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Silica uptake from nanoparticles and silica condensation state in different tissues of *Phragmites australis*

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HIGHLIGHTS

- Silicon precipitation occurs in tissues with transpiration function.
- Silica condensation state in grass depends on dominant function of the tissue.
- Nanosilica is mainly dissolved prior to uptake.

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ABSTRACT

Silicon is described as beneficial for grasses by enhancing yield and fitness via a considerable contribution to pathogen, drought, and pest resistance. Silicic acid is the predominant form for uptake and transport within the plant and will precipitate in leaves. But it is unknown whether polymeric nanosilicon compounds in its synthetic form, with an increasing concentration in aquatic environments, can be suitable for plant nutrition. Therefore, we investigated the uptake, transport, and deposition of silicic acid/silica within plants using synthetic nanosilica. Our results show a significant difference in silicon (Si) content within the different tissues of *Phragmites australis*. The nanosilica had been dissolved prior to the uptake by plants. The chemical form of Si during uptake was not traceable. A significant enhancement in the condensation state of the silica was found from root to leaves especially from culm to leaf tips visible by the increasing content of Q^4-groups in the NMR spectra. We conclude that synthetic nanosilica has the same quality as source for the beneficial element Si like natural silica. Since the condensation state is described to control silica solubility, we suggest that different condensation states within the plant may result in different remobilization of silicon during decomposition of the plant material.

1. Introduction

Silicon as the second most abundant element on the Earth's crust is known to be beneficial or even essential for many organisms. Many terrestrial (e.g. grasses) and aquatic (emergent and submerged macrophytes) plants accumulate high amounts of amorphous Si in their tissues (Schoelynck et al., 2010; Struyf et al., 2007). In ecosystems dominated by Si-accumulating species like grasses, high amounts of Si are cycled each year via plant root uptake and litter fall (Cornelis et al., 2010; Melzer et al., 2010). A plant species likely to be important in Si cycling is common reed (*Phragmites australis* TRIN. ex Steudt.), which is one of the most abundant wetland plants worldwide. Amorphous silicon is beneficial with positive effects on biomass production of grasses (Eneji et al., 2008; Schaller et al., 2012), as well on pathogen and herbivore defense (Fawe et al., 1998) and amelioration of abiotic stress (Ma, 2004).

Silicon is immobilized by biomineralization as plant opals (phytoliths) in cell walls (Epstein, 1999; Sangster, 1970) and as amorphous silica forming a Si double layer immediately beneath the cuticle (Currie and Perry, 2007; Schaller et al., 2012). The uptake of Si mainly in the form of amorphous silica is either passive (unspecific) or active by ATP mediated transporters via (special groups of) aquaporins with subsequent genetic control (Ma et al., 2004; Ma and Yamaji, 2006).

Current findings have shown that *P. australis* cannot avoid the surplus uptake of Si into aboveground biomass (Schaller et al., 2012). Hereby Si seems to be transported within plants by transpiration driven water transport and is precipitated mainly in tissues with photosynthesis (transpiration) function. It was shown that the speciation of Si in grasses differs between uptake/transportation tissues and tissues with transpiration function. In roots and culm, Si exists mainly as silicic acid [$Si(OH)_4$], an uncharged monomeric molecule (at pH values below 9) whereas in leaf blades and leaf sheath, $Si(OH)_4$

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mainly polymerizes forming silica gel [$SiO_2 \cdot nH_2O$] (higher condensation state) (Ma and Yamaji, 2006). A more precise determination of the change in Si speciation within different plant tissues (fine roots, coarse roots, rhizomes, culms, leaf sheath, leaf blades and leaf tips) has not been done so far. Furthermore, nothing is known about the uptake and transport of pyrogenic synthetic nanoparticles of amorphous silica within the different tissues, whereas an uptake via aquaporins can be neglected considering the particle size (Liu and Zhu, 2010). The production and application of synthetic silica nanoparticles increased during the last years (Som et al., 2011). It is known that silica nanoparticles occurring in the air can easily enter the lungs of mammals by air inhalation and will be distributed within their body by lymph and blood (Silva et al., 2012). While air-born silica nanoparticles washed out by rain result in higher concentrations of silica nanoparticles in the aquatic environment. Silica nanoparticles may also enter the aquatic environments directly from textiles by washing (Som et al., 2011). These particles are in turn difficult to remove from the water pathway (Chang et al., 2007; Chin et al., 2006), which points to the higher availability of silica nanoparticles for plants growing in aquatic environments.

Consequently, the uptake of Si from pyrogenic artificial amorphous nanosilica and at the same time the condensation state of Si within the different plant tissues were investigated in a pot experiment using *P. australis*.

2. Material and methods

2.1. Plant material and experimental conditions

Rhizomes of *P. australis* were collected at the beginning of March 2009 from a permanently submerged littoral stand in Lake Großteich 10 km northeast of Dresden, Germany (51°08′N, 13°43′E). The rhizomes were carefully washed with tap water to remove attached sediment before choosing pieces of about 15 cm length with a diameter of about 5 cm and the same morphology. Three pieces of rhizome were planted at 10 cm depth in each of thirty-six 15-L polyethylene buckets containing 1 kg of peat (Borena, Cologne, Germany) with 10% Si by dry weight. This peat has further a pH = 4.0, a carbon content of 42%, a nitrogen content of 1% and a phosphorus content of 0.01%. A molar N:P supply ratio of about 9 (weak nitrogen limitation according to Güsewell and Freeman (2005)) was chosen to provide maximum yield (Rejmankova, 2005; Struyf et al., 2007) and a nitrogen content comparable to that observed in natural reed stands (Kohl et al., 1998). The buckets were fertilized in April and July by adding nutrient solutions per pot each time containing $CaCl_2 \cdot 2H_2O$ (0.83 mmol), K_2HPO_4 (2.30 mmol), NH_4NO_3 (10 mmol), $MgSO_4 \cdot 7H_2O$ (2.03 mmol), H_3BO_3 (0.24 mmol), $Na_2MoO_4 \cdot 2H_2O$ (0.10 mmol), $ZnSO_4 \cdot 7H_2O$ (0.22 mmol), $CuSO_4 \cdot 5H_2O$ (0.02 mmol), $CoSO_4 \cdot 7H_2O$ (0.0007 mmol), $MnCl_2 \cdot 4H_2O$ (0.18 mmol), $FeSO_4 \cdot 7H_2O$ (0.09 mmol), and $Na_2EDTA \cdot 2H_2O$ (1.34 mmol). Evaporated water was replaced every day with deionized water.

