

Silicon fertilizer application promotes phytolith accumulation in rice plants

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Silicon fertilizer application promotes phytolith accumulation in rice plants

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Abstract

In this study, a pot experiment was designed to elucidate the effect of varying dosages of silicon(Si) fertilizer application in Si-deficient and enriched paddy soils on rice phytolith and the carbon (i.e., C) bio-sequestration within phytoliths (PhytOC). The maximum silicon fertilizer dosage treatment(XG3) in the Si-deficit paddy soil resulted in an increase in the rice phytolith content by 100.77% in the stem, 29.46% in the sheath and 36.84% in the leaf compared with a lack of silicon fertilizer treatment(CK). However, the maximum silicon fertilizer dosage treatment(WG3) in the silicon-enriched soil increased the rice phytolith content by only 32.83% in the stem, 27.01% in the sheath and 32.06% in the leaf. Overall, Si fertilizer application significantly ($p<0.05$) increased the content of the rice phytoliths in the stem, leaf and sheath in both the Si-deficient and enriched paddy soils, and the statistical results showed a positive correlation between the amount of Si fertilizer applied and the rice phytolith content with correlation coefficients of 0.998 ($p<0.01$) in the Si-deficient soil and 0.952 ($p<0.05$) in the Si-enriched soil. In addition, the existence of phytoliths in the stem, leaf and sheath of rice and its content in the Si-enriched soil were markedly higher than that in the Si-deficient soil. Therefore, silicon fertilizer application helped to improve the phytolith content of the rice plant.

Key Words: Si fertilizer, phytolith accumulation, Si-deficient paddy soils, PhytOC, Rice organs

Introduction

Phytoliths derive from bio-mineralization in plants and usually take the shape of the plant cell or cell spatium where Si is deposited. Phytoliths content of plants ranges from less than 50 g kg^{-1} to as high as 150 g kg^{-1} (Epstein, 1994; Ji et al, 2017; Parr et al, 2010; Song et al, 2013; Song et al,

2017), mainly due to phylogenetic differences in Si requirements of most dicotyledons and some Gramineae (Hodson et al, 2005), as well as the amount of available silica in the environment (Guo et al, 2015; Seyfferth et al, 2013; Si et al, 2018; Wen et al, 2018).

Rice is a staple crop, with a global planting area of approximately 1.64×10^8 ha as of 2014 (Prajapati et al, 2016). When rice is harvested, the rice straw and husks are removed from the paddy field and used for other purposes, including animal feeding and firewood, or simply incinerated (Savant et al, 1996). Thus, most of the Si taken up by rice is removed from a field when the rice straw is removed, and the loss of SiO_2 is from 75 kg hm^{-2} to 130 kg hm^{-2} every production season (Zhang et al, 2014). Such large losses of Si make it difficult to maintain the balance of Si in soils from natural weathering alone. Currently, most paddy soils in China are Si-deficient. For example, 73% of paddy soils in Zhejiang Province, and approximately 60% in Henan Province are Si-deficient (Cai, 2015). Some research has shown that Si fertilizer application can significantly increase the biomass of rice (Wu et al, 2014; Zhang et al, 2014).

In plants, monosilicic acid is taken up from the soil by a specific transporter (Ma et al, 2006; Song et al, 2014a) and deposited throughout the cellular structures, thereby forming amorphous Si particles known as 'phytoliths' (Pearsall, 1989; Piperno, 1988). There is a significant correlation between the Si content and the phytolith content of crop materials, including the leaves, stems and sheaths, the Si concentration of the plant phytoliths is approximately 90%. (Song et al, 2014a)

Phytoliths can occlude small amounts of many elements, such as C, N, S, and so on (Anala and Nambisan, 2015; Kameník et al, 2013; Li et al, 2014). The C-occluded content of phytoliths ranges from less than 1 g kg^{-1} to as high as 100 g kg^{-1} . (Clarke, 2003). This PhytOC can be stored in

the soil for thousands of years(Parr and Sullivan, 2005). Thus, it plays a vital role in global carbon pools(Song et al, 2014a). The Si cycle is tightly coupled to the C cycle, and this interaction is relevant for research on climate change(Chadwick et al, 1994).The formation of phytoliths in rice plants depends not only on the crops(Guo et al, 2015; Li et al, 2013a), but also on the plant cultivars(Henriet et al, 2008; Hodson et al, 2005; Yang et al, 2015)and soil Si availability(Henriet et al, 2008; Klotzbücher et al, 2018), and so on.

The application of silicon fertilizer on soils with different available silicon content needs further study on the accumulation of Phytolith in rice. Thus, in this work, we designed a pot experiment to elucidate the effect of varying dosages of Si fertilizer application on the rice phytolith and PhytOC contents of plants grown in Si-deficient and enriched paddy soils.

1.Methods

1.1 Experimental Soils and Rice cultivar

The Si-deficient paddy soil (red paddy soil) was obtained from Yangliu Town, Xuanchen City, Anhui Province, P.R. China. The Si-enriched paddy soil (Wushan soils) was obtained from the Changshu Agroecological Experimental Station, Chinese Academy of Sciences. The base is located in Xinzhuang County, South Changshu, Suzhou, Jiangsu Province, P.R. China. The physicochemical properties of two soils were showed in Table 1.

