Originally published as:


DOI: http://doi.org/10.1111/ggr.12243
Laser ablation in situ silicon stable isotope analysis of phytoliths

Daniel A. Frick¹, †, Jan A. Schuessler¹*, Michael Sommer²,³, Friedhelm von Blanckenburg¹,⁴

¹ GFZ German Research Centre for Geosciences, Earth Surface Geochemistry, Potsdam, Germany.
² Leibniz-Centre for Agricultural Landscape Research (ZALF) e. V., Working Group Si Biogeochemistry, 15374 Müncheberg, Germany.
³ University of Potsdam, Institute of Earth and Environmental Sciences, Karl-Liebknecht-Str. 24-24, 14476 Potsdam, Germany.
* present address: Thermo Fisher Scientific GmbH, 28199 Bremen, Germany

† dfrick@gfz-potsdam.de, +49 331 288 28963

Abstract
Silicon is a beneficial element for many plants, and is deposited in plant tissue as amorphous bio-opal called phytoliths. The biochemical processes of silicon uptake and precipitation induce isotope fractionation: the mass-dependent shift in the relative abundances of the stable isotopes of silicon. At the bulk scale, δ³⁰Si ratios span from -2 to +6 ‰. To further constrain these variations in situ, at the scale of individual phytolith fragments, we used femtosecond laser ablation multicollector inductively coupled plasma mass spectrometry (fsLA-MC-ICP-MS).

A variety of phytoliths from grasses, trees and ferns were prepared from plant tissue or extracted from soil. Good agreement between phytolith δ³⁰Si ratios obtained by bulk solution MC-ICP-MS analysis and in situ isotope ratios from fsLA-MC-ICP-MS validates the method. Bulk solution analyses result in at least two-fold better precision for δ³⁰Si (2SD on reference materials ≤0.11 ‰) over that found for the means of in situ analyses (2SD typically ≤0.24 ‰).

We find that bushgrass, common reed, and horsetail show large internal variations up to 2 ‰ in δ³⁰Si, reflecting the various pathways of silicon from soil to deposition. fsLA provides a means to identify the underlying processes involved in the formation of phytoliths using silicon isotope ratios.

Keywords
in situ silicon isotope ratios analysis, phytolith, laser ablation inductively coupled plasma mass spectrometry, biogenic silica

Introduction
Silicon is the second most abundant element in the Earth’s crust, and considered a beneficial element for plants (Epstein 1999, Marschner and Marschner 2012). Silicon can become highly enriched in plant
tissues (up to 40 mg·g⁻¹) where it mainly promotes structural rigidity, pathogen resistance and plant health (Ma and Yamaji 2015, Rodrigues and Datnoff 2015, Deshmukh et al. 2017), and serves to promote to salinity stress resistance (Haghighi et al. 2012, Rios et al. 2017). Silicon is taken up from the soil solution by plant roots exclusively as monosilicic acid (H₄SiO₄) via aquaporin membrane transporters, and is then transported passively along the transpiration gradient towards the plant’s organs (Ma et al. 2006, 2011, Delvigne et al. 2009). Silicon is eventually deposited in the form of phytoliths or other amorphous silica precipitates within cell lumina, cell walls or intercellular spaces (Watteau and Villoin 2001, Piperno 2006, Ma et al. 2011). The structure, form, and sizes of phytoliths differ greatly between plant species and can be used to identify their presence from phytoliths preserved in soil and sediment (Piperno 2006). There is an ongoing debate on the stability of phytoliths after their deposition in soil. Fraysse and co-worker showed that the stability of the phytoliths depends on the availability of water, temperature, microbial activity and the acidity of the soil solution, where dissolution is lowest at low pH and low temperature (Fraysse et al. 2006, 2009, 2010). The experimentally determined phytolith dissolution time constant is just slightly lower than that of amorphous silicon. Based on these estimates the half-life of a phytolith with respect to dissolution is between six months and three years (Fraysse et al. 2009). Prentice and Webb showed that during the dissolution of phytoliths, oxygen and silicon isotope are fractionated (Prentice and Webb 2016). The magnitude of the fractionation depends on the pH, and temperature of the solution. The authors observed a two-stage dissolution process. A first, very fast stage that is attributed to the removal of a reactive surface, and a second stage involves re-precipitation of silicon. Under favourable conditions, phytoliths persist dissolution in soil and can be used to reconstruct past vegetation, or the use and domestication of plants during prehistoric settlements (Piperno 2006, Hodson et al. 2008, Hodson 2016).

As is the case for all elements that contain more than one stable isotope, the biogeochemical reactions at the Earth’s surface induce a mass-dependent shift in the relative abundance of the three stable silicon isotopes (²⁹Si, ³⁰Si and ³¹Si). The shift in the resulting isotope ratios can be used to study the biogeochemical cycle of silicon (Bouchez et al. 2013, Sutton et al. 2018). Traditionally, silicon isotope ratios were determined after fluorination of the silicon (either by treatment with a fluorine source (Allenby 1954) or by laser fluorination (De La Rocha et al. 1996) on a gas isotope ratio mass spectrometer (IRMS). Since the 2000s, silicon is analysed in solution using high-resolution MC-ICP-MS. (De La Rocha 2002, Georg et al. 2006). The sample preparation used for the gas IRMS and MC-ICP-MS are designed for bulk analysis and not for the analysis of single phytoliths. The isotopic composition of silicon in plants thus investigated resulted in a large isotopic range of -2.7 ‰ (rice stem) to 6.1 ‰ (rice grains) (Douthitt 1982, Ding et al. 2005, 2008b, 2008a, Ziegler et al. 2005, Opfergelt et al. 2006, 2008, 2010, Engström et al. 2008, Sun et al. 2008, 2016b, 2016a, Hodson et al. 2008, Delvigne et al. 2009,
Köster et al. 2009, Bern et al. 2010, Cornelis et al. 2010, Steinhoefel et al. 2011, Opfergelt and Delmelle 2012, Prentice and Webb 2016, Riotte et al. 2018). Only a few studies investigated the silicon isotope fractionation between plants and soil. Plants preferentially incorporate light silicon, and the isotopic fractionation (reported as \( \Delta^{30}\text{Si}_{\text{Plant-Solution}} \)) for banana, rice and bamboo are between -0.5 % to -1.6 % (Ziegler et al. 2005, Opfergelt et al. 2006, Ding et al. 2008b, 2008a, Sun et al. 2008, 2016b, Delvigne et al. 2009). Apart from the silicon in phytoliths, oxygen has been successfully used as paleoenvironmental proxy. Alexandre and co-worker showed a temperature dependency on the oxygen isotope signature of phytoliths. Changes in the mean annual temperature lead to a shift in the oxygen isotope composition of rainwater, and ultimately those changes are recorded in the silica deposits (Alexandre et al. 2012).