The Si uptake experiment was set up in four open-top-chambers (OTC) at a field station near Dresden (Germany, 50°57′40.07″N, 13°33′32.59″E) using filtered ambient air and ambient temperature conditions. The chambers had a roof to prevent nutrient inputs by dry or wet deposition, a volume of 22.8 m^3, and an air exchange rate of 50 $m^3\ h^{-1}$. The buckets received 100 g silica (46.6 g Si) per pot and were replicated 12 times. Silicon was added in the form of low acidic (pH ~ 4.7), synthetic amorphous silica (Aerosil 300; Evonik Industries AG, Essen, Germany). The pH of the substrate was adjusted to 5.3 by mixing 10 g of $CaCO_3$ per pot with the peat before planting the rhizomes. This pH is close to the pH of the silica species used and within the natural pH range of soils and sediments in which *P. australis* grows in the field. No differences in pH (measured using Electrode: Mettler Toledo INLAB 414 and unit: Delta 320, Germany) and conductivity (530 μS cm^{-1}) (measured using LF 39, Meinsberg, Germany) were observed during the experiment. At the start of experiment the silicon water concentration was 35 mg L^{-1}, whereas at the end of the experiment a mean silicon concentration of 25.3 (22.6–28.4) mg L^{-1} was found in the pore water.

2.2. Sampling, sample preparation and analysis

Standing-dead fully brown plant shoots were clipped just above the sediment surface on 19 November 2009 when nutrients and carbon had been translocated to the rhizomes as shown in other experiments (Tylova et al., 2008). The harvested shoots were separated into leaf tips, leaf blades, leaf sheaths and culms, and subsequently dried at 50 °C to constant weight. At the same time fine roots (without aeration parenchyma) and coarse roots (with aeration parenchyma) and rhizomes, without evidence of senescence, were sampled, carefully washed with deionized water and afterwards dried at 105 °C to constant weight. A CEM Mars5 microwave digestion system (CEM Corporation, Matthews, NC, USA) was used to digest the ground plant material (particle size < 0.5 mm) at 180 °C in 3 mL of HNO_3, 1.5 mL of HF and 3 mL of H_3BO_3. Standard reference material (poplar leaves, GBW7604; Office of Certified Reference Material, Langfang, China) that had been ground and digested as above was used for calibration. Silicon was determined by ICP-OES (Optima 7000DV, Perkin Elmer) with UV detection and quantification at 251.6 nm (Si).

Solid-state ^{29}Si NMR measurements were performed on a Bruker Avance 300 spectrometer operating at a resonance frequency of 59.6 MHz using a commercial double resonance 4 mm MAS NMR probe. Single-pulse excitation spectra were recorded at a MAS spinning rate of 14,000 Hz. During the signal acquisition ^1H decoupling (Fung et al., 2000) was applied. The spectra were referenced relative to tetramethylsilane (TMS). For quantitative ^{29}Si NMR measurements DSS (natrium-3-trimethylsilyl-1-propansulfonat, $\delta = 0$ ppm) was used as reference substance.

A part of the leaf blade was examined with scanning electron microscopy (SEM, JEOL T 330A) equipped with an element detector (EDR 288; Röntec, Berlin, Germany) and run at 15 kV. Plant sample for electron microscopy was critical-point dried and subsequently coated with carbon (Zimmermann et al., 2000). An area of about 0.09 mm^2 in the middle of the leaf blade was scanned. All scan was run at a magnification of 350×.

Kruskal–Wallis-test and Mann–Whitney-test were applied for comparison of the data of the different tissues using the statistic program SPSS version 14.0 (IBM, U.S.).

3. Results and discussion

3.1. Distribution of silica within the plant

Silica uptake in the root system is reported to be either unspecific or mediated by special groups of aquaporins with subsequent genetic control (Ma et al., 2004; Ma and Yamaji, 2006). Once taken up, silica is transported within the plant precipitating mainly in tissues with high photosynthetic activity and related transpiration (Ma and Yamaji, 2008). Our results reveal significant differences ($p < 0.001$, Kruskal–Wallis-test) in Si content between different tissues at the end of the experiment (Fig. 1). As shown previously, significant differences (Mann–Whitney-test) for adjacent tissues were found between culm and leaf sheath ($p < 0.005$), between leaf sheath and leaf blades ($p < 0.005$) (Schaller et al., 2012). This can be explained by a permanent supply due to dissolution of the nanosilica in the rhizosphere and an immobilization in low amounts in the roots and a transport in low concentrations within the different plant tissues, which may differ between plant species (Mitani and Ma, 2005). Silica precipitates in tissues with photosynthesis activity (Schaller et al., 2012) as a possible result of Si supersaturation due to boundary layer water loss via stomata (Meinzer et al., 1990). No differences in the amount and distribution of silica in plant tissues were found comparing with plants