The rice cultivar (*Oryza sativa*) Nanjing 46 was achieved from the Changshu Agroecological Experimental Station, Chinese Academy of Sciences.

1.2 Pot Experiment

Two soils (Si-deficient and enriched paddy soils) were selected from Xuanchen City and the Changshu Agroecological Experimental Station, Chinese Academy of Sciences, respectively. Four

available Si dosages were designed in the pot experiments: 1. CK (Si fertilizer not applied); 2. low slag Si fertilizer I (SiO_2 150 kg ha⁻¹); 3. high slag silicon fertilizer II (300 kg ha⁻¹), and 4. high slag silicon fertilizer III (600 kg ha⁻¹). Thus, this experiment comprised 8 treatments repeated 3 times. Two soils were placed in the pot bowl for a total volume of 0.0175 m³; each pot contained N 46%, P₂O₅ 13.5% and K₂O 60%, Si fertilizer as base fertilizer was applied, 3 rice plants were planted in every pot. Pots were placed in greenhouse of the Changshu Agroecological Experimental Station, Chinese Academy of Sciences in June 2014, and the whole rice growth period was maintained using conventional management.

1.3 Sample preparation

After the rice cultivar harvest, each rice plant was separated into five different organs: sheath, leaf, root, stem and grain. All rice samples were rinsed twice in distilled water, placed in an ultrasonic bath for 20 min and subsequently dried in oven at 70°C for 24 h. After hulling, the rice organs samples were stored for phytolith extraction and PhytOC determination.

1.4 Phytolith extraction from rice organs and PhytOC analysis

The phytolith extraction was used for a revised wet digestion measurement previously described by Sun et al. (Sun et al., 2016; Zuo and Lü, 2011). Phytolith extraction samples assemblages were installed on glass slides in Balsam Canada mounting medium. The slides were viewed at 400 × magnification using a microscope (Jiangnan XP-213, China) fitted with a polarizing filter and a 5.0 MP color CCD camera to ensure that no organic material residue as showed by Parr et al. (Parr et al., 2010) (Fig.1). The PhytOC was measured using an Elemental Analyzer 3000 (GmbH Company, Germany).

1.5 Statistical Analyses

The mean values of all parameters were calculated from the determination of three replicates, and the standard errors of the means were determined. A one-way ANOVA was used to measure the significance of the results between different varieties, and Tukey's multiple range tests ($p < 0.05$) were subsequently performed. All of the statistical analyses were performed using SPSS v.17 for Windows.

2 Results

2.1 Phytolith and C contents of the phytoliths in the rice organs

With the increase in the application of the Si fertilizer dosages, the content of the phytoliths in the rice organs could be increased in the Si-deficient red paddy soil (Table 2). For example, the content of the phytoliths in the XG3 (26.10 g kg⁻¹) and XG2 (18.50 g kg⁻¹) stems was significantly ($p < 0.05$) higher than that of the control (13.00 g kg⁻¹), and the rate increased by 100.7% and 42.3%, respectively. In addition, the content of the phytoliths of XG1 in the stem was not significantly ($p > 0.05$) different than that of the control. However, the content of the phytoliths in the rice sheath and leaf could be significantly ($p < 0.05$) increased by the application of all the Si fertilizer dosages. The content of the phytoliths in the XG3 treatment rice grains could only be increased by a high dose of Si fertilizer application. However, the content of the phytoliths in all the root treatments was not significantly ($p > 0.05$) different from that of the control.

With the increase in the application of the Si fertilizer doses, the content of the phytolith in the rice organs could be increased in the Si-enriched Wushan paddy soil (Table 2). For example, the content of the phytoliths of the WG3 (100.60 g kg⁻¹) and WG2 (93.13 g kg⁻¹) leaves was significantly ($p < 0.05$) higher than that of the control (76.18 g kg⁻¹), and the increase in the rate

was 32.06% and 22.25%, respectively. In addition, the content of the phytoliths of WG1 in the stem was not significantly ($p>0.05$) different from that of the control. However, the content of the phytoliths in the other rice organs could be significantly ($p<0.05$) increased by the application of high Si fertilizer dosages.

Thus, different Si fertilizer doses might increase the content of the phytoliths in the rice organs in either Si-deficient red paddy soil or Si-enriched Wushan paddy soil. The C content in the phytoliths in the organs was not affected by the increase in the Si fertilizer dose. However, the content of the C in the phytoliths was different in all the organs. Generally the content of the C of the leaf phytoliths was higher than that of the other organs (Table 3).