The stable silicon isotope analyses of phytoliths from living plants potentially offers insight into the biogeochemical transformations of silicon between soil and plant organs, whereas the analyses of phytoliths found in soils offer to provide information on their origin and preservation history. An in situ analysis technique with a high spatial resolution could in addition provide the possibility to assess phytolith heterogeneity, and thus provide supplemental information on the spatial and temporal variations of the silicon cycles involved. However, to date the application of an in situ technique to study stable silicon isotopes of phytoliths is still limited. Steinhoefel and co-worker characterised the different silicon pools in two weathered soils in situ to establish an approach to quantify silicon transport processes during weathering. Their investigation included the analysis of the phytolith pool.

However, an independent verification of the analytical approach was not performed at that time (Steinhoefel et al. 2011).

To determine in situ the silicon isotopic composition of a variety of phytoliths from different plants and thus close this gap, we developed an analytical routine based on femtosecond laser ablation multi-collector inductively coupled plasma mass spectrometry (fsLA-MC-ICP-MS). The use of fsLA has been demonstrated to be advantageous for the analysis of stable silicon isotope ratios, in particular when using non-matrix matched reference materials, e.g. (Schuessler and von Blanckenburg 2014). To evaluate our analytical routine, we used a set of natural phytolith samples extracted and purified from soil and a set of phytolith samples extracted directly from plant leaves and stems. Different sample mounting and laser ablation strategies were evaluated to optimise analytical precision. Due to the phytolith’s high water and carbon contents matrix effects on instrumental mass bias could potentially arise in the ICP (Jochum et al. 2014, Zheng et al. 2018). Yet no phytolith reference material with a characterised silicon isotope composition is available that would allow the test for such effects. Thus to assess the accuracy of the fsLA-MC-ICP-MS analytical method, comparative values for the natural phytoliths were obtained after bulk digestion, chromatographic silicon purification, and analysis by solution MC-ICP-MS.
Materials and Methods

Phytolith samples

Two different sources for phytoliths were selected. The first source is directly from living plants, and the second source are phytoliths separated from the organic-rich topsoil horizon (O). In total 7 different phytolith samples from 6 different species were collected.

Phytoliths from common reed (Phragmites australis) and bushgrass (Calamagrostis epigejos) were extracted directly from living plants from the “Hühnerwasser” (“Chicken Creek”) catchment, the former lignite opencast mine “Welzow-Süd” in northeast Germany (Puppe et al. 2017). Additionally, common and rough horsetail (Equisetum arvense, Equisetum hyemale) were obtained from a location in northern Germany (samples provided by Prof. Peter Behrens, Institute for Inorganic Chemistry, Leibniz University of Hannover). Two different parts of rough horsetail were sampled separately, from annual plants only nodes were calcinated, and from perennial plants the entire stem - containing nodes and internodes were calcinated. Two phytolith samples were extracted from O-horizons of forest soils, i.e. from European beech (Fagus sylvatica L.) collected in Beerenbusch, a study site in northern Brandenburg, Germany, close to the village Rheinsberg (Sommer et al. 2013). Norway spruce (Picea abies) was collected from the Wildmooswald, in the southern Black Forest (Steinhoefel et al. 2011).

With these 7 samples we can investigate the homogeneity of phytolith silicon isotope composition within single plant species. We expect that isotopically homogeneous plant parts will result in a narrow distribution of isotope ratios determined in situ, and close agreement between the averages of fsLA with the isotope ratio obtained by solution analysis after bulk sample digestion. Potential heterogeneity within the phytoliths is expected to result in a broader silicon isotope ratio distribution obtained from fsLA. Depending on how representative the phytolith fragments selected for in situ analyses are, a deviation in the isotope averages of fsLA ratio from the isotope ratio from bulk solution will emerge. Such a deviation can also be caused by samples that contain sub-micrometre sized silicate precipitates (Sommer et al. 2006). These would be contained in bulk analyses, but not in the larger fragments mounted for fsLA. For phytoliths extracted from soil (Norway spruce and European beech) such differences are not expected, as during the sample preparation an amalgamation of fragments is taking place which leads to an artificial homogenisation of Si pools and their of isotope ratios.

Phytolith extraction

Phytoliths sampled directly from the plant parts were ashed at 600°C (6 – 24h). European beech and Norway spruce phytoliths were extracted from the organic-rich forest soil layer by combined chemical and physical separation procedure. Those phytoliths were extracted by oxidising the organic matter using H₂O₂ (35 vol%), HNO₃ (65 vol%), HClO₄ (70 vol%) at 80 °C until reaction subsides. Subsequently,
Fe oxides and carbonates are dissolved by boiling the sample in HCl (10 vol%) for 30 min. Finally, the phytoliths are separated by shaking the remaining solid phase with 30 mL of sodium polytungstate (Na₆(H₂WO₄)₀·H₂O, density of 2.3 g cm⁻³), centrifugation at 3000 rpm for 10 min, carefully pipetting the supernatant, and filtering by 5µm Teflon filter. This step was repeated three times. The filter residue was washed with water, and dried at 105 °C. (Alexandre et al. 1997, Sommer et al. 2013). The obtained phytolith separates are compacted into a loosely bound pellet (see Phytolith imaging section) and might contain minor amounts of residual quartz, clay, or particles from aeolian dust (Steinhoefel et al. 2011).

Phytolith imaging

For sample characterisation, phytoliths were imaged using a scanning electron microscope (SEM, Zeiss Ultra Plus, working distance 7.6 mm, acceleration voltage 15 kV) and a light microscope (Leica EZ4D) at GFZ Potsdam. The phytoliths imaged by SEM were fixed on an aluminium holder using carbon-conductive adhesive tape and were carbon-coated. For light microscopy the phytoliths were mounted with the same method as used for LA, fixed with lacquer on a microscope slide.

Sample preparation for laser ablation

Three different strategies for introducing mounted phytoliths into the ablation cell were evaluated. In the first strategy phytolith fragments were imbedded in epoxy resin. The mount was carefully polished to expose the fragments. In the second method phytoliths were directly fixed by carbon-conductive adhesive tape onto an aluminium SEM holder. In the third method phytolith samples were fixed onto microscope slides using clear nail lacquer.

The preparation of the epoxy mounts is time consuming, and part of the sample is lost in the polishing process. The resulting thin sample will eventually lead to shorter measurement duration, and thus to lower precision in isotope ratios. Sample preparation on adhesive tape is faster. Phytoliths immediately adhere to the aluminium holder and can be analysed directly by LA. However, adhesive tape tends to attract dust and laser debris. Nail lacquer performs similarly to adhesive tape, dries in 40 s, and the entire phytolith can be analysed. To minimise possible contamination from the support, microscope slides made from poly(methyl methacrylate) (PMMA) were used throughout the study. Several commercial nail polishes and lacquers were bought in a local German chemist’s shop and tested for blank contamination, impact and scrub resistance. An advantage of nail lacquer is that phytoliths can be recovered by re-dissolving the lacquer.

Method validation strategy and uncertainty evaluation

No phytolith reference material with a characterised silicon isotope composition is available for use as external reference material. Since phytoliths are made up of bio-opal, they contain water (<9%) and to
some extent also carbon (Piperno 2006). The presence of carbon potentially leads to matrix effects in the plasma, which would not be accounted for using the standard sample bracketing approach with a dry reference material containing no carbon (Fliegel et al. 2011, Frick and Günther 2012). Moreover, the material embedding the phytoliths (epoxy resin or nail laquer) might get partially ablated. To evaluate the performance of the silicon isotope measurements obtained by fsLA-MC-ICP-MS, several reference materials (BHVO-2, NBS28) and a homogenised phytolith sample (European beech) were analysed by both fsLA-MC-ICP-MS and bulk solution MC-ICP-MS, respectively. Additionally, reference materials for which published silicon isotope data exists (ATHO-G, BHVO-2, GOR132-G, and NIST612) were analysed in each fsLA session for quality control and to verify that the target measurement uncertainty is met. Thereby uncertainties in the measurement results of these homogenous materials can be evaluated and then used as a benchmark to assess micro-scale silicon isotope heterogeneity in the unknown phytolith samples detected by in situ fsLA-MC-ICP-MS analysis.

The silicon isotope results are reported in the δ-notation as permil deviation relative to an international measurement standard (Coplen et al. 2002).

\[ \delta^{30}Si = \left( \frac{\frac{^{30}Si}{^{28}Si}}{\frac{^{30}Si}{^{28}Si}} \right)_{\text{sample}} - 1 \times 10^3 \]  

(1)

All δ²⁸Si and δ³⁰Si from bulk solution MC-ICP-MS analysis are reported relative to NBS28 (quartz NIST SRM8546 alias NBS28, δ²⁸Si ≡ 0 and δ³⁰Si ≡ 0; directly used as bracketing measurement standard) and in situ fsLA-MC-ICP-MS analysis are reported relative to NIST SRM610 (used as bracketing measurement standard) which is isotopically indistinguishable from NBS28, and also identical to NIST SRM612 (Schuessler and von Blanckenburg 2014, and Table S7).

All δ³⁰Si and δ²⁸Si results are accompanied with uncertainties at the 95% confidence level, exemplified for δ³⁰Si as follows. Individual δ³⁰Si values obtained by in situ analysis by fsLA-MC-ICP-MS contain mainly three uncertainty contributions, i.e. from the analysis of the sample and from the two bracketing standard measurements (NIST SRM610). For each of these, an expanded standard measurement uncertainty (U) (JCGM 2008) is obtained by calculating the standard deviation (s) of n silicon isotope ratio integration cycles acquired during < 3 min of analysis (18 > n < 94 for samples given in Frick et al. 2018, Table S1). Count- statistical uncertainties at the 95% confidence level can be expressed as the standard deviation of the mean \( 2 \cdot s_m = \frac{k \cdot s}{\sqrt{n}} \) with a coverage factor k = 2 (Ellison and Williams 2012). Here, instead of k we use t derived from student-t distribution at 95% confidence to account for variable n \( 2SE = \frac{t \cdot s}{\sqrt{n}} \); this is also sometimes called the standard error of the mean at 95% confidence. By propagation of these individual counting statistical uncertainties through equation 1,
we obtain a combined expanded standard uncertainty (U.) for each individual in situ fsLA-MC-ICP-MS δ30Si result. We note that other components of variance contributing to the uncertainty (e.g., mass bias stability, blank correction, ...) are already included in this statistical evaluation.

To evaluate the measurement precision the repeatability was calculated as 2 times the standard deviation (2SD) of n individual δ-values obtained by independent sample-standard bracketing measurements. We followed this procedure on reference materials of which the average results and their uncertainties were compared to independently obtained δ30Si values, and to verify the previously established target uncertainty of the fsLA-MC-ICP-MS method of better than ±0.23‰ (2SD) and ±0.15‰ (2SD) for δ30Si and δ29Si, respectively (Schuessler and von Blanckenburg 2014, Frick et al. 2016).

The latter approach is also used for bulk solution MC-ICP-MS silicon isotope analysis, where repeat measurements of samples and reference materials (after dissolution and silicon chromatographic purification) are obtained and average δ30Si and δ29Si values and associated standard deviations are calculated (Table 1 and S6).

To obtain reference silicon isotope ratios for our laser ablation study we have measured bulk phytolith samples after dissolution using an established alkali fusion method (Georg et al. 2006, Zambardi and Poitrasson 2011, Schuessler and von Blanckenburg 2014). Briefly, 5 to 15 mg phytoliths were fusion melted in an Ag crucible with NaOH (p.a. grade, tested for silicon background levels) at 750°C for 15 min. The fusion cake was dissolved in ultra-pure water (18 MΩ·cm) followed by mild 0.03 M HCl. The obtained solution was acidified to pH 1.5 with HCl. An aliquot of the solution was loaded onto chromatography columns (Spectrum 104704 PP containing 1.5 ml AG50W-X8) and silicon was eluted with 5 ml H2O. Complete silicon recovery and purity (> 95% Si) was verified by ICP-OES analysis. Before MC-ICP-MS analysis, the purified silicon solutions were acidified to 0.1 M HCl and diluted to a concentration of 0.6 μg·g⁻¹. Solutions of samples and bracketing measurement standards (NIST SRM8546, NBS28) were both doped with 0.6 μg·g⁻¹ Mg to use the 25Mg/24Mg ratio for mass bias drift correction during the analysis. The solutions were introduced into the ICP using an ESI ApexHF desolvator and a self-aspirating PFA nebuliser (measured uptake 160 μL min⁻¹). Measurements were done in dynamic mode, alternating between silicon and magnesium isotopes, each for 30 cycles at 4 s integration time per cycle. The Neptune cup configuration and instrument settings can be found in the supplement, Table S1 and Table S2. BHVO-2 (USGS, basalt powder) is a well-characterised reference material for silicon isotopes and was prepared and analysed together with the phytoliths to verify no bias is introduced during sample preparation and measurement. Additionally, ERM-CD281 (IRMM, rye grass) was analysed using the same method; unfortunately up-to-now, there are no other published silicon isotope data available for comparison. All δ28Si and δ30Si from bulk analysis are reported relative
to NBS28. Since NIST SRM610 and NBS28 are isotopically indistinguishable in their silicon composition, the bulk and in situ data can be directly compared (Schuessler and von Blanckenburg 2014, Frick et al. 2016, Table S7).

\textit{In situ} silicon isotope analysis by femtosecond laser ablation MC-ICP-MS

\textit{In situ} silicon isotope ratios were measured using a custom-built deep-UV (196 nm) femtosecond laser ablation system (GFZ \textit{fem}2, containing a frequency-quadrupled Spectra Physics Solstice femtosecond laser, see (Schuessler and von Blanckenburg 2014) for technical details) in combination with an inductively coupled plasma multi-collector mass spectrometer (Thermo Fisher Scientific Neptune MC-ICP-MS, equipped with the Neptune Plus Jet Interface) at the Helmholtz Laboratory for the Geochemistry of the Earth Surface (von Blanckenburg et al. 2016). The ion optics were operated at medium mass resolution with a typical mass resolving power $m/\Delta m > 6000$, to resolve isobaric interferences (mainly $^{14}\text{N}^{16}\text{O}^+$ on $^{30}\text{Si}^+$). Faraday detectors (equipped with $10^{11}$ Ω amplifiers) were positioned to measure on interference-free, flat top peak shoulders. The Faraday cup configuration is shown in Table S3. The gas flows, torch position, and ion optics were tuned daily for highest intensity and mass bias stability, while maintaining the $^{14}\text{N}^{16}\text{O}^+$ interference on $^{30}\text{Si}^+$ at resolvable levels (<10 V).

The typical laser and ICP-MS settings can be found in Table S4 and Table S5.

fsLA has proven to be advantageous over nsLA for non-matrix matched silicon isotope ratio standardisation of different sample matrices (Janney et al. 2011, Schuessler and von Blanckenburg 2014). To further improve the mass bias stability during non-matrix matched calibration using the standard-sample-bracketing approach, wet plasma conditions were used by admixture of water from spray chamber nebulisation to the laser aerosol before entering the ICP. The addition of water stabilises the position of the highest analyte ionisation density in the plasma regardless of the matrix, which leads to an improved accuracy in non-matrix matched quantification (Flamigni et al. 2012, 2014).

Both measures, fsLA and wet plasma lead to less mass bias drift between bracketing standards and samples and thus improves the precision of the isotope ratio determination. Instrumental mass bias was corrected for by standard-sample-bracketing using NIST SRM610 as measurement standard (Frick et al. 2016). The laser beam (ca. 25 μm in diameter) on the sample was continuously scanned over the sample - the paths were adapted to the irregular shapes of phytolith samples. To account for the fragile nature and sometimes also porous structure of phytoliths, the laser frequency (pulse repetition rate) during phytolith analysis was dynamically adjusted to maintain a signal intensity as similar as possible to that obtained during ablation of the bracketing standard (4 - 7 V on $^{28}\text{Si}$). Moreover, every analytical session contained a range of reference materials (ATHO-G, BHVO-2, GOR132-G, NIST SRM612, and NBS28) that were repeatedly analysed in between the phytolith measurements for comparison to published values (see compilations in (Jochum et al. 2005, Schuessler and von Blanckenburg 2014).
Raw data processing and background corrections were made according to the protocol described in Schuessler and von Blanckenburg (2014) that also involves application of several data rejection/acceptance criteria. Of these, the most important ones are that A) only $^{30/28}$Si and $^{29/28}$Si ratios are used for the calculation which deviate less than 3SD from the mean and B) only results which follow the mass-dependent terrestrial fractionation line in a three-isotope-plot of $\delta^{29}$Si vs. $\delta^{30}$Si within analytical uncertainties and C) have a mass bias drift between the two bracketing standards of less than 0.30‰ in $^{30/28}$Si are accepted and reported in this study.

Results and Discussions

Phytolith imaging

In Figure 1 SEM and optical microscopic images of the phytolith samples are shown with typical laser ablation paths. Phytoliths from rough horsetail look like sheet structures. Figure 1A and 1B show SEM images of the epoxy embedded (A) and carbon tape-affixed (B) perennial horsetail sample. In Figure 1A the thin nature of the sheet structures is visible. In Figure 1B a group of intact stomatal phytoliths after the separation process are shown. For comparison, Figure 1C shows the optical microscopic image of the perennial horsetail phytoliths sample affixed using nail lacquer.

The annual and perennial rough horsetail phytolith samples differ by the age of the plants and by the plant parts from which they were extracted. The perennial sample consist of stems and nodes, and the annual sample are from nodes only. No visual difference between the nodes of the annual and perennial sample was visible. Common horsetail phytoliths (not shown) form also sheet-like structures.

European beech and Norway spruce phytolith samples were separated from forest soils. Figure 1D shows the optical microscopic image of the Norway spruce phytolith sample and Figure 1E-F of European beech. Figure 1E reveals a compacted particle structure that resulted from the chemical and physical sample separation employed. The sample comprises a mixture of phytolith fragments rather than a single phytolith.

Common reed phytoliths form are also sheet-like structures (Figure 1G), however the sheets tend to be smaller and thinner compared to the rough and common horsetail samples. Typically, only one to two laser ablation passes were possible before exhausting the sample. To ensure sufficient analysis time nevertheless the size of the ablation area was increased. The bushgrass phytolith sample (Figure 1H) is an assembly of small sheet like structures (300-400 µm) with much smaller fragments (10 µm) of various sizes and shapes.
Figure 1: SEM (A,B,E, and G) and optical microscopy (C,D,F, and H) images of the phytolith samples. (A-C) rough horsetail, (D) Norway spruce, (E-F) European beech, (G) common reed, and (H) bushgrass phytoliths. (A-C) shows the different fixation techniques used in this study, (A) embedded into epoxy, (B) affixed using carbon sticky tape, (C) affixed using nail lacquer. (D-F) shows the compact, pellet-like nature of the Norway spruce and European beech phytoliths separated from the forest soil. Common reed (G), and (H) bushgrass phytoliths show a sheet-like structure. Scale bars are 100 µm for SEM, and 500 µm for the light microscopy images. Apart from (B) and (E) the images show typical laser ablation traces which are highlighted with white arrows.
Bulk phytolith silicon isotope analysis results

The bulk silicon isotope ratio composition of the 7 samples measured by solution MC-ICP-MS (after fusion digestion and chromatographic silicon purification) ranges from $\delta^{29}$Si = -1.12 ‰ to +1.27 ‰ (see Table 1). This is within the range previously reported in the literature for phytoliths ($\delta^{29}$Si -2.3 to +6.1 ‰) (Douthitt 1982, Ding et al. 2005, 2008b, 2008a, Ziegler et al. 2005, Opfergelt et al. 2006, 2008, 2010, Engström et al. 2008, Sun et al. 2008, 2016b, Hodson et al. 2008, Köster et al. 2009, Bern et al. 2010, Cornelis et al. 2010, Steinhofel et al. 2011, Opfergelt and Delmelle 2012, Prentice and Webb 2016). The values reported in the literature however, comprise not only of samples from natural ecosystems, but also from laboratory growth experiments, and included samples from roots to seeds, and some examples were analysed by fluorination and gas MS which might have resulted in lower accuracy than modern solution MC-ICP-MS techniques.

Table 1: Silicon isotope ratios from bulk phytolith digestion, with 2 standard deviations based on n replicate mass spectrometer measurements. For in situ fsLA, standard deviation is based on n measurement of individual phytoliths. *Bushgrass was digested and analysed in two independent bulk solution MC-ICP-MS analytical sessions (each n=3), †Norway spruce sample was analysed previously by (Steinhofel et al. 2011) and resulted in $\delta^{29}$Si = -0.36 ± 0.09 ‰. The measurement standards NBS-28 and NIST SRM610 are indistinguishable in their silicon isotope composition and therefore delta values from both analytical techniques can be directly compared.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Bulk digestion solution MC-ICP-MS</th>
<th>In situ fsLA-MC-ICP-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\delta^{29}$Si (‰)</td>
<td>2SD</td>
</tr>
<tr>
<td>Bushgrass*</td>
<td>0.50</td>
<td>0.07</td>
</tr>
<tr>
<td>Common horsetail</td>
<td>-0.56</td>
<td>0.15</td>
</tr>
<tr>
<td>Common reed</td>
<td>0.66</td>
<td>0.07</td>
</tr>
<tr>
<td>European beech</td>
<td>0.08</td>
<td>0.10</td>
</tr>
<tr>
<td>Norway spruce†</td>
<td>-0.21</td>
<td>0.03</td>
</tr>
<tr>
<td>Rough horsetail (annual)</td>
<td>0.06</td>
<td>0.03</td>
</tr>
<tr>
<td>Rough horsetail (perennial)</td>
<td>0.32</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Two different reference materials, BHVO-2 and ERM-CD281, were analysed using the same procedure along with the phytolith samples. The results are shown in the supplement, Table S6. Within two standard deviations there is no difference between the measured and the published values (Jochum et al. 2005, Schuessler and von Blanckenburg 2014). The repeatability of all solution MC-ICP-MS measurements on samples and reference materials is similar to what was previously obtained using this method (Tatzel et al. 2015, Oelze et al. 2016, Frick et al. 2016).

Results of in situ phytolith analysis

Evaluation of the laser ablation parameters and sample preparation methods

The fsLA system employed in this study is characterised by two features that differ from those common to ns lasers. The achievable spot size is limited to 15-30 µm, and the laser beam has a Gaussian energy
distribution. Both the small spot size and the Gaussian intensity distribution result in rapid increase in laser crater depth which is accompanied by fast decrease in ion beam intensity. We circumvent this effect by three means. A) We typically perform a small-size raster (50 - 100 µm) analysis instead of a single spot analysis; B) in contrast to nsLA we use a faster scan speed (40 µm/s); and C) we employ a higher laser pulse repetition rate (frequency 20-40 Hz). During the optimisation of the analytical routine we evaluated laser parameters optimised for the different phytolith types. For the sheet-like structured phytoliths from common and rough horsetail, a larger raster or line scan across selected features was chosen. During the first pass on those sample, a low laser pulse repetition rate (15-20 Hz) was applied to obtain signal intensities in the range of 4-7 V for $^{28}$Si. On the subsequent passes the laser frequency was increased. The ablation characteristics of the compacted phytolith samples, European beech and Norway spruce, resembled closely those encountered on silicate glass reference materials.

These samples were analysed with a small raster size, with up to 10 passes and without the need to adjust the laser frequency. Generally, we have chosen analysis parameters similar to those outlined by Schuessler and von Blanckenburg, with laser ablation durations of 100 s, and on-peak background measurements after each sample (Schuessler and von Blanckenburg 2014).

Three different strategies for fixing the phytolith samples in the ablation cell were evaluated. Embedding samples into epoxy resin typically led to short data acquisition periods at ion beam intensities that provide good counting statistics with high temporal variations in signal intensity (Figure 2). This is mainly due to the relatively thin samples left after polishing the epoxy mounts that are consumed while the laser ablation pit deepens with every pass of the line scan and also ablates areas consisting predominately of epoxy resin where the porous sample has been removed by polishing. In contrast, affixing phytoliths using adhesive tape or clear nail lacquer does not lead to a loss of material. This leads to longer data acquisition periods at a relatively constant signal intensity, resulting in a better precision of the silicon isotope ratios. An example for this different ablation behaviour is shown in Figure 2. Figure 2A-B is a rough horsetail (perennial) phytolith imbedded into epoxy, and Figure 2C-D fixed with nail lacquer on a PMMA slide.

The signal on the epoxy-embedded phytoliths appears only after the third pass of the laser ablation raster (see Figure 2A). During the first two passes the phytolith was still partially covered with epoxy. The laser pulse repetition rate (frequency) was thus increased to accelerate the ablation. Once the phytolith was fully uncovered, very high signal intensities were recorded. For the nail lacquer-fixed phytolith, such initial uncovering was not required, and the ion signal was detected shortly upon ablation began and remained stable throughout the analysis. The signal on the epoxy-embedded phytolith lasted for about 50 seconds, whereas for the phytolith fixed with nail lacquer the full ablation period of 100 s was used for data acquisition. The epoxy-embedded perennial rough horsetail analysis resulted in a $\delta^{30}$Si = 0.87 ± 0.25 ‰ (n = 56, 2 SE) and for the nail lacquer-fixed phytolith 0.86 ± 0.18 ‰
(n = 81, 2 SE). The average δ^{30}Si by bulk solution MC-ICP-MS result is 0.61 ± 0.13 ‰ (n = 3, 2SD) and
the average fsLA-MC-ICP-MS result for this sample is 0.43 ± 1.40 ‰ (n = 21, 2SD).

Figure 2: Typical fsLA-MC-ICP-MS signal from rough horsetail (perennial) phytoliths, left (A-B) embedded in epoxy resin, and
right (C-D) glued with clear nail lacquer. {superscript}29Si intensity (A, C), {superscript}30/28Si ratio (B, D), and calculated mean (solid black line) with 3
SD (dashed grey line) of the {superscript}30/28Si ratio (black dots). Only {superscript}30/28Si ratios that are deviating less than 3 SD from the mean in their
{superscript}30/28Si and {superscript}29/28Si ratio are used for the data evaluation and calculation of delta values (grey circles). For clarity, background
ratios (acquired beyond 120 cycles) are not shown. One cycle is equal to an integration time of 1.049 seconds.

Carbon-adhesive tape and nail lacquer are our preferred sample preparation methods. The
preparation is faster, and a smaller number of preparation steps are involved. However, when handling
the sample, the epoxy-embedded and nail lacquer-fixed samples are favoured, since no dust or laser
ablation debris can adhere to the sample after the epoxy or lacquer has dried. Based on these
considerations the best applicable sample preparation method is using clear nail lacquer to affix the
phytolith samples onto PMMA slides.

Evaluation and comparison of fsLA with the bulk solution silicon isotope measurements

The results of the in situ measured phytoliths by fsLA-MC-ICP-MS are shown in Figure 3, and the
individual results are reported in Table S1 in the accompanying data publication (Frick et al. 2018).
Each phytolith sample was analysed in at least three independent measurement sessions. Data in
Figure 3 are sorted according to the measurement session.

Comparing δ^{30}Si values from bulk solution MC-ICP-MS silicon isotope with the averages from in situ
fsLA-MC-ICP-MS analysis, three different cases can be identified. A) A heterogeneous silicon isotope
composition of phytoliths within a single plant species (bushgrass, common reed, rough horsetail, and
common horsetail), B) a homogeneous composition from the soil-extracted phytoliths (European
beech) and C) a heterogeneous composition from the soil-extracted phytoliths of Norway spruce. The
potentially existing fourth case, a homogenous distribution of phytoliths from a single plant species,
was not found. The wide variation in silicon isotope ratios of more than 2‰ observed within a single plant indicates that different silicon translocation and utilisation mechanisms in the plant are traced by silicon isotopes. The soil water silicon isotopic composition as well as the cell environment, where silicic acid is dehydrated and phytoliths are formed, influence the isotopic composition of the formed phytoliths. For common reed, rough horsetail (perennial) and common horsetail no systematic bias between the bulk solution and in situ fsLA data is observed. For bushgrass and rough horsetail (annual) a systematic difference between results from both measurement methods is apparent. Closer inspection of the distribution of the single bushgrass phytolith analysis indicates, however, that this difference is not due to a systematic analytical bias. Rather, while the large spread is due to inter-phytolith fragment isotopic heterogeneity, the small number of phytoliths analysed by fsLA-MC-ICP-MS did not result in representative sampling of the distribution. For the annual rough horsetail phytolith sample, the individual micro-scale analysis overlaps with the bulk analysis within uncertainties. A systematic offset between the two analytical routines is therefore unlikely. These results together with the results obtained on reference materials (Table S7, supplement) verify the previously established target uncertainty of the fsLA-MC-ICP-MS method of better than ±0.23‰ (2SD) and ±0.15‰ (2SD) for δ$_{30}$Si and δ$_{28}$Si, respectively (Schuessler and von Blanckenburg 2014, Frick et al. 2016).

For European beech and Norway spruce an amalgamation of the phytoliths has taken place during the extraction procedure from the soil horizon and separation. The phytolith samples of European beech show a very narrow silicon isotope ratio distribution, indicating either a homogenisation of the silicon isotopic composition during the sample preparation, or a homogeneous initial phytolith composition. The electron microscope image showed finely-ground material (see Figure S1A in the supplement). Thus, homogenisation during sample preparation is the most likely explanation. The average silicon isotope composition obtained by fsLA-MC-ICP-MS (δ$_{30}$Si = 0.10 ± 0.05 ‰, combined expanded standard uncertainty at 95% CI for n = 24) overlaps within the confidence interval of the average bulk solution MC-ICP-MS result (δ$_{30}$Si = 0.13 ± 0.11 ‰, n = 3) for this sample. For Norway spruce the measured micro-scale silicon isotopic variability is higher (δ$_{30}$Si = -0.88 to +1.39 ‰), and here, phytoliths fragments observed by SEM are larger (see Figure S1B in the supplement), indicating that a small-scale homogenisation of the fragments has not been attained. Close inspection of the individual fsLA-MC-ICP-MS results for Norway spruce reveals that a small number of phytoliths in the sample population are significantly heavier in their silicon isotope composition than the bulk composition (up to δ$_{30}$Si = 1.39 ‰). The detection of such small sub-groups within a sample population is one of the strengths of the in situ technique and would not have been noticed in the bulk solution MC-ICP-MS analysis. The average Norway spruce fsLA-MC-ICP-MS result (δ$_{30}$Si = -0.15 ± 0.30 ‰, n = 25) and the bulk silicon isotope composition (δ$_{30}$Si = -0.42 ± 0.09 ‰, n = 3) are indistinguishable from each other.
within their uncertainties. We conclude that for the two most homogeneous samples (Norway spruce and European beech) no systematic difference between the two analytical methods is apparent. Thus, the fsLA-MC-ICP-MS analytical routine can provide accurate in situ stable silicon isotope ratios of plant and soil extracted phytoliths. Any systematic differences between in situ and bulk silicon isotope ratio signatures thus arises from non-representative sampling, such as the possible inclusion of sub-micrometre sized silicate precipitates in bulk samples and their exclusion from larger phytolith samples which were not amalgamated.

![Graph](image)

Figure 3: $\delta^{30}\text{Si}$ values measured by in situ fsLA-MC-ICP-MS on phytolith samples (bushgrass, European beech, common reed, rough horsetail, annual and perennial, common horsetail and Norway spruce). Uncertainty on each individual data point is the combined expanded standard uncertainty at the 2SE level for $\delta^{30}\text{Si}$ (see method section for definition). Light grey area is the average $\delta^{30}\text{Si}$ value (relative to NIST SRM610) for each sample from in situ fsLA-MC-ICP-MS analysis with 95% confidence interval. The dark grey shaded area is the average $\delta^{30}\text{Si}$ composition (relative to NBS28) with 95% confidence interval obtained by bulk solution MC-ICP-MS. The size of the data points is indicative of the number of measurements cycles used for data acquisition (1 cycle = 1.049 s integration time). Only where few cycles were acquired (<30 integrations) the statistical uncertainty expressed as $\frac{1}{\sqrt{n}}$ increases. The measurements are arranged according to the temporal sequence in which they were measured. The results include phytoliths from epoxy-embedded, nail lacquer-, and carbon-adhesive fixed specimens.

Analytically the in situ analysis of the phytolith samples is more challenging due to non-uniform ablation behaviour resulting in higher uncertainties compared to the analysis of glass or rock reference standards. The non-uniform ablation causes individual integration cycles to strongly deviate from the mean. These can be identified by a statistical outlier test and rejected. For analysis where more than forty 1-second-integration cycles were averaged, no systematic variation with the standard deviation of the mean is observed. For very short data acquisition periods ($n < 30$, see dot size in Figure 3) we observe an increase in the standard deviation of the mean in silicon isotope ratios for an individual analysis, and thus an increase in the combined expanded standard uncertainty of $\delta^{30}\text{Si}$ values. Some
of the results are subject to a large analytical uncertainty, which span almost the entire reported silicon isotopic range of biogenic opal. For completeness those data are included in Figure 3, in the following discussion those inferior data are however omitted.

Implications for silicon biogeochemistry

The in situ fsLA-MC-ICP-MS results allow to develop a more detailed insight into the biogeochemistry of silicon in plants. The first significant finding is the small-scale heterogeneity in the silicon isotope composition within a given plant species (Figure 4). Not only does this heterogeneity imply that a variety of processes are involved in the formation of phytoliths in plants. The obtained isotope ratios potentially also aide to identify these processes.

Results from both annual and perennial rough horsetail samples are good examples of this potential application. The bulk analyses reveal a significant difference between the annual and the perennial sample. The annual phytoliths show a lower δ30Si signature (0.14 ± 0.06 ‰, 2SD) than the perennial phytoliths (0.61 ± 0.13 ‰, 2SD). For these differences three explanations are possible: A) differences in soil chemistry can result in a compositional change in the plants’ silicon source; B) the isotope fractionation during uptake varies, depending on environmental conditions; and C) the removal of stems in the annual sample shifts the isotopic composition. All three explanations will result in differences in the silicon isotope composition of the formed phytoliths. Note that explanations A) and B) might also include temporal variations, as the isotope composition of silicon in the source may shift between years but also between seasons (Steinhoefel et al. 2017), and so may the isotope fractionation factors depending on, for example, silicon saturation in fluids or pH. Thus δ30Si of individual fragments might depend on their age. Given the experimental design, a differentiation between the three mechanisms are not possible.

We also find specific silicon isotope ratios that appear to correlate to morphological features of analysed phytoliths. Optical microscopy imaging of laser ablation paths in the perennial horsetail sample revealed that the two analyses with the horsetails’ lowest δ30Si (-0.79 ‰ and -0.85 ‰) were measured in a region lacking visible features of stomata. In all other measurements, yielding higher δ30Si, the laser ablation path included stomata features. This distinction suggests differences in the depositional processes of bio-silica close to stomata, or that these particular phytoliths were formed at a different stage of the plants growth and thus from a different source of silica.
The second finding that highlights the future scientific potential of this method is a higher $\delta^{30}\text{Si}$ in some of the phytoliths (Figure 4) compared to the $\delta^{30}\text{Si}$ of typical soil solutions that range from -1 to +2 ‰ (Opfergelt and Delmelle 2012). Plants preferentially incorporate light silicon isotopes from the soil solution, with a $^{30}\text{Si}/^{28}\text{Si}$ fractionation between plant and soil solution of -0.5 ‰ to -1.6 ‰ (Ziegler et al. 2005, Opfergelt et al. 2006, Ding et al. 2008b, Sun et al. 2008, 2016b, 2016a, Hodson et al. 2008, Delvigne et al. 2009, Köster et al. 2009, Bern et al. 2010, Cornelis et al. 2011, Steinhoefel et al. 2011, Opfergelt and Delmelle 2012, Prentice and Webb 2016, Röttte et al. 2018)

![Diagram](https://via.placeholder.com/150)

**Figure 4:** $\delta^{30}\text{Si}$ values of the analysed phytoliths. Black circles denote bulk solution MC-ICP-MS results, error bars are 2SD of repeated measurements. Grey diamonds denote single values determined by fsLA-MC-ICP-MS. Box plots depict mean (black dot) and median (dotted line) of the in situ fsLA-MC-ICP-MS measurements, with 2 standard error (box size) and 2 standard deviation (whisker). For comparison the top row contains the Si isotope range of published soil solutions (from Opfergelt and Delmelle 2012) and published values for phytoliths and biogenic opal are shown in black (measured by gas phase IRMS) and grey (measured by MC-ICP-MS). (Douthitt 1982, Ding et al. 2005, 2008b, 2008a, Ziegler et al. 2005, Opfergelt et al. 2006, 2008, 2010, Engström et al. 2008, Sun et al. 2008, 2016b, 2016a, Hodson et al. 2008, Delvigne et al. 2009, Köster et al. 2009, Bern et al. 2010, Cornelis et al. 2011, Steinhoefel et al. 2011, Opfergelt and Delmelle 2012, Prentice and Webb 2016, Röttte et al. 2018)

such that $\delta^{30}\text{Si}$ of around 0 ‰ is expected for whole plants. Bushgrass and common reed were harvested from the same catchment, and both yielded a significantly heavier silicon isotope signature ($\delta^{30}\text{Si}_{\text{bushgrass}} = 0.99 \pm 0.12$ ‰, and $\delta^{30}\text{Si}_{\text{common reed}} = 1.27 \pm 0.05$ ‰, with an outlier of 3.85 ± 0.69 ‰ in bushgrass, and another outlier of 4.26 ± 0.58 ‰ in common reed, Figure 4). This observation is intriguing because a (bio)chemical process enriching heavy silicon isotopes has not been found to date.

Precipitation and adsorption of both abiogenic and biogenic silicon is known to enrich the light isotopes in the precipitate (Oelze et al. 2014, Geilert et al. 2015, Frings et al. 2016). Thus, the solution remaining after precipitation process will yield high $\delta^{30}\text{Si}$. There are two possible pathways of such residual silicon
high in $\delta^{30}\text{Si}$ into phytoliths. Either the soil solutions’ silicon available to the plants was previously depleted in light isotopes by, for example, neo-formation of secondary minerals, or these phytoliths formed from an intra-plant silicon pool that was depleted in the light isotope by previous precipitation of bio-silica within the plant.

Conclusions

We have established an analytical routine based on femtosecond laser ablation inductively coupled plasma mass spectrometry (fsLA-MC-ICP-MS) for in situ analysis of stable silicon isotope composition of phytoliths.

In terms of sample preparation, the lowest uncertainties associated with the $\delta^{30}\text{Si}$ results were achieved by mounting phytolith samples with clear nail lacquer onto a PMMA sample holder. The traditional approach, using epoxy resin for embedding, impaired the measurements by loss of phytolith material during polishing and thus to low and highly variable ion beams signals during laser ablation and data acquisition.

Good agreement between phytolith $\delta^{30}\text{Si}$ results obtained by bulk solution MC-ICP-MS analysis after total sample decomposition and silicon purification with in situ determined silicon isotope ratio by fsLA-MC-ICP-MS validates the analytical method described in this study.

In terms of uncertainty, bulk analysis resulted in at least two-fold better precision for $^{30/28}\text{Si}$ (typically 2SD on reference materials was better than 0.11 ‰) over that found for the means of in situ analyses (2SD typically better than 0.24). If differences were found between results from in situ analyses and those from bulk solution MC-ICP-MS, we identified the origin from sample heterogeneity combined with incomplete capture of a phytolith population in the analysis of single fragments. Phytolith samples extracted from the soil horizons were more amalgamated (i.e., homogenisation of the phytoliths’ fragments) due to the preparation technique, leading to easier analyses, and also to isotopic homogenisation on the scale of fsLA-MC-ICP-MS.

In terms of advantages, fsLA-MC-ICP-MS is the better-suited method for screening and characterising heterogeneous phytolith populations. We found that certain investigated species (bushgrass, common reed, and horsetail) show large internal variations amounting to up to 2 ‰ in $\delta^{30}\text{Si}$ between different phytolith fragments. Capturing such heterogeneity thus potentially opens the possibility to investigate pathways of silicon of plants grown in vivo, after removal of only small parts of plant tissues. Furthermore, using phytoliths from geo-archives could enable the exploration of past silicon biogeochemical and climate cycles.
Supplementary information

All data used in this study are available online as an open access supplementary dataset at GFZ Data Services http://doi.org/10.5880/gfz.3.3.2018.002. (Frick et al. 2018)

Conflict of Interest

The authors have no conflict of interest to declare.

Acknowledgements

D.A.F. thanks the Swiss National Science Foundation for an Early Postdoc.Mobility fellowship (P2EZP2_168836), and the GFZ German Research Centre for Geosciences, the Helmholtz Laboratory for the Geochemistry of the Earth Surface (HELGES) and the Leibniz Centre for Agricultural Landscape Research for providing excellent (laboratory) infrastructure. The authors would like to thank Josefine Buhk and Jutta Schlegel for their support in HELGES, Danuta Kaczorek, ZALF, for the extraction of phytoliths and two anonymous reviewers for their helpful reviews that improved this manuscript.

References


Figure 1: SEM (A,B,E, and G) and optical microscopy (C,D,F, and H) images of the phytolith samples. (A-C) rough horsetail, (D) Norway spruce, (E-F) European beech, (G) common reed, and (H) bushgrass phytoliths. (A-C) shows the different fixation techniques used in this study, (A) embedded into epoxy, (B) affixed using carbon sticky tape, (C) affixed using nail lacquer. (D-F) shows the compact, pellet-like nature of the Norway spruce and European beech phytoliths separated from the forest soil. Common reed (G), and (H) bushgrass phytoliths show a sheet-like structure. Scale bars are 100 µm for SEM, and 500 µm for the light microscopy images. Apart from (B) and (E) the images show typical laser ablation traces which are highlighted with white arrows.

Figure 2: Typical fsLA-MC-ICP-MS signal from rough horsetail (perennial) phytoliths, left (A-B) embedded in epoxy resin, and right (C-D) glued with clear nail lacquer. $^{28}$Si intensity (A, C), $^{30/28}$Si ratio (B, D), and calculated mean (solid black line) with 3 SD (dashed grey line) of the $^{30/28}$Si ratio (black dots). Only $^{30/28}$Si ratios that are deviating less than 3 SD from the mean in their $^{30/28}$Si and $^{29/28}$Si ratio are used for the data evaluation and calculation of delta values (grey circles). For clarity, background ratios (acquired beyond 120 cycles) are not shown. One cycle is equal to an integration time of 1.049 seconds.

Figure 3: $\delta^{30}$Si values measured by in situ fsLA-MC-ICP-MS on phytolith samples (bushgrass, European beech, common reed, rough horsetail, annual and perennial, common horsetail and Norway spruce). Uncertainty on each individual data point is the combined expanded standard uncertainty at the 2SE level for $\delta^{30}$Si (see method section for definition). Light grey area is the average $\delta^{30}$Si value (relative to NIST SRM610) for each sample from in situ fsLA-MC-ICP-MS analysis with 95 % confidence interval. The dark grey shaded area is the average $\delta^{30}$Si composition (relative to NBS28) with 95 % confidence interval obtained by bulk solution MC-ICP-MS. The size of the data points is indicative of the number of measurements cycles used for data acquisition (1 cycle = 1.049 s integration time). Only where few cycles were acquired (<30 integrations) the statistical uncertainty expressed as $t \cdot s_n$ increases. The measurements are arranged according to the temporal sequence in which they were measured. The results include phytoliths from epoxy-embedded, nail lacquer-, and carbon-adhesive fixed specimens.

Figure 4: $\delta^{30}$Si values of the analysed phytoliths. Black circles denote bulk solution MC-ICP-MS results, error bars are 2SD of repeated measurements. Grey diamonds denote single values determined by fsLA-MC-ICP-MS. Box plots depict mean (black dot) and median (dotted line) of the in situ fsLA-MC-ICP-MS measurements, with 2 standard error (box size) and 2 standard deviation (whisker). For comparison the top row contains the Si isotope range of published

List of Tables

Table 1: Silicon isotope ratios from bulk phytolith digestion, with 2 standard deviations based on n replicate mass spectrometer measurements. For in situ fsLA, standard deviation is based on n measurement of individual phytoliths. *Bushgrass was digested and analysed in two independent bulk solution MC-ICP-MS analytical sessions (each n=3). †Norway spruce sample was analysed previously by (Steinhoefel et al. 2011) and resulted in \( \delta^{30} \text{Si} = -0.36 \pm 0.09 \%\). The measurement standards NBS-28 and NIST SRM610 are indistinguishable in their silicon isotope composition and therefore delta values from both analytical techniques can be directly compared.
Supplement to “Laser ablation in situ silicon stable isotope analysis of phytoliths”

Daniel A. Frick¹,†, Jan A. Schuessler³, Michael Sommer²,³, Friedhelm von Blanckenburg¹,⁴

¹ GFZ German Research Centre for Geosciences, Earth Surface Geochemistry, Potsdam, Germany.
² Leibniz-Centre for Agricultural Landscape Research (ZALF) e. V., Working Group Si Biogeochemistry, 15374 Müncheberg, Germany.
³ University of Potsdam, Institute of Earth and Environmental Sciences, Karl-Liebknecht-Str. 24-24, 14476 Potsdam, Germany.

+4933128828963

Content

Table S1: Instrument configuration of Faraday cup and amplifier settings for bulk solution MC-ICP-MS Si isotope measurements. There is a 3 s delay time between line 1 and line 2. .......................... 2
Table S2: Instrument settings for bulk solution MC-ICP-MS Si isotope measurements. *Parameters tuned for best sensitivity, stability and peak shape before each measurement session. ................. 2
Table S3: Instrument configuration of Faraday cup and amplifier settings for fsLA MC-ICP-MS Si isotope analysis. .......................................................... 2
Table S4: Typical laser settings used throughout the study. *Laser repetition rate was adjusted to attain uniform 28Si ion beam intensities during measurements (matched to bracketing standard signal intensity). .......................................................... 2
Table S5: Instrument settings used for in situ fsLA fsLA MC-ICP-MS Si isotope analysis. *Parameters tuned for best sensitivity, stability and peak shape before each measurement session. Auxiliary and nebuliser gas flows were within 0.1 L min⁻¹ for the different measurement sessions. .......................................................... 2
Table S6: Silicon isotope ratio results from solution MC-ICP-MS analysis (after alkali fusion and Si purification) of international reference materials. Mean δ-values with their 2 standard deviