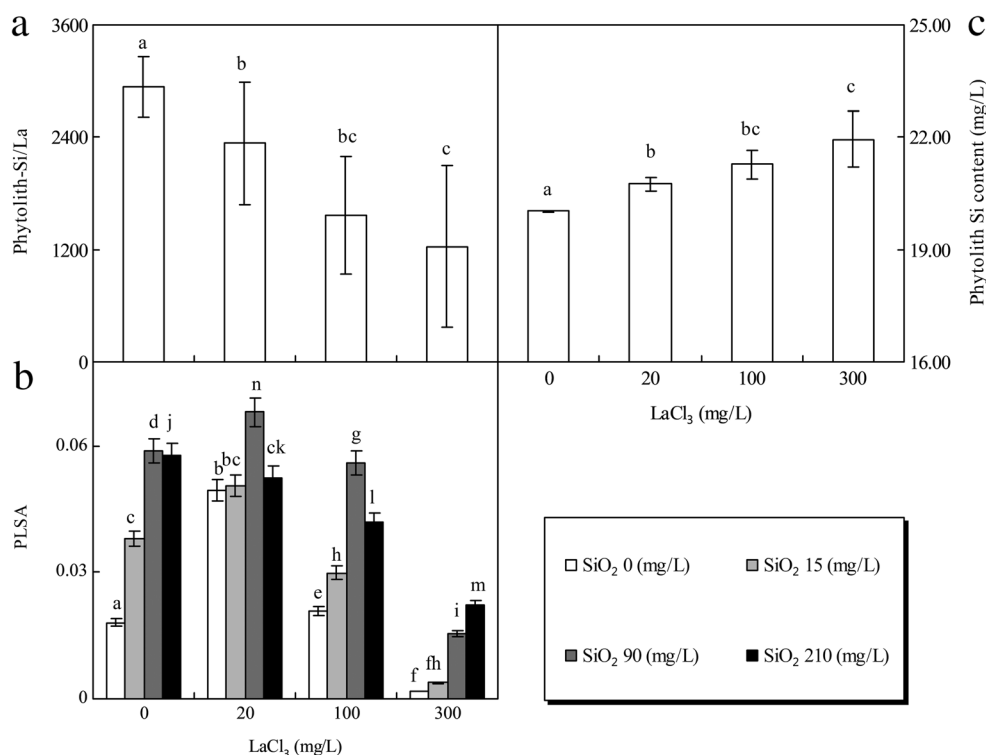
Fig. 5 **a** The average ratio of Si/La in leaf phytoliths of rice seedlings in the pretreatments with different La level regardless of Si level. Means with various letters are significantly different at the $P < 0.05$ level of confidence according to Duncan's multiple range test. Error bars represent the standard deviations of the mean. $n = 4$. **b** The PLSA in leaves of rice seedlings with the pretreatments with different La and Si levels. Means with various letters are significantly different at the $P < 0.05$ level of confidence according to Duncan's multiple range test. Error bars represent the standard deviations of the mean. $n = 3$. **c** The average content of Si in leaf phytoliths of rice seedlings in the pretreatments with different La level regardless of Si level. Means with various letters are significantly different at the $P < 0.05$ level of confidence according to Duncan's multiple range test. Error bars represent the standard deviations of the mean. $n = 4$

shows that compared with the control, the PLSA in leaves treated with 20, 100, and 300 mg/L La increased by 175.44, 15.74, and − 89.89%. The PLSA in leaves treated with 15, 90, and 210 mg/L Si increased by 111.11, 227.78, and 221.61%, respectively. Under the combined treatment with 20 mg/L La and Si at 15 mg/L (90 and 210 mg/L), compared with the control and the treatment of 20 mg/L La alone or Si alone at 15 mg/L (90 and 210 mg/L), the PLSA increased by 181.11% (1.98%, 32.91%), 5.01% (37.27%, 15.65%), and 191.72% (5.91%, − 9.30%), respectively. When rice seedlings were treated with 100 mg/L La and Si at 15 mg/L (90 and 210 mg/L), compared with that of the control and the single treatment of 100 mg/L La or Si at 15 mg/L (90 and 210 mg/L), the PLSA increased by 66.39% (43.76%, − 21.27%), 210.98% (168.68%, − 4.89%), 133.23% (101.51%, − 1.59%), respectively. When rice seedlings were treated with 300 mg/L La and Si at 15 mg/L (90 and 210 mg/L), compared with that of the control and the single treatment of 300 mg/L La or Si at 15 mg/L (90 and 210 mg/L), the PLSA increased by − 78.45% (113.13%, − 89.80%), − 14.93% (741.37%, − 73.98%), 22.93% (1115.82%, − 61.78%), respectively. Moreover, in Table 3, with the increase in Si concentration, regardless of La concentration, the PLSA demonstrated an increasing trend in leaves and stems and dropped-and-raised trend in roots, and the

mean of the PLSA in various organs followed a decreasing order: stems > leaves > roots (Table 3).

In addition, we also investigated the variation of phytolith Si/La and phytolith Si content in the treatments with different La concentration. The statistical results showed that compared with the treatment unaffected with La, the value of phytolith Si/La in rice leaves decreased along with the increase of exogenous La concentration (Fig. 5a), while the content of phytolith Si was the adverse, and it increased along with the increase of La (Fig. 5c).

Photosynthesis parameters in rice seedlings subject to different Si and La concentrations

The net photosynthetic rate (Pn) represents the photosynthesis intensity of rice leaves, and the higher the value is, the greater the photosynthesis intensity of rice is. Figure 6a shows the variation of leaf Pn of rice seedlings affected by different Si and La concentration. Firstly, compared with the CK, the Pn of leaves in the single treatment with Si reached maximum when Si was 90 mg/L, but it decreased drastically when adding excessive Si (210 mg/L). Secondly, compared with the CK, the Pn of leaves in the single treatment with 20 or
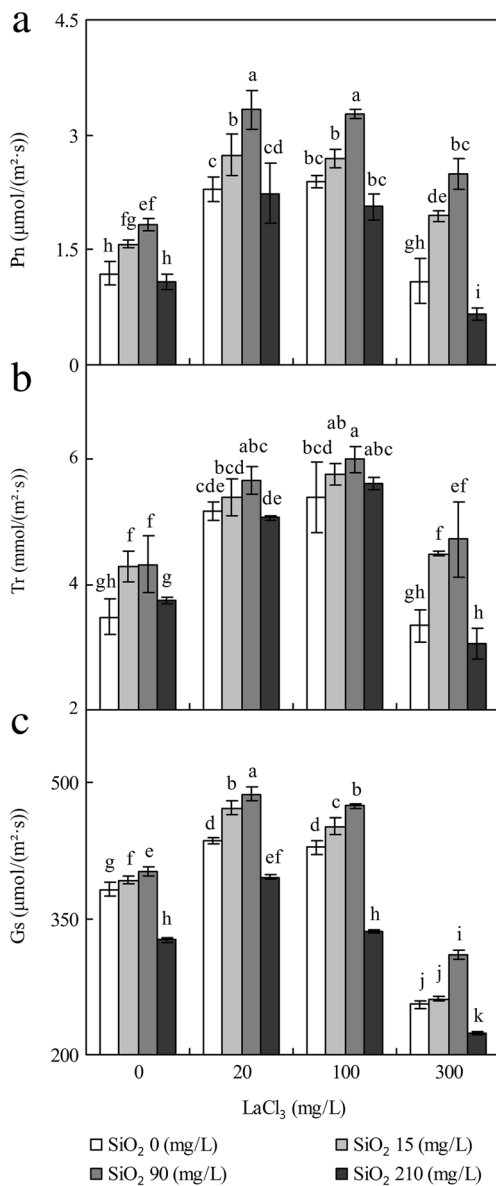
Fig. 6 **a** Net photosynthetic rate (Pn), **b** transpiration rate (Tr), and **c** stomatal conductance (Gs) under pretreatments with different levels of La and Si. Significant differences at the $P < 0.05$ level are shown with different letters. $n = 3$

100 mg/L La increased significantly, while it was inhibited significantly at 300 mg/L La. Thirdly, compared with the CK and the single treatment with Si level (15, 90, or 210 mg/L), the Pn increased significantly in the combined treatments with low La and different Si, and it increased first then decreased compared with the single treatment with low La along with the increase of Si level. Compared with the CK and the single treatment with different Si concentration (15, 90, or 210 mg/L) or 100 mg/L La, the variation of Pn in the combined treatments with 100 mg/L La and different Si level was similar to that of the combined treatments with low La and different Si level, but its Pn value was lower. While the Pn in the combined treatments with 300 mg/L La and 15 or

90 mg/L Si was significantly higher than that in the CK, the single treatment with 15 or 90 mg/L Si or 300 mg/L La. Compared with the CK, the single treatment with 210 mg/L Si or 300 mg/L La, the Pn was significantly lower in the combined treatments with 300 mg/L La and 210 mg/L Si, when the Pn reached minimum and the photosynthetic rate was inhibited significantly.

Leaves are important functional organs for water loss in rice, and they are closely related with the transpiration of whole plant. In our study, we used transpiration rate (Tr) to estimate the transpiration of rice leaves, the higher the Tr, the more water loss and the stronger transpiration of rice. Stoma of rice leaves is an important channel for water vapor loss, and the stomatal conductance (Gs) can reflect the size and density of stoma. Gs can be influenced by light and water status in rice leaves, it is sensitive to the variation of environment and is closely related with the transpiration of rice leaves. Figure 6b, c shows the variation of Tr and Gs affected by different Si and La concentration. Firstly, compared with the CK, the Tr and Gs both increased in the single treatment with 20 or 100 mg/L La, while the Tr under 20 mg/L La level was lower than that under 100 mg/L La, but the Gs was higher, and the Tr and Gs in the treatment with 300 mg/L La both decreased significantly compared with the CK. Besides, compared with the CK, the Tr and Gs both increased in the treatment with 15 or 90 mg/L Si concentration, and the increase was more significant when Si level was 90 mg/L, but the Tr and Gs decreased significantly in the treatment with 210 mg/L Si, especially the decrease of Gs was more significant.

In addition, In the combined treatments with different La and Si concentration, we found two phenomena as following: firstly, compared with the CK, the Tr and Gs all increased significantly in the combined treatments with appropriate Si (15 or 90 mg/L) and La (20 or 100 mg/L), and they also increased significantly compared with those of the single treatment with 20 or 100 mg/L La, particularly, the increase was more obvious in the combined treatments with 90 mg/L Si and 20 or 100 mg/L La. However, compared with the CK and the single treatment with 20 or 100 mg/L La, the Tr increased but the Gs decreased in the combined treatments with 210 mg/L Si and 20 or 100 mg/L La. Secondly, compared with the CK, the Tr increased but Gs decreased in the combined treatments with appropriate Si (15 or 90 mg/L) and 300 mg/L La, while they both increased compared with single treatment with 300 mg/L La, particularly, the increase was more significant in the combined treatments with 90 mg/L Si and 300 mg/L La; compared with the CK and the single treatment with 300 mg/L La, the Tr and Gs both decreased significantly in the combined treatments with 210 mg/L Si and 300 mg/L La, and the transpiration of rice leaves was inhibited obviously.

Therefore, transpiration and photosynthesis were inhibited by high La concentrations but was promoted by low La level, and high concentration of La and Si deteriorated the inhibited effects

of both La and Si on the growth of rice seedlings. Moreover, our correlation analysis showed that the PLCO and Tr were positively correlated in the leaves and stems ($P < 0.001$ in leaves and $P < 0.01$ in stems), but were not significantly correlated in the roots ($P > 0.05$) (Fig. 7d). Moreover, there were positive correlations between Pn and Tr under the influence of various concentrations of La in the leaves of rice seedlings ($P < 0.0005$ in the La concentration of 20 and 300 mg/L, $P < 0.01$ in non-La, and $P < 0.05$ in 100 mg/L La) (Fig. 7e).

Chlorophyll content in rice seedlings in response to different La and Si levels

Compared with the control, leaf chlorophyll a + b (Ca + b) and chlorophyll a/b (Ca/b), respectively, increased by 63.45 and 42.54% with 20 mg/L La, increased by 35.03 and 24.79% with 100 mg/L La, and decreased by 21.32 and 16.39% with 300 mg/L La. Similarly, compared with the control, leaf Ca +

b and Ca/b increased by 16.75 and 9.66% with 15 mg/L Si, increased by 29.44 and 20.17% with 90 mg/L Si, and decreased by 4.57 and 3.78% with 210 mg/L Si (Table 4). Thus, single applications of La or Si at low concentrations were beneficial for the growth of rice seedlings, which promoted the photosynthetic efficiency of leaves via an increase in chlorophyll a, but the high concentration of La or Si, in turn, inhibited leaf photosynthesis via the decrease in chlorophyll a. Moreover, when the combined treatment of La and Si were used, compared with the control, leaf Ca + b and Ca/b, respectively increased by 73.60 and 42.44% with a combination treatment of 15 mg/L Si and 20 mg/L La, increased by 52.28 and 34.45% with the treatment of 15 mg/L Si and 100 mg/L La, decreased by 7.11 and 5.04% with the treatment of 15 mg/L Si and 300 mg/L La; increased by 86.29 and 50.00% with a combination treatment of 90 mg/L Si and 20 mg/L La, increased by 62.94 and 42.44% with the treatment of 90 mg/L Si and 100 mg/L La, increased by 8.00 and

Fig. 7 Correlations of **a** the Si content with the phytolith content, **b** the La content with the PhytLa content, **c** the phytolith content with the PhytLa content, **d** the PLCO with the transpiration rate (Tr), and **e** the net photosynthetic rate (Pn) with the Tr in rice seedlings under treatments with La and Si, and $n = 16$
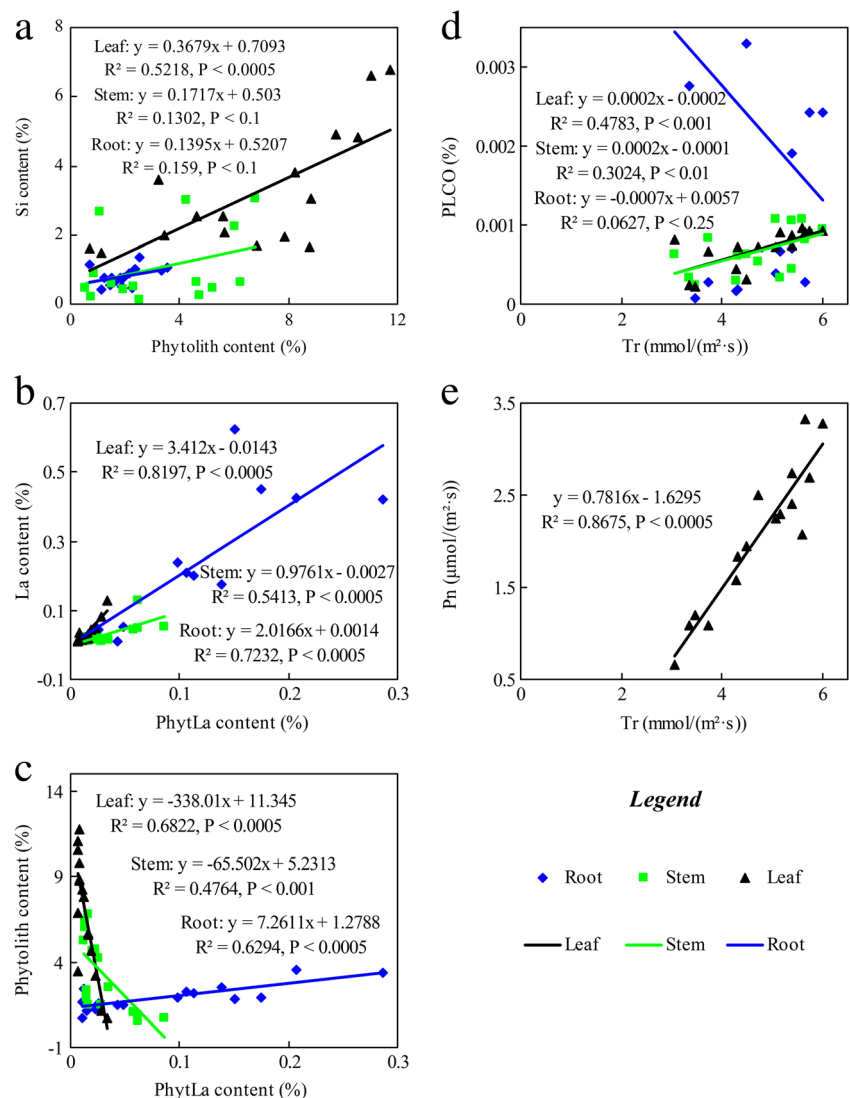
Table 4 Chlorophyll content in the leaves of rice seedlings at different La and Si levels

Treatments	Ca + b mg/L	Ca/b	Treatments	Ca + b mg/L	Ca/b
T1 (CK)	1.97 ± 0.01f	2.38 ± 0.08a	T9	2.55 ± 0.07c	2.86 ± 0.13d
T2	3.22 ± 0.06b	3.25 ± 0.07e	T10	3.67 ± 0.09a	3.57 ± 0.12f
T3	2.66 ± 0.07 cd	2.97 ± 0.06 cd	T11	3.21 ± 0.05b	3.39 ± 0.15e
T4	1.55 ± 0.06f	1.99 ± 0.07a	T12	2.05 ± 0.02 cd	2.39 ± 0.12 cd
Means	2.35	2.65	Means	2.87	3.05
T5	2.30 ± 0.09de	2.61 ± 0.16bc	T13	1.88 ± 0.11f	2.29 ± 0.06a
T6	3.42 ± 0.08b	3.39 ± 0.09e	T14	3.12 ± 0.06b	2.99 ± 0.11e
T7	3.00 ± 0.12b	3.20 ± 0.26e	T15	2.61 ± 0.07 cd	2.95 ± 0.05 cd
T8	1.83 ± 0.10ef	2.26 ± 0.06ab	T16	1.41 ± 0.15f	1.84 ± 0.16a
Means	2.64	2.87	Means	2.26	2.52

Means with various letters are significantly different at the $P < 0.05$ level of confidence according to Duncan's multiple range test. Values are means ± standard deviation error, $n = 3$; C represented chlorophyll content

0.42% with the treatment of 90 mg/L Si and 300 mg/L La; increased by 58.38 and 25.63% with a combination treatment of 210 mg/L Si and 20 mg/L La, increased by 32.49 and 23.95% with the treatment of 210 mg/L Si and 100 mg/L La, decreased by 28.43 and 22.69% with the treatment of 210 mg/L Si and 300 mg/L La. Maximum Ca + b and Ca/b were recorded in response to a combined treatment of 90 mg/L Si and 20 mg/L La, and minimum Ca + b and Ca/b was observed under combined treatment of 210 mg/L Si and 300 mg/L La. Obviously, low concentrations of Si and La improved the photosynthetic efficiency of leaves as a result of an increase in chlorophyll a; however, a high concentration of Si and La aggravated the inhibition of photosynthesis of leaves via the decrease in chlorophyll a levels, in contrast to the effects by a single application of La or Si.

Discussion

Mechanism of phytolith La sequestration in rice

Based on previous studies and the results of this study, we proposed a possible mechanism for phytolith La sequestration: Silicic acid derived from the soil solution is passively and actively transported to the xylem by Si transporter proteins located at the symplasm of roots (Ma et al. 2006; Mitani et al. 2005). Thus, the silicic acid in the xylem passively moves upwards via the transpiration stream (Mitani et al. 2005). The transport of silicic acid by the transpiration stream, with a strong transpiration pull, results in the extensive distribution of silicic acid in the apoplast of the aerial parts of rice. The concentration of silicic acid increases to a level where it polymerizes as silica gel (Carter 2009). Newly-formed silica gel encloses the organelles that internalize La via the La^{3+} anchor on the surface of the cell wall or cell membrane (Ye et al. 2008) and/or enter into the cell nucleus and cytoplasm by endocytosis of the cell (Wang et al. 2014), while the silica gel takes shape derived from the various cell types of rice. Water removal from silica gel results in an amorphous phytolith silica with La inclusion forms.

The sequestration ability of phytoliths on La may be due to the solubility of phytoliths in plants. The study conducted by Dove and Crerar (1990) indicated that the dissolution of silica could be promoted by water molecules that break Si-O linkages based on Lewis acids and bases theory. However, trivalent La may reduce the solubility of phytoliths. This is because trivalent metals can be strongly adsorbed on the surfaces of phytoliths (Dove 1995). The previous experiment conducted by Nguyen et al. (2014) indicated that Al application reduced the solubility of phytolith. In this study, the results derived from Fig. 5a show that the Si/La ratios of phytoliths in the leaves decreased with increase in the external La concentration, suggesting that La application not only reduced the release of phytolith silica (because the phytolith Si content increased with the increase of the external La level, see Fig. 5c) but also promoted the sequestration of La by phytoliths. Thus, the solubility of the phytolith silica reduced with the increase in the supply of La. Trivalent La enhanced the stability of Si-O-Si bonds via the formation of Si-O-La-O-Si bonds and reduced the solubility of phytolith silica; this further cut down the risk that the La source incorporated into phytoliths was released anew into living organisms in plants. Therefore, the absorption of La ions on phytoliths inhibited the interaction of La with other organelles and further promoted the function and efficiency of photosynthesis and transpiration via the reduction of La in plants. It is logical to assume that the biogenic sequestration of La by phytoliths is a basal physiological and biochemical defense response against La uptake in plants.

The role of phytolith La sequestration on the adverse effects of La on rice seedling growth

The previous studies have reported that the contents of phytoliths and PhytC of rice can be promoted by supplying

Si nutrition (Guo et al. 2015). However, in this study, the excessive Si supply inhibited the growth and development of rice seedlings by lowering photosynthesis and transpiration (Fig. 6 and Table 4), although high Si concentration still increased the phytolith content (Table 3). Thus, it is important to maintain the growth and development of rice seedlings for phytolith La/C sequestration on a biological time scale. Figure 5b shows similar variations as the results from Fig. 6 and Table 4 along with the change in external Si concentration, indicating that excessive Si (210 mg/L Si) reduced the PLSA. Moreover, the presence of excessive phytoliths due to high Si concentration may deteriorate this process in the leaves, which may cause an increase in residual leaf La (because of the decrease in PLSA) and inhibit the Pn, Ca + b, Ca/b, Tr, and Gs (and F/B, WUE, Tr, Gs, WP, and OP in Table S4). However, suitable Si supply (15 and 90 mg/L Si) would increase the PLSA indicating that leaf residual La decrease via PhytLa, and reduce the adverse effects of La on the rice seedlings via PhytLa because of the increase in Pn, Ca + b, Ca/b, Tr, and Gs (and F/B, WUE, Tr, Gs, WP, and OP in Table S4), which confirmed that phytoliths via La sequestration alleviates La stress on rice seedling growth.

Apart from external Si, external La concentration is also a primary factor controlling the production of phytolith La sequestration in rice. Low La concentration (20 mg/L La) promoted photosynthesis in rice seedlings, which was inhibited by high La concentration (100 and 300 mg/L La) (Fig. 6 and Table 4). These results are consistent with our previous study in rice (Hu et al. 2016). Thus, La at low concentration might coordinate with proteins on the chloroplast membrane, promote chlorophyll synthesis, and improve plant photosynthesis. However, excessive La could interact with the proteins in chloroplast outer membrane and damage the ultrastructure of the chloroplast and further inhibited chlorophyll synthesis, leading to the inhibition of plant photosynthesis and threaten the physiological growth and development of rice.

The results derived from Fig. 5b also exhibited that the PLSA was promoted by low La concentration but reduced by high La concentration, suggesting that low La concentration promote the ameliorating effect of phytoliths on La stress, and indicating that leaf residual La reached a minimum via PhytLa and reduced the adverse effects of La in rice seedlings. Many previous reports indicated that low La concentration in species was beneficial to the growth of the plant, but they neglected the important sequestration function of phytoliths. La damage to plants should be an important consideration in phytolith studies.

Notably, increasing the La and Si concentrations further increased the combined stress effect of La and Si on photosynthesis (Fig. 6 and Table 4). Moreover, the PLSA under combined La and Si stress decreased with increasing concentration of La but increased with increasing concentration of Si (Fig. 5b). These results suggest that the inhibitory effects of the combined

La and Si stress on photosynthesis with increasing La concentration may be due to the decrease in PLSA, which results in the increase in leaf residual La, disrupting protein synthesis in the chloroplast outer membrane, thus, inhibiting plant photosynthesis. The decrease in PLSA in turn is due to the decrease in the slope (derived from Fig. 3a) and the increase in the slope (derived from Fig. 4a) with the increase in La concentration. However, the increase in the PLSA under the combined effect of La and Si stress, with increasing Si concentration, indicates that the available Si supply could help rice overcome the adverse effects of La by enhancing the production of phytoliths at both low and high La concentrations.

Factors from the accumulation of phytoliths affecting bio-sequestration of La within phytoliths

The previous study shows that phytolith content in different plants and different parts of the same plant vary significantly, ranging from 0.3~12% (Li et al. 2013a; Song et al. 2012b; Piperno 2006), which is consistent with the results derived from this study that showed the phytolith content ranged from 0.8~11% (Fig. 2 and Table 3). The present study shows that phytoliths are formed in different parts of rice seedlings, with the highest phytolith formation in leaves and the lowest in roots, which is consistent with the results of mature rice derived from Li et al. (2013a). Our correlation analysis showed that the phytolith content in different parts was closely correlated to the PhytLa content and the linear slope was the smallest and negative in leaves, greater in stems, and was positive in roots (Fig. 7c). These results indicated that fewer phytoliths would occlude more La in aerial parts than in the roots, suggesting that the phytoliths of aerial parts had a better La sequestering ability, being the highest in leaves; however, roots showed the lowest ability for sequestering La, which was consistent with the result of the PLSA derived from Table 3. Our study further showed that the La content in different parts was significantly positively correlated with the PhytLa content (Fig. 7b), and the slope of equation in leaves had the highest value of all parts, which indicated that the efficiency of phytolith La sequestration depends on the actual accumulation of La within rice and the highest ability of phytolith La sequestration was in leaves. Our previous study had shown a significant correlation between Si content and phytolith formation in the grasses (Song et al. 2012b), which is similar to the result obtained in rice leaves in this study (Fig. 7a). However, the correlation of Si content with phytolith content in other parts (stems and roots in rice seedlings) was not significant. As mentioned above, the average content of phytolith in leaves was the highest in all parts of rice seedlings, and the slope of regression equation derived from the correlation between the content of phytolith and PhytLa in leaves was the lowest in all parts and was a negative value, and the slope of regression equation derived from the

correlation between the content of La and PhytLa in leaves was the highest in all parts and was a positive value, suggesting that leaves are the most important contributor to the formation of phytoliths and La sequestration within phytoliths, which play a crucial role in La bio-sequestration within phytoliths in rice seedlings.

The results derived from Table 3 show that the distribution pattern of PhytLa and La varies greatly within different parts in rice seedlings. The average content of La in rice organs was the highest in roots followed by leaves and the lowest in stems, which was consistent with the previous study for maize (*Zea mays* L.) indicating the highest content of La in roots (Xu et al. 2003). In contrast to roots that showed the highest average content of PhytLa and La, acrial parts showed different distribution of the content of PhytLa and La between leaves and stems. Moreover, PLSA varied significantly in different plant parts, being higher in the stems and leaves than root tissues, which is due to the differential distribution of the PhytLa and La content between different parts. The discrepancy of phytolith La sequestration in different parts may be due to different Si functions of organs. For example, the roots can actively supply abundant Si for other parts and result in less cell silicification and the lowest concentration of phytoliths; however, leaves and stems attained higher concentration of phytoliths compared with roots via the transpiration stream. Higher phytoliths production in different parts may play an important role in augmenting the phytolith La sequestration in rice seedlings.

According to the variations of phytolith content, PhytLa content, La content and PLSA in leaves of rice seedlings affected by different Si and La concentrations, we concluded the following four phenomena: firstly, compared with the CK, the treatments with 20 or 100 mg/L La both promoted the phytolith content, PhytLa content, La content and PLSA in rice leaves, proving that 20 or 100 mg/L La not only promoted the production of phytoliths in rice leaves but also improved the efficiency of La sequestration by phytolith and the accumulation of La and ultimately increased PLSA and strengthened the ability of phytolith La sequestration. In addition, compared with the CK, the treatments with 300 mg/L La decreased the phytoliths' production in rice leaves, but improved the efficiency of La sequestration by phytoliths and the accumulation of La significantly, showing that more La was absorbed in the solution with 300 mg/L La, thus making the La sequestration by phytolith much more easier, so more La was sequestrated by less phytolith; meanwhile, 300 mg/L La inhibited the formation of phytoliths, so more La could not be sequestrated by phytoliths, and the ability of phytolith La sequestration was inhibited. Secondly, compared with the CK, the phytolith content and PLSA of leaves increased significantly along with the increase of Si level, but the content of PhytLa and La changed unconspicuously, indicating that the increase of the production of phytoliths improved the ability

of phytolith La sequestration in rice leaves, the increase of bulk phytoliths resulted in the drastic decrease of La, thus reduced the adverse effects of La on rice. Thirdly, compared with the single treatment with 20 mg/L La or different Si concentration (15, 90, or 210 mg/L), the phytolith content and PLSA both tended to increase in the combined treatments with 20 mg/L La and different Si, while the content of PhytLa and La both tended to increase or decrease respectively. These indicated that the formation of phytoliths and the ability of phytolith La sequestration in rice leaves were improved more significantly in the combined treatment with 20 mg/L La and different Si concentration (15, 90, or 210 mg/L) than in the single treatment with La or Si, though the efficiency of La sequestration by phytoliths and the accumulation of La were inhibited, more production of phytoliths improved the ability of phytolith La sequestration significantly. Fourthly, with the increase of La concentration, the promotion of the phytolith content and the ability of phytolith La sequestration in rice leaves were weakened in the combined treatment with Si and La. Specially, compared with the single treatment with Si concentration (15, 90, or 210 mg/L), the phytolith content and PLSA both decreased in the combined treatment with 300 mg/L La and different Si, while the content of PhytLa and La both increased significantly; besides, the phytolith content and PLSA both increased, but the content of PhytLa and La both decreased compared with those in the single treatment with 300 mg/L La. These implied that compared with the single treatment with different Si level, though the accumulation of La and the efficiency of La sequestration by phytolith were improved, in the combined treatment with high La and various Si level, the accumulation of phytolith was inhibited; thus, the ability of phytolith La sequestration decreased, while the accumulation of La and the efficiency of La sequestration by phytolith were inhibited in the combined treatment with 300 mg/L La and various Si level compared with those in the single treatment with 300 mg/L La, but the accumulation of phytolith was improved, ultimately the ability of phytolith La sequestration was strengthened.

Role of phytolith morphology on the bio-sequestration of La within phytoliths

The shape of the plant cells vary greatly, which are the basic structural and functional units. The distribution of phytolith morphotypes derived from the shapes of cells vary greatly in different parts of plants. Our results (Table S1) showed that concave dumbbells and tubes accounted for the majority of phytoliths in the leaves, while panicoid dumbbells and crenates accounted for the most in stems. Moreover, the concave dumbbells, panicoid dumbbells, complex rondels, and bulliforms were distributed in the roots. As mentioned before, the PLSA in the aerial parts was higher than that in roots, and a higher phytolith content was noted in aerial parts, suggesting

that concave dumbbells, panicoid dumbbells, tubes and cre-nates are the main contributors to La sequestration within phytoliths in aerial parts of rice. We speculate that large amounts of phytoliths accumulate to provide a greater surface area for anchoring La.

Tubes were the most common morphotypes in our study. Our results showed that low concentration of La promoted the production of tubes and high Si concentration improved the production of tubes (Table 2). Moreover, the tube production was promoted by Si and La alone or the combined effect of Si and La compared with the control. The correlation analysis derived from Table S2 showed a significant correlation be-tween the concentration of tubes and the phytolith content in leaves ($P < 0.05$). These suggested that the production of tubes depended on the application of La and Si, which was closely related with the formation of phytoliths. This could be attrib-uted to the important transport function of the vessel cells, which exposed the tube phytoliths to abundant La dissolved in the water transported in vascular plants. Excessive La ac-celerated cell death and reduced cell division, thus, resulting in the formation of larger phytoliths. Moreover, absorption of abundant La on the surface of phytoliths strengthened the stability of tubes by decreasing their solubility of phytoliths (Strömberg 2004). Alternatively, the dissolution rate of differ-ent phytolith morphotypes in the same plant is different and influenced by many factors, including pH, phytolith shape, surface area, and water content (Piperno 2006). These may impact the distribution of various phytolith morphotypes and further determine phytolith La sequestration. In this study, bulliforms, elongate smooths, and tabular psilates did not con-tribute to the main morphology that affected the production of phytoliths owing to their fewer quantity, the reasons may be inferred that their larger bulk can be broken and dissolved easily in the phytolith extraction process, whereas their small-er surface area derived from their fewer quantity would result in a poorer effect of La sequestration by phytoliths.

Mentioned above, tube phytolith is an important phytolith morphotype affecting the La sequestration by phytoliths, and in contrast to Fig. 5b, we found the following four phenomena, which further confirmed that the quantity variation of the tube phytoliths influenced the leaf phytolith La sequestration signifi-cantly: firstly, compared with the CK, the quantity of tubes in rice leaves in the treatment with 20 or 100 mg/L La increased, and the increase in the treatment with 20 mg/L La was more significant, while the PLSA in rice leaves increased significantly and the increased was much more obvious in the treatment with 20 mg/L La. Besides, compared with the CK, the quantity of tubes in rice leaves in the treatment with 300 mg/L La did not change obvi-ously, but it was significantly inhibited compared with that of the treatment with 20 or 100 mg/L La. Meanwhile, compared with the CK, the PLSA in the treatment with 300 mg/L La was inhibited significantly. These indicated that the tubes in rice leaves were closely related with the La sequestration by

phytoliths in the treatments with low La or high La. In addition, compared with the CK, the quantity of tubes in rice leaves in-creased significantly in the treatment with different Si level (15, 90, or 210 mg/L), and the PLSA also tended to increase, while the increase of the quantity of tubes and the PLSA was more obvious in the treatment with 90 or 210 mg/L Si. These proved that in the treatment with Si, the tubes in rice leaves affected the phytolith La sequestration significantly, and the formation of tubes in rice leaves determined the phytolith La sequestration.

Secondly, compared with the CK and the single treatment with 15 or 90 mg/L Si, the quantity of tubes and the PLSA both increased evidently in the combined treatment with low La and appropriate Si concentration (15 or 90 mg/L), suggesting that the influence of tubes on the phytolith La sequestration was enhanced in this combined treatment. Compared with the CK, the quantity of tubes and the PLSA both increased significantly in the combined treatment with low La and 210 mg/L Si, and they both tended to decrease compared with the single treatment with 210 mg/L Si. These indicated that in the combined treat-ment with low La and 210 mg/L Si, the phytolith La sequestra-tion was still affected by the tubes. In addition, compared with the single treatment with low La, the quantity of tubes did not change and the PLSA increased slightly in the combined treat-ment with low La and 15 mg/L Si, while the quantity of tubes increased and the PLSA increased significantly in the combined treatment with low La and 90 mg/L Si, and they both increased in the combined treatment with low La and 210 mg/L Si. These further proved that in the combined treatment with low La and various Si concentration, the tubes were correlated closely with the phytolith La sequestration.

Thirdly, compared with the CK and the single treatment with 100 mg/L La, the quantity of tubes did not change or increased respectively, while the PLSA both increased significantly in com-bined treatment with 100 mg/L La and 15 mg/L Si, and the quan-tity of tubes and PLSA all increased evidently in the combined treatment with 100 mg/L La and 90 or 210 mg/L Si. These indi-cated that in the combined treatment with 100 mg/L La and low Si, the correlation between the tubes and the phytolith La seques-tration was weakened, while in the combined treatment with 100 mg/L La and high Si (90 or 210 mg/L), the influence of tubes on the phytolith La sequestration was strengthened. In addition, compared with the single treatment with 15 or 210 mg/L Si, the quantity of tubes and the PLSA both decreased in the combined treatment with 100 mg/L La and 15 or 210 mg/L Si. Compared with single treatment with 90 mg/L Si, the quantity of tubes increased significantly while the PLSA did not change obviously in the combined treatment with 100 mg/L La and 90 mg/L Si. These suggested that compared with the combined treatment with 100 mg/L La and 15 or 90 mg/L Si, the influence of tubes on phytolith La sequestration was strengthened more significant-ly in the combined treatment with 100 mg/L La and 210 mg/L Si.

Fourthly, compared with the single treatment with 300 mg/L La, the quantity of tubes and the PLSA both increased

obviously in the combined treatment with 300 mg/L La and 15 or 90 mg/L Si, and the quantity of tubes increased but the PLSA decreased compared with those of the single treatment with 15 or 90 mg/L Si. These indicated that the correlation between the tubes and the phytolith La sequestration was weakened in rice leaves. In addition, compared with the CK and the single treatment with 300 mg/L La, the quantity of tubes and the PLSA increased significantly in the combined treatment with 300 mg/L La and 210 mg/L Si, and they both decreased evidently compared with those of the single treatment with 210 mg/L Si. These indicated that when affected by excessive Si and La, the correlation between the tubes in rice leaves and the phytolith La sequestration became much more close. Therefore, when affected by 300 mg/L La, 210 mg/L Si significantly strengthened the influence of the leaf tubes on the phytolith La sequestration, but 15 or 90 mg/L Si weakened it.

Role of transpiration in the bio-sequestration of La within phytoliths

Many factors affect the absorption of Si in plants, such as species (interspecies differences), climate (temperature, humidity, evaporation), and soil conditions (pH and available Si) (Kameník et al. 2013; Piperno 2006; Epstein 1999). The high concentration of Si in plants is attributed to the highly available Si concentration in the soil (Ma et al. 2006). Plant roots take up Si, through Casparian strips, from the soil in the form of monosilicic acid ($Si(OH)_4$) and deposit it in the leaves through the xylem in apoplast (Ma et al. 2006). Mitani et al. (2005) showed that Si exists mainly in the form of hydrated amorphous silica ($SiO_2 \cdot nH_2O$) in leaves and monosilicic acid in stems and roots. Si is transformed by migration in plant-soil systems and is ultimately deposited as phytoliths, which is the final silicified form of Si. Our results show that the formation of phytoliths was closely related to Si, which is primarily described as a process to use cell wall and cell membrane as templates for polymerization and accumulation on the surface of variously shaped cells via the evaporation of water and transpiration stream (Ma and Yamaji 2006). Transpiration regulates the physiological activities of a plant and helps it to adapt to the environment, which affects the formation and production of phytoliths in various plant tissues (Song et al. 2012a). Moreover, it increases the resistance to diseases that are important for the healthy growth of rice. This study showed that the PLCO in the above-ground parts exhibited a significant positive correlation to Tr, indicating that the transpiration affected the final production of Phytolith La sequestration in leaves and stems ($P < 0.001$ in leaves; $P < 0.01$ in stems) (Fig. 7d). However, the Tr and PLCO showed a weak negative correlation in roots ($P > 0.05$). The study conducted by Okuda and Takahashi (1962) inferred that the Si uptake by roots is not related with transpiration in rice, which is accordance with this study. This can be attributed to the fact that

monosilicic acid uptake by roots is an active process via a type of transporter protein, which contains cysteine residues (Tamai and Ma 2003). The Si uptake by roots provides a Si source for the other aerial parts by a transpiration stream, resulting in fewer phytoliths in the roots as mentioned above. Our study further showed that the PhytLa content in leaves was closely related to the phytolith content, which was in turn correlated to the Si content in leaves, suggesting that leaf is an important organ (a transpiration stream end) for the production of phytoliths and the efficiency of phytolith La sequestration via Si accumulation is achieved by passive monosilicic acid absorption in the transpiration stream (Handreck and Jones 1968). These results further confirmed the reports derived from the study conducted by Okuda and Takahashi (1962) that indicated that transpiration could only affect the Si deposit in the aerial parts of rice. Moreover, a significant correlation was demonstrated between Pn and Tr (Fig. 7e). Thus, apart from transpiration, this result revealed that photosynthesis indirectly affects the formation of phytoliths and the final production of phytolith La sequestration because light provides sufficient energy for transpiration, making transpiration stream the main dynamic of Si transport in plant body (Jones and Handreck 1965).

Implications of phytolith La sequestration in agriculture

The intact silica taken up by the plant is in the form of silicic acid, which is derived from a biogenic Si (BSi) pool that has been considered to be predominant soluble silica source in agricultural soils (Guntzer et al. 2012; Sommer et al. 2006). This BSi is a major factor controlling Si concentrations in soil solutions and effective for plant uptake (Haynes 2017). The BSi pool is derived from plant litters and residues, and phytolith silica accounts for the vast bulk of the pool of BSi (Struyf and Conley 2012). It seems likely that the entire pool of BSi will be replenished equivalently by new phytolith silica via plant litter returns (Haynes 2017). However, in agricultural soils, the loss of BSi in the soil occurs due to the removal of harvested products (phytolith Si contained in it) (Keller et al. 2012), which is most pronounced under Si accumulating crops such as rice. This loss would be enhanced by the removal of crop residues, which often occur in rice fields from which the straw of rice is removed as well as the grains (Haynes 2017). The long-term field trial conducted by Guntzer et al. (2012) and Keller et al. (2012) indicated that removal of harvested products would inevitably result in a decline in the size of BSi pool in the soil, despite the routine application of Si fertilizers.

The fact that the agricultural field located in rare earth mineralized areas such as the agricultural soil of Hetian Town in Southeast China (where the concentration range of bioavailable REE reached 58–159 mg/kg (ppm, w/w)) (Li et al. 2013b), means that high concentration of La will not only

inhibit the growth of crops but also impair the sequestration ability of phytoliths on La by decreasing the PLSA of phytoliths. The decrease in the size of BSi pool in rare earth mineralized agricultural areas due to less return of phytolith silica in crop biomass results in the decrease of Si concentration in soil solution which adversely affects the sequestration ability of phytolith on La. The decrease in the sequestration ability of phytoliths can magnify the amplitude of damage inflicted by La on crops.

In another agricultural field, far away in the rare earth mineralized areas, the fact that low La concentration (due to REE micro-fertilizer application) in fields can promote crop growth and enhance the return of crop biomass, means that the increase in the size of the BSi pool in agricultural soil would result in an increase in the Si concentration in the soil solution. The factors including low La concentration and increasing Si concentration promote the La sequestration ability of the phytoliths in crops, which can magnify the amplitude of La sequestration by phytoliths within crops and further lower the quantity of La residues in crops. Phytolith silica induced breakage of linkages between La and crop uptake would reduce the risk of La entering the human food chain in both low and high La concentration agriculture area via the return of PhytLa in the soil, which is likely to be a persistent diminution of the risk of La over time. However, this hypothesis should be confirmed by further studies.

Conclusion

In summary, our study demonstrated three viewpoints as follows:

(1) The growth of rice seedlings was controlled by La and Si, and their threshold values were 20 and 90 mg/L, respectively, which were inhibited by La and Si above their threshold values and promoted by La and Si below their threshold values. The combined effects of La and Si inhibited growth of rice seedlings either under Si concentration above its threshold value or under La concentration above its threshold value, and it in turn exerted promotion on the growth of rice seedlings either under Si concentration below its threshold value or under La concentration below its threshold value.

(2) The damage of La on the rice seedlings can be alleviated by phytoliths, of which the La sequestration ability can be promoted either by the increase in Si concentration or by using low La concentration; however, it can be inhibited either by high La concentration or decreasing Si concentration. The assuaging effects of phytoliths on the damage by La depend on the sequestration ability of phytoliths on La.

(3) Phytolith La sequestration ability is also affected by changes in the leaf physiological and biochemical characteristics such as Pn, Tr, Gs, and chlorophyll, as well as phytolith characteristics such as phytolith morphology, phytolith content, PhytLa content, and the solubility of phytolith.

Author contributions Y.S. performed the experiment and wrote the paper. Y.S. and L.H.W. analyzed the data. Q.Z., X.H.H. and Y.S. designed the study. All authors discussed the results and commented on the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

Aragay G, Pons J, Merkoci A (2011) Recent trends in macro-, micro-, and nanomaterial-based tools and strategies for heavy-metal detection. Chem Rev 111:3433–3458

Biasioli M, Fabietti G, Barberis R, Ajmone-Marsan F (2012) An appraisal of soil diffuse contamination in an industrial district in northern Italy. Chemosphere 88(10):1241–1249. https://doi.org/10.1016/j.chemosphere.2012.03.081

Blecker SW, Mcculley RL, Chadwick OA, Kelly EF (2006) Biologic cycling of silica across a grassland bioclimosequence. Glob Biogeochem Cycles 20:4253–4274

Carter JA (2009) Atmospheric carbon isotope signatures in phytolith-occluded carbon. Quat Int 193(1-2):20–29. https://doi.org/10.1016/j.quaint.2007.11.013

Dove PM (1995) Kinetic and thermodynamic controls on silica reactivity in weathering environments. Rev Mineral Geochem 31:235–290

Dove PM, Crerar DA (1990) Kinetics of quartz dissolution in electrolyte solutions using a hydrothermal mixed flow reactor. Geochim Cosmochim Acta 54(4):955–969. https://doi.org/10.1016/0016-7037(90)90431-J

EPA/600/R-12/572 (2012) Rare earth elements: a review of production, processing, recycling, and associated environmental issues. Office of Research and Development EPA/600/R-12/572

Epstein E (1999) Silicon. Annu Rev Plant Physiol Plant Mol Biol 50(1):641–664. https://doi.org/10.1146/annurev.arplant.50.1.641

Epstein E (2001) Silicon in plants: facts vs concepts. In: Datnoff LE, Snyder GH, Korndorfer GH (eds) Silicon in agriculture. Elsevier, Amsterdam, pp 1–15. https://doi.org/10.1016/S0928-3420(01)80005-7

García-Jiménez A, Gómez-Merino FC, Tejeda-Sartorius O, Trejo-Téllez LI (2017) Lanthanum affects bell pepper seedling quality depending on the genotype and time of exposure by differentially modifying plant height, stem diameter and concentrations of chlorophylls, sugars, amino acids, and proteins. Front Plant Sci 8:308

Guntzer F, Keller C, Poulton PR, Mcgrath SP, Meunier JD (2012) Long-term removal of wheat straw decreases soil amorphous silica at Broadbalk, Rothamsted. Plant Soil 352(1-2):173–184. https://doi.org/10.1007/s11104-011-0987-4

Guo F, Song Z, Sullivan L, Wang H, Liu X, Wang X et al (2015) Enhancing phytolith carbon sequestration in rice ecosystems through basalt powder amendment. Chin Sci Bull 60:591–597

Handreck KA, Jones LHP (1968) Studies of silica in the oat plant. IV. Silica content of plant parts in relation to stage of growth, supply of silica, and transpiration. Plant Soil 29(3):449–459. https://doi.org/10.1007/BF01348976

Haynes RJ (2017) The nature of biogenic Si and its potential role in Si supply in agricultural soils. Agric Ecosyst Environ 245:100–111. https://doi.org/10.1016/j.agee.2017.04.021

Hodson MJ, Sangster AG (1999) Aluminium/silicon interactions in conifers. J Inorg Biochem 76(2):89–98. https://doi.org/10.1016/S0162-0134(99)00119-1

Hu ZY, Richter H, Sparovek G, Schnug E (2004) Physiological and biochemical effects of rare earth elements on plants and their agricultural significance: a review. J Plant Nutr 27(1):183–220. https://doi.org/10.1081/PLN-120027555

Hu Z, Haneklaus S, Sparovek G, Schnug E (2006) Rare earth elements in soils. Commun Soil Sci Plant Anal 37(9-10):1381–1420. https://doi.org/10.1080/00103620600628680

Hu H, Wang L, Li Y, Sun J, Zhou Q, Huang X (2016) Insight into mechanism of lanthanum (III) induced damage to plant photosynthesis. Ecotoxicol Environ Saf 127:43–50. https://doi.org/10.1016/j.ecoenv.2016.01.008

Huang ZT, Jiang PK, Chang SX, Zhang Y, Ying YQ (2014) Production of carbon occluded in phytolith is season-dependent in a bamboo forest in subtropical china. PLoS One 9(9):e106843. https://doi.org/10.1371/journal.pone.0106843

Ingamels CO (1964) Rapid chemical analysis of silicate rocks. Talanta 11(3):665–666. https://doi.org/10.1016/0039-9140(64)80083-7

Jones LHP, Handreck KA (1965) Studies of silica in the oat plant III. Uptake of silica from soils by plant. Plant Soil 23(1):79–96. https://doi.org/10.1007/BF01349120

Kameník J, Mizera J, Řanda Z (2013) Chemical composition of plant silica phytoliths. Environ Chem Lett 11:189–195

Keller C, Guntzer F, Barboni D, Labreuche J, Meunier JD (2012) Impact of agriculture on the Si biogeochemical cycle: input from phytolith studies. Compt Rendus Géosci 344(11-12):739–746. https://doi.org/10.1016/j.crte.2012.10.004

Li Z, Song Z, Parr JF, Wang H (2013a) Occluded C in rice phytoliths: implications to biogeochemical carbon sequestration. Plant Soil 370(1-2):615–623. https://doi.org/10.1007/s11104-013-1661-9

Li X, Chen Z, Chen Z, Zhang Y (2013b) A human health risk assessment of rare earth elements in soil and vegetables from a mining area in Fujian Province, Southeast china. Chemosphere 93(6):1240–1246. https://doi.org/10.1016/j.chemosphere.2013.06.085

Lichtenthaler HK (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol 148:350–382. https://doi.org/10.1016/0076-6879(87)48036-1

Liu RQ, Xu XJ, Wang S, Shan CJ (2016) Lanthanum improves salt tolerance of maize seedlings. Photosynthetica 54(1):148–151. https://doi.org/10.1007/s11099-015-0157-7

Lu H, Zhang J, Wu N, Liu KB, Xu D, Li Q (2009) Phytoliths analysis for the discrimination of foxtail millet (Setaria italica) and common millet (Panicum miliaceum). PLoS One 4(2):e4448. https://doi.org/10.1371/journal.pone.0004448

Ma JF, Yamaji N (2006) Silicon uptake and accumulation in higher plants. Trends Plant Sci 11(8):392–397. https://doi.org/10.1016/j.tplants.2006.06.007

Ma JF, Tamai K, Yamaji N, Mitani N, Konishi S, Katsuhara M, Ishiguro M, Murata Y, Yano M (2006) A silicon transporter in rice. Nature 440:688–691

Meunier JD, Colin F, Alarcon C (1999) Biogenic silica storage in soils. Geology 27:835–838

Mitani N, Ma JF, Iwashita T (2005) Identification of the silicon form in xylem sap of rice (Oryza sativa L.) Plant Cell Physiol 46(2):279–283. https://doi.org/10.1093/pcp/pci018

Mortlock RA, Froelich PN (1989) A simple method for the rapid determination of biogenic opal in pelagic marine sediments. Deep Sea Res A Oceanogr Res Pap 36(9):1415–1426. https://doi.org/10.1016/0198-0149(89)90092-7

Nguyen NM, Dultz S, Guggenberger G (2014) Effects of pretreatment and solution chemistry on solubility of rice-straw phytoliths. J Plant Nutr Soil Sci 17:349–359

Okuda A, Takahashi E (1962) Studies on the physiological role of silicon in crop plant. Part 8. Some examination on the specific behavior of low land rice in silicon uptake. J Sci Soil Manure Jpn 33:217–221

Parr JF, Sullivan LA (2011) Phytolith occluded carbon and silica variability in wheat cultivars. Plant Soil 342(1-2):165–171. https://doi.org/10.1007/s11104-010-0680-z

Parr JF, Dolic V, Lancaster G, Boyd WE (2001) A microwave digestion method for the extraction of phytoliths from herbarium specimens. Rev Palaeobot Palynol 116(3-4):203–212. https://doi.org/10.1016/S0034-6667(01)00089-6

Parr J, Sullivan L, Quirk R (2009) Sugarcane phytoliths: encapsulation and sequestration of a long-lived carbon fraction. Sugar Technol 11(1):17–21. https://doi.org/10.1007/s12355-009-0003-y

Piperno DR (2006) Phytoliths: a comprehensive guide for archaeologists and paleoecologists. Econ Bot 60:391–392

Shan X, Wang H, Zhang S, Zhou H, Zheng Y, Yu H, Wen B (2003) Accumulation and uptake of light rare earth elements in a hyperaccumulator dicropteris dichotoma. Plant Sci 165(6):1343–1353. https://doi.org/10.1016/S0168-9452(03)00361-3

Sommer M, Kaczorek D, Kuzyakov Y, Breuer J (2006) Silicon pools and fluxes in soils and landscapes—a review. J Plant Nutr Soil Sci 169(3):310–329. https://doi.org/10.1002/jpln.200521981

Song Z, Wang H, Strong PJ, Li Z, Jiang P (2012a) Plant impact on the coupled terrestrial biogeochemical cycles of silicon and carbon: implications for biogeochemical carbon sequestration. Earth Sci Rev 115(4):319–331. https://doi.org/10.1016/j.earscirev.2012.09.006

Song Z, Liu H, Si Y, Yin Y (2012b) The production of phytoliths in China's grasslands: implications to the biogeochemical sequestration of atmospheric CO_2. Glob Chang Biol 18(12):3647–3653. https://doi.org/10.1111/gcb.12017

Strömberg CAE (2004) Using phytolith assemblages to reconstruct the origin and spread of grass-dominated habitats in the great plains of North America during the Late Eocene to Early Miocene. Palaeogeogr Palaeoclimatol Palaeoecol 207(3-4):239–275. https://doi.org/10.1016/j.palaeo.2003.09.028

Struyf E, Conley D (2012) Emerging understanding of the ecosystem silica filter. Biogeochemistry 107(1-3):9–18. https://doi.org/10.1007/s10533-011-9590-2

Tamai K, Ma JF (2003) Characterization of silicon uptake by rice roots. New Phytol 158(3):431–436. https://doi.org/10.1046/j.1469-8137.2003.00773.x

Taylor SR, Mclennan SM (1985) The continental crust: its composition and evolution. Blackwell Scientific, Oxford 312

Walkley A, Black IA (1934) An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci 37(1):29–38. https://doi.org/10.1097/00010694-193401000-00003

Wang C, Shi C, Liu L, Wang C, Qiao W, Gu Z, Wang X (2012) Lanthanum element induced imbalance of mineral nutrients, HSP 70 production and DNA-protein crosslink, leading to hormetic response of cell cycle progression in root tips of vicia faba L. seedlings. Dose Response 10(1):96–107. https://doi.org/10.2203/dose-response.11-041.Wang

Wang L, Li J, Zhou Q, Yang G, Ding XL, Li X et al (2014) Rare earth elements activate endocytosis in plant cells. Proc Natl Acad Sci U S A 111(35):12936–12941. https://doi.org/10.1073/pnas.1413376111

Wei Z, Hong F, Yin M, Li H, Hu F, Zhao G, Wong JWC (2005) Subcellular and molecular localization of rare earth elements and structural characterization of yttrium bound chlorophyll a, in naturally grown fern dicranopteris dichotoma. Microchem J 80(1):1–8. https://doi.org/10.1016/j.microc.2004.07.005

Xu X, Zhu W, Wang Z, Witkamp GJ (2003) Accumulation of rare earth elements in maize plants (*Zea mays*, L.) after application of mixtures of rare earth elements and lanthanum. Plant Soil 252(2):267–277. https://doi.org/10.1023/A:1024715523670

Ye Y, Wang L, Huang X, Lu T, Ding X, Zhou Q, Guo S (2008) Subcellular location of horseradish peroxidase in horseradish leaves treated with La(III), Ce(III) and Tb(III). Ecotoxicol Environ Saf 71(3):677–684. https://doi.org/10.1016/j.ecoenv.2007.11.020

Zeng Q, Zhu JG, Cheng HL, Xie ZB, Chu HY (2006) Phytotoxicity of lanthanum in rice in haplic acrisols and cambisols. Ecotoxicol Environ Saf 64(2):226–233. https://doi.org/10.1016/j.ecoenv.2005.03.016

Zuo XX, Lü HY (2011) Carbon sequestration within millet phytoliths from dry-farming of crops in china. Chin Sci Bull 56(32):3451–3456. https://doi.org/10.1007/s11434-011-4674-x

Zuo XX, Lu HY, Gu ZY (2014) Distribution of soil phytolith-occluded carbon in the Chinese Loess Plateau and its implications for silica–carbon cycles. Plant Soil 374(1-2):223–232. https://doi.org/10.1007/s11104-013-1850-6