2.2 Phytolith content and the estimated PhytOC fluxes in whole rice plants

Compared with the control treatment, the content of phytoliths in the whole rice plant was significantly ($p<0.05$) increased by the use of a high Si fertilizer dose in the two types of soils (Table 4). The C content of the phytoliths and the PhytOC content of the dry organ weights were not significantly ($p>0.05$) different in the rice plant. In Si-deficient red paddy soil, the estimated PhytOC fluxes were calculated by the content and proportion of the phytoliths and the C content of the phytoliths in each part of the rice plant. The results showed that the application of Si fertilizer could significantly ($p<0.05$) increase the content of the estimated PhytOC fluxes in the whole plant with the increase in the Si fertilizer dosage. The estimated PhytOC fluxes of the XG2 ($11.36 \text{ kg-CO}_2 \text{ ha}^{-1} \text{ yr}^{-1}$) and XG3 ($12.93 \text{ kg-CO}_2 \text{ ha}^{-1} \text{ yr}^{-1}$) treatments were 43.04% and 49.70%, respectively, and were significantly ($p<0.05$) higher than those of the control treatment ($8.41 \text{ kg-CO}_2 \text{ ha}^{-1} \text{ yr}^{-1}$). In the Si-enriched soil, the phytolith content of all the Si fertilizer treatments in the rice plants was higher than that of the control treatment, but it was not significantly ($p>0.05$)

different in all the Si fertilizer treatments compared with the control treatment. The estimated PhytOC fluxes of WG1 were 1.3% lower than those of the control.

2.3 The correlation coefficients between the six variables of the red paddy soil

As shown in Table 5, the coefficient of variation in the different factors in the Si-deficient red paddy soils was high, illustrating considerable variation among these different Si fertilizer dosages. The results demonstrated that there was a significant correlation ($R=0.998$, $p<0.01$) between the phytolith content and the silicon fertilizer dose. The C contents of the phytoliths were not correlated ($R=-0.177$, $p>0.05$) with the phytolith content in the rice plants treated with different fertilizer doses. The correlation coefficient was 0.986, indicating a significant relationship ($p<0.05$) between the phytolith content and the estimated PhytOC fluxes. The biomass of the rice was significantly related to the phytolith content ($R=0.972$, $p<0.05$) and the estimated PhytOC fluxes ($R=0.994$, $p<0.01$).

2.4 The Correlation coefficients between the six variables of the Wushan soil

As shown in Table 6, the coefficient of variation in the different factors in the Si-deficient red paddy soils was high, illustrating considerable variation among the different Si fertilizer doses. The results demonstrated that there was a significant correlation ($R=0.952$, $p<0.05$) between the phytolith content and the silicon fertilizer dose. The C contents of the phytoliths were not correlated ($R=-0.035$, $p>0.05$) with the phytolith content in the rice plants of different fertilizer treatments. The correlation coefficient was 0.598 and was significantly correlated ($p>0.05$) between the phytolith content and the estimated PhytOC fluxes. The biomass of the rice was significantly correlated with the phytolith content ($R=-0.890$, $p>0.05$) and the estimated PhytOC fluxes ($R=0.076$, $p>0.05$).

3 Discussion

Rice accumulates Si (Seyfferth et al, 2013), and the Si concentration is approximately 10%-15% in the rice plant (Marschner, 1995) with approximately 90% of the Si present in the phytolith (Wang, 1998). There was a significant correlation between the Si content and the phytolith content of the crop materials, such as the phytolith contents of the rice leaves, stems, and sheaths (Song et al, 2014a). The shape of the phytoliths in the different rice organs varied (e.g., double-peaked, bulliform and parallel dumbbell phytoliths) (Prajapati et al, 2016). Prajapati et al. reported that the phytolith content in the different rice organs (stem, sheath, leaf and grain) ranged from 0.14 g kg⁻¹ to 26.4 g kg⁻¹ (Prajapati et al, 2016). Similar results and trends were reported by other researchers (Guo et al, 2015; Li et al, 2013c). Our results showed that whether the paddy soil was Si-deficient or Si-enriched, the utilization of Si fertilizer could significantly ($p < 0.05$) improve the phytolith content of the rice organs (Tables 2, 3), such as the stem, sheath, leaf, grain and root. According to formation mechanism of phytolith, the soil available Si is taken up by rice plant the roots, usually take the shape of the plant cell or cell spatium where Si is deposited (Ma, 2003; Neumann, 2003; Piperno, 1988; Song et al, 2016). Thus, the use of Si fertilizer increased the content of effective Si in the soil (Cai, 2015; Liu et al, 2006; Ma et al, 2004) and increased the absorption capacity of Si in the rice (Guo et al, 2015; Huan et al, 2018; Li et al, 2013c; Seyfferth et al, 2013; Zuo et al, 2016), therefore increasing the phytolith content of the rice plant (Table 4).

A substantial amount of research reported that the factors of the PhytOC content were as follows: different varieties (Li et al, 2013b; Parr et al, 2010; Parr et al, 2009; Parr and Sullivan, 2011; Song et al, 2017; Sun et al, 2017), pest and disease resistances (Ma et al, 2002), nitrogen utilization (Zhao et al, 2016), basalt powder (Guo et al, 2015), soil-effective Si content (Klotzbücher

et al, 2018; Song et al, 2014b) and net production on the ground(Blecker et al, 2006). It has been shown that Si is an important element for rice growth and the deficiency of plant-available Si may exert an adverse effect on the rice yield through biotic stresses, disease and pests, and so on(Ma et al, 2004; Ma, 2004). Our results also showed that the content of phytolith in rice plants were different in Si-deficient and Si-enriched paddy soil. The content of Phytolith in rice plants with Si-enriched paddy soils was higher than that in rice plants with Si-deficient paddy soil (Table 2, 4). Moreover, whether in silicon-deficient soil or in silicon-deficient soil, The phytolith content of rice plants was positive correlation($p<0.05$) with the Si fertilizer dose in two types paddy soil((Table 5, 6). Previous studied have demonstrated that the content of the Si (phytoliths) in crops may be promoted through the Si fertilizers application (Alvarez and Datnoff, 2001; Liang et al, 2010; Mecfel et al, 2010). Further, in the Si-deficient paddy soil, the estimated PhytOC fluxes was significantly related to the Si fertilizers ($R=0.973, p<0.05$), and the phytolith content ($R=0.986, p<0.05$)and the biomass of the rice ($R=0.994, p<0.01$)(Table 5). However, in the Si-enriched paddy soil, the estimated PhytOC fluxes was not correlation($P>0.05$) with these factors. Zhang et al showed (Zhang et al, 2014)that the yield of rice was increased 14.5% by the use of 225 kg ha^{-1} Si fertilizer; when the application of Si fertilizer was increased to 375 kg ha^{-1} , the yield of the rice was only increased by 10.1%. Similarly, Wu et al also recommended the use of 225 kg ha^{-1} Si fertilizer as the most economical measure(Wu et al, 2014). We also obtained the same results. The application of Si fertilizer to the Si-enriched paddy soil did not increase the biomass of the rice but reduced it. In particular, when the amount of the Si fertilizer reached 600 kg ha^{-1} , the rice biomass decreased significantly by 29.10% compared with the control treatment (Table 4). Therefore, excessive Si fertilizer not only has no benefit to the accumulation of estimated PhytOC

fluxes in rice plant, but also reduces the yield of rice. However, for Si-deficient soils, the application of Si fertilizer can not only increase rice yield, but also increase phytoliths content of rice plants and the estimated PhytOC fluxes (Table 4). Thus, different Si fertilizer doses were one of measures to improve the phytolith content and the biomass of the rice plant. Thus, how to promote the phytolith content and C content of phytoliths will require further in-depth study.

The global rice cultivation area was approximately 1.64×10^8 ha in 2014 (Prajapati et al, 2016); When rice is harvested, the rice straw and husks are removed from the paddy field and used for other purposes, including animal feeding and firewood, or simply incinerated (Savant et al, 1996). Thus, most of the Si taken up by rice is removed from a field when the rice straw is removed, and the loss of SiO_2 is from 75 kg ha^{-1} to 130 kg ha^{-1} every production season (Zhang et al, 2014). Such large losses of Si make it difficult to maintain the balance of Si in soils from natural weathering alone. Appropriate dosages of Si fertilizer could solve the problem of Si deficiency in soil, and increase the biomass of rice and the content of phytolith in rice plants, and indeed result in the occlusion of increased CO_2 in the rice plants (Liang et al, 2010; Mecfel et al, 2010). The estimated PhytOC fluxes increased from $0.49 \text{ Kg-e-CO}_2 \text{ ha}^{-1} \text{ yr}^{-1}$ to $4.52 \text{ Kg-e-CO}_2 \text{ ha}^{-1} \text{ yr}^{-1}$ (Table 4). More than $8.04 \times 10^4 \text{ Mg-e-CO}_2$ to $7.41 \times 10^5 \text{ Mg-e-CO}_2$ would have been occluded within the phytolith of the rice plants per year globally. Considering that the highest estimated PhytOC flux of the rice plants, $2.12 \times 10^6 \text{ Mg-e-CO}_2$, would have been occluded within the phytolith of rice plants every year. Although the annual CO_2 bio-sequestration within the rice phytoliths of the unit area is likely to be lower than that of other plants, such as bamboo leaf litter ($1.56 \times 10^7 \text{ Mg-e-CO}_2 \text{ yr}^{-1}$) (Parr, 2006), wetland plants ($4.39 \times 10^7 \text{ Mg-e-CO}_2 \text{ yr}^{-1}$) (Guo et al, 2015), grasslands ($4.14 \times 10^7 \text{ Mg-e-CO}_2 \text{ yr}^{-1}$) (Song et al, 2012), millet ($2.37 \times 10^6 \text{ Mg-e-CO}_2 \text{ yr}^{-1}$) (Pan et al, 2017) and sugarcane

leaf(0.72×10^7 Mg-e-CO₂yr⁻¹) (Parr et al, 2009). In this study, we showed that Si fertilizer application could promote the phytolith content and biomass of rice plants and further improve the estimated PhytOC flux of rice plants. Thus, the measure provided a theoretical basis for the bio-carbon sequestration of the rice plant and laid a foundation for PhytOC fixation in paddy soil by the return of straw.

Conclusion

The use of silicon fertilizer could significantly increase the phytolith content of rice plants in Si-deficient red paddy soil or Si-enriched Wushan soil. The phytolith content of rice plants was positive correlation with the silicon fertilizer dose in two types paddy soil. The estimated PhytOC fluxes in Si-deficient red paddy soil was a positive correlation with the phytolith content, the biomass of the rice and the silicon fertilizer dose, respectively. In this study, we estimated that the PhytOC fluxes increased from $0.49 \text{ Kg-e-CO}_2 \text{ ha}^{-1} \text{ yr}^{-1}$ to $4.52 \text{ Kg-e-CO}_2 \text{ ha}^{-1} \text{ yr}^{-1}$. More than 8.04×10^4 Mg-e-CO₂ to 7.41×10^5 Mg-e-CO₂ would have been occluded within the phytoliths of the rice plants per year globally. Therefore, Si fertilizer application might provide a new approach to increase the atmospheric CO₂ occluded within the phytoliths, offering a potential method.

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References

252 Alvarez, J. and Datnoff, L. E. (2001). The economic potential of silicon for integrated management and
 253 sustainable rice production *Crop Prod* 20, 43-48.

254 Anala, R. and Nambisan, P. (2015). Study of morphology and chemical composition of phytoliths on
 255 the surface of paddy straw. *Paddy & Water Environment* 13, 521-527.

256 Blecker, S. W., Mcculley, R. L., Chadwick, O. A. and Kelly, E. F. (2006). Biologic cycling of silica across a
 257 grassland bioclimosequence. *Global Biogeochemical Cycles* 20, 4253-4274.

258 Cai, D. (2015). Silicon fertilizer: a new resource-saving and environment-friendly fertilizer. *China*
 259 *Agri-Production News*, 23-23.

260 Chadwick, K. M., Johnson, R. G., Dhaul, A. K., Steinmeyer, R. D. and Halm, R. L. (1994). Conversion of
 261 direct process high-boiling component to chlorosilane monomers in the presence of chlorine.

262 Clarke, J. (2003). The occurrence and significance of biogenic opal in the regolith. *Earth Science*
 263 *Reviews* 60, 175-194.

264 Epstein, E. (1994). The anomaly of silicon in plant biology. *Proceedings of the National Academy of*
 265 *Sciences of the United States of America* 91, 11-17.

266 Guo, F., Song, Z., Sullivan, L., Wang, H., Liu, X., Wang, X., Li, Z. and Zhao, Y. (2015). phytolith carbon
 267 sequestration in rice ecosystems through basalt powder amendment. *Science Bulletin* 60,
 268 591-597.

269 Henriët, C., Bodarwé, L., Dorel, M., Draye, X. and Delvaux, B. (2008). Leaf silicon content in banana
 270 (*Musa* spp.) reveals the weathering stage of volcanic ash soils in Guadeloupe. *Plant and Soil* 313,
 271 71-82.

272 Hodson, M., White, P., Mead, A. and Broadley, M. (2005). Phylogenetic variation in the silicon
 273 composition of plants. *Annals of Botany* 96, 1027-1046.

274 Huan, X., Lu, H., Zhang, J. and Wang, C. (2018). Phytolith assemblage analysis for the identification of
 275 rice paddy. *Scientific Reports* 8

276 Ji, Z., Yang, X., Song, Z., Liu, H., Liu, X., Qiu, S., Li, J., Guo, F., Wu, Y. and Zhang, X. (2017). Silicon
 277 distribution in meadow steppe and typical steppe of northern China and its implications for
 278 phytolith carbon sequestration. *Grass & Forage Science*, DOI: 10.1111/gfs.12316.

279 Kameník, J., Mizera, J. and Řanda, Z. (2013). Chemical composition of plant silica phytoliths.
 280 *Environmental Chemistry Letters* 11, 189-195.

281 Klotzbücher, T., Klotzbücher, A., Kaiser, K., Merbach, I. and Mikutta, R. (2018). Impact of agricultural
 282 practices on plant-available silicon. *Geoderma* 331, 15-17.

283 Li, B., Song, Z., Li, Z., Wang, H., Gui, R. and Song, R. (2014). Phylogenetic variation of phytolith carbon
 284 sequestration in bamboos. *Sci Rep* 4, 4710.

285 Li, Z., Song, Z. and Li, B. (2013a). The production and accumulation of phytolith-occluded carbon in
 286 Baiyangdian reed wetland of China. *Applied Geochemistry* 37, 117-124.

287 Li, Z., Song, Z., Li, Beilei and Cai, Y. (2013b). Phytolith production in wetland plants of the Hangzhou Xixi
 288 Wetlands ecosystem. *Journal of Zhejiang A & F University* 30, 470-476.

289 Li, Z., Song, Z., Parr, J. F. and Wang, H. (2013c). Occluded C in rice phytoliths: implications to
 290 biogeochemical carbon sequestration. *Plant and Soil* 370, 615-623.

291 Liang, Y., Haixia, H., Yong-Guan, Z., Jie, Z., Chunmei, C. and Volker, R. M. (2010). Importance of plant
 292 species and external silicon concentration to active silicon uptake and transport. *New Phytologist*
 293 172, 63-72.

294 Liu, M. d., Zhang, Y. l., Meng, X. f., Li, J., Yang, D., Wang, Y.-j. and Yu, N. (2006). Eva lua tion of Silicon
 295 Supply ing Capac ity of Paddy Soil by Silicon Libera tion Dynamics Equa tion

296 I The Relationship between Parameter of Soil Silicon Liberation Dynamics Equation and Relative Yield.

297 *Chinese Journal of Soil Science* 37, 107-110.

298 Ma, F., Namiki, M., Sakiko, N., Saeko, K., Kazunori, T., Takashi, I. and Masahiro, Y. (2004).

299 Characterization of the Silicon Uptake System and Molecular Mapping of the Silicon Transporter

300 Gene in Rice. *Plant Physiology* 136, 3284-3289.

301 Ma, F. J. (2004). Role of Silicon in Enhancing the Resistance of Plants to Biotic and Abiotic Stresses. *Soil*

302 *Sci Plant Nutr* 50, 11 - 18.

303 Ma, J. F. (2003) *Functions of Silicon in Higher Plants*: Springer Berlin Heidelberg.

304 Ma, J. F., Kazunori, T., Masahiko, I. and Guo Feng, W. (2002). A rice mutant defective in Si uptake. *Plant*

305 *Physiology* 130, 2111-2117.

306 Ma, J. F., Tamai, K., Yamaji, N., Mitani, N., Konishi, S., Katsuhara, M., Ishiguro, M., Murata, Y. and Yano,

307 M. (2006). A silicon transporter in rice. *Nature* 440, 688-691.

308 Mecfel, J., Hinke, S., Goedel, W. A., Marx, G., Fehlhaber, R., Bäucker, E. and Wienhaus, O. (2010). Effect

309 of silicon fertilizer on silicon accumulation in wheat. *Journal of Plant Nutrition and Soil Science* =

310 *Zeitschrift fuer Pflanzenernaehrung und Bodenkunde* 170, 769-772.

311 Neumann, D. (2003) *Silicon in Plants*: Springer Berlin Heidelberg.

312 Pan, W., Song, Z., Liu, H., Zwieten, L. V., Li, Y., Yang, X., Han, Y., Liu, X., Zhang, X. and Xu, Z. (2017). The

313 accumulation of phytolith-occluded carbon in soils of different grasslands. *Journal of Soils &*

314 *Sediments* 17, 1-8.

315 Parr, J., Sullivan, L., Chen, B., Gongfu, Y. E. and Zheng, W. (2010). Carbon bio-sequestration within the

316 phytoliths of economic bamboo species. *Global Change Biology* 16, 2661-2667.

317 Parr, J., Sullivan, L. and Quirk, R. (2009). Sugarcane phytoliths: encapsulation and sequestration of a

318 long-lived carbon fraction. *Sugar Tech* 11, 17-21.

319 Parr, J. F. (2006). Effect of fire on phytolith coloration. *Geoarchaeology-an International Journal* 21,

320 171-185.

321 Parr, J. F. and Sullivan, L. A. (2005). Soil carbon sequestration in phytoliths. *Soil Biology & Biochemistry*

322 37, 117-124.

323 Parr, J. F. and Sullivan, L. A. (2011). Phytolith occluded carbon and silica variability in wheat cultivars.

324 *Plant and Soil* 342, 165-171.

325 Pearsall, D. M. (1989) Paleoethnobotany. *A Handbook of Procedures*. Academic Press. , pp. 1-470.

326 Piperno, D. R. (1988) *Phytolith Analysis: An Archaeological and Geological Perspective*: San Diego:

327 Academic Press.

328 Prajapati, K., Rajendiran, V. Coumar, Dotaniya, Ajay, Kundu and Patra (2016). Carbon occlusion

329 potential of rice phytoliths: Implications for global carbon cycle and climate change mitigation.

330 *Applied Ecology and Environmental Research* 14, 265-281.

331 Savant, N. K., Snyder, G. H. and Datnoff, L. E. (1996). Silicon Management and Sustainable Rice

332 Production. *Advances in Agronomy* 58, 151-199.

333 Seyfferth, A. L., Kocar, B. D., Lee, J. A. and Fendorf, S. (2013). Seasonal dynamics of dissolved silicon in

334 a rice cropping system after straw incorporation. *Geochimica Et Cosmochimica Acta* 123,

335 120-133.

336 Si, Y., Wang, L., Zhou, Q. and Huang, X. (2018). Effects of lanthanum and silicon stress on

337 bio-sequestration of lanthanum in phytoliths in rice seedlings. *Environmental Science & Pollution*

338 *Research* 25, 10752-10770.

339 Song, Z., Liu, H., Li, B. and Yang, X. (2013). The production of phytolith-occluded carbon in China's

340 forests: implications to biogeochemical carbon sequestration. *Global Change Biology* 370,
341 615-623.

342 Song, Z., Liu, H., Si, Y. and Yin, Y. (2012). The production of phytoliths in China's grasslands:
343 implications to the biogeochemical sequestration of atmospheric CO₂. *Global Change Biology* 18,
344 3647-3653.

345 Song, Z., Liu, H., Strömberg, C., Yang, X. and Zhang, X. (2017). Phytolith carbon sequestration in global
346 terrestrial biomes. *Science of the Total Environment* 603-604, 502-509.

347 Song, Z., Müller, K. and Wang, H. (2014a). Biogeochemical silicon cycle and carbon sequestration in
348 agricultural ecosystems. *Earth-Science Reviews* 139, 268-278.

349 Song, Z., Mcgrouter, K. and Wang, H. (2016). Occurrence, turnover and carbon sequestration
350 potential of phytoliths in terrestrial ecosystems. *Earth-Science Reviews* 158, 19-30.

351 Song, Z., Wang, H., Strong, P. J. and Guo, F. (2014b). Phytolith carbon sequestration in China's
352 croplands. *European Journal of Agronomy* 53, 10-15.

353 Sun, X., Liu, Q., Zhao, G., Chen, X., Tang, T. and Xiang, Y. (2017). Comparison of phytolith-occluded
354 carbon in 51 main cultivated rice (*Oryzasativa*) cultivars of China. *Rsc Advances* 7, 54726-54733.

355 Sun, X., Qin, L., Xiang, C. and Keya, Z. (2016). Evaluation of the occluded carbon within husk phytoliths
356 of 35 rice cultivars. *Frontiers of Earth Science* 10, 683-690.

357 Wang, Y. J. (1998). A study on the chemical composition of phytoliths. *J Oceano Huanghai Bohai Seas* 16,
358 33-38.

359 Wen, C., Lu, H., Zuo, X. and Ge, Y. (2018). Advance of research on modern soil phytolith. *Science China*
360 *Earth Sciences* 61, 1-14.

361 Wu, H.-b., Shen, J.-n. and Hu, S.-s. (2014). Effect of Silicon on Plant Traits and Yield of Cold Region Rice.

362 *North Rice(in chinese)* 44, 12-15.

363 Yang, X., Song, Z., Liu, H., Bolan, N. S., Wang, H. and Li, Z. (2015). Plant silicon content in forests of

364 north China and its implications for phytolith carbon sequestration. *Ecological Research* 30,

365 347-355.

366 Zhang, L., Chen, X., Chang, B., Gu, X., Li, W., wang, S. and Wang, X. (2014). Application effect of silicon

367 fertilizer on rice. *Heilongjiang Agricultural Sciences (in Chinese)*, 43-46.

368 Zhao, Y., Song, Z., Xu, X., Liu, H., Wu, X., Li, Z., Guo, F. and Pan, W. (2016). Nitrogen application

369 increases phytolith carbon sequestration in degraded grasslands of North China. *Ecological*

370 *Research* 31, 117-123.

371 Zuo, X. and Lü, H. (2011). Carbon sequestration within millet phytoliths from dry-farming of crops in

372 China. *Science Bulletin* 56, 3451-3456.

373 Zuo, X., Lu, H., Li, Z., Song, B., Xu, D., Zou, Y., Wang, C., Xiuja, H. and He, K. (2016). Phytolith and

374 diatom evidence for rice exploitation and environmental changes during the early mid-Holocene

375 in the Yangtze Delta. *Quaternary Research* 86, 304-315.

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392 **Figure captions**

393 **Figure 1.** Optical microscope images of phytoliths extracted from the rice samples using the wet ashing Method
394 according to Zuo (2011) and Sun(2016), Magnification 400×, scale bar 30 μm.

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Table 1 Basic chemical properties of the two soils

Experimental Soils	NH ₄ OAc -extractable Si (mg kg ⁻¹)	pH	total N (g kg ⁻¹)	total P (g kg ⁻¹)	total K (g kg ⁻¹)	organic matter (mg kg ⁻¹)	Na ₂ CO ₃ - extractable P (mg kg ⁻¹)	NH ₄ OAc- extractable K (mg kg ⁻¹)
Si-deficient paddy soil (red paddy soil)	5.67	4.62	1.20	0.18	52.49	28.89	17.44	210.0
Si-enriched paddy soil (Wushan soils)	252.3	7.54	2.40	0.73	20.16	39.89	34.27	101.7

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401 Table 2 Effect different of silicon fertilizers on rice organs content of phytoliths,(W: Wushan soil; X:
402 Red paddy soil;G: Leg silicon fertilizer)

Treatment s	stem(g kg ⁻¹)	sheath(g kg ⁻¹)	leaf(g kg ⁻¹)	grain(g kg ⁻¹)	root(g kg ⁻¹)
XCK	13.00±1.44c	36.01±1.45c	34.72±1.50c	11.59±2.41b	80.66±25.81a
XG1	16.84±1.07bc	42.73±2.74b	41.37±2.25b	13.86±1.61b	117.20±18.58a
XG2	18.50±0.91b	49.37±0.89a	48.72±2.09a	12.96±3.49b	92.87±36.86a
XG3	26.10±4.41a	46.62±2.38a	47.51±3.08a	19.37±1.38a	84.78±30.35a
WCK	37.22±4.19b	75.31±4.68bc	76.18±4.44b	16.60±3.29b	67.46±22.70b
WG1	38.57±4.63b	74.18±3.21c	76.14±8.10b	17.26±1.92b	56.09±1.76b
WG2	42.09±3.23b	84.00±5.19b	93.13±11.68a	21.69±1.83ab	96.66±14.10a
WG3	49.44±0.74a	95.72±5.59a	100.60±7.98a	24.72±4.96a	82.03±5.32ab

403 Different lowercase letters after the data means that the difference between different type of
404 Si-fertilizers dosage treatments is significant (p<0.05)

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Table 3 Effect different of silicon fertilizers on rice organs content of C content of phytoliths, (W: Wushan soil; X: Red paddy soil; G: Leg silicon fertilizer)

Treatments	stem(g kg ⁻¹)	sheath(g kg ⁻¹)	leaf(g kg ⁻¹)	grain(g kg ⁻¹)	root(g kg ⁻¹)
XCK	8.60±1.34a	5.80±0.75a	8.02±1.81a	4.78±0.61a	1.67±0.01a
XG1	4.85±1.20b	6.09±0.92a	9.21±3.84a	6.79±1.87a	1.88±0.31a
XG2	5.28±1.20b	4.82±1.20a	9.47±1.75a	7.08±1.43a	1.68±0.24a
XG3	4.64±0.38b	4.61±0.97a	7.23±1.20a	5.10±1.90a	2.19±0.34a
WCK	2.28±0.36a	2.28±0.39ab	7.41±2.81a	6.53±2.91a	4.98±1.42a
WG1	2.59±0.55a	2.16±0.11ab	3.22±0.82a	3.39±0.45a	4.79±2.22a
WG2	4.13±2.0a	2.82±0.54a	5.58±3.37a	4.34±0.92a	4.68±0.59a
WG3	2.23±0.67a	1.90±0.09b	4.31±2.57a	4.42±1.18a	4.03±1.12a

Different lowercase letters after the data means that the difference between different type of Si-fertilizers dosage treatments is significant (p<0.05)

413 Table 4 Effect different of silicon fertilizers on rice plants content of phytoliths, C content of
 414 phytoliths, PhytOC content of dry organs weight, and the estimated PhytOC fluxes per ha in kg of
 415 CO₂ equivalents (kg~e~ CO₂) for rice (W: Wushan soil; X: Red paddy soil;G: Leg silicon fertilizer)

Treatment s	Phytolith content(g kg ⁻¹)	C content of phytoliths(g kg ⁻¹)	PhytOC content of dry organs weight (g kg ⁻¹)	Estimated PhytOC fluxes (kg-CO ₂ ha ⁻¹ yr ⁻¹)	Biomass (t ha ⁻¹)
XCK	24.23±0.39b	5.74±0.52a	0.13±0.01b	8.41±0.99b	16.38±2.34b
XG1	26.64±1.02a	6.61±1.48a	0.16±0.04a	10.38±2.43a	18.43±1.74a
XG2	28.32±2.06a	6.56±1.04a	0.17±0.03a	11.36±2.34a	18.90±1.67a
XG3	31.94±2.18a	5.14±1.05a	0.16±0.03a	12.93±0.54a	20.33±0.77a
WCK	34.96±0.88b	5.44±1.80a	0.17±0.05a	8.74±0.29a	17.94±0.46a
WG1	36.22±1.06b	3.21±0.15a	0.11±0.01a	7.92±0.95a	17.42±1.61a
WG2	48.06±4.34a	4.29±1.09a	0.21±0.08a	11.11±1.93a	17.78±4.38a
WG3	53.85±0.79a	4.39±0.68a	0.21±0.07a	9.23±0.09a	14.76±1.78b

416 Different lowercase letters after the data means that the difference between different type of
 417 Si-fertilizers dosage treatments is significant (p<0.05)
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Table 5 The Correlation coefficients between the six variables of the red paddy soil

Variables	Silicon fertilizer	Phytolith content	C content of phytoliths	PhytOC content of dry organs weight	Estimated PhytOC fluxes	Biomass
Silicon fertilizer	1					
Phytolith content	0.998**	1				
C content of phytoliths	-0.238	-0.177	1			
PhytOC content of dry organs weight	0.620	0.665	0.612	1		
Estimated PhytOC fluxes	0.973*	0.986*	-0.008	0.795	1	
Biomass	0.953*	0.972*	0.041	0.799	0.994**	1

420 * Correlation is significant at the 0.05 level (2-tailed).

421 ** Correlation is significant at the 0.01 level (2-tailed).

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Table 6 The Correlation coefficients between the six variables of the Wushan soil

Variables	Silicon fertilizer	Phytolith content	C content of phytoliths	PhytOC content of dry organs weight	Estimated PhytOC fluxes	Biomass
Silicon fertilizer	1					
Phytolith content	0.952*	1				
C content of phytoliths	-0.209	-0.035	1			
PhytOC content of dry organs weight	0.599	0.796	0.526	1		
Estimated PhytOC fluxes	0.333	0.598	0.229	0.800	1	
Biomass	-0.890	-0.746	0.100	-0.393	0.076	1

425 * Correlation is significant at the 0.05 level (2-tailed).

426 ** Correlation is significant at the 0.01 level (2-tailed).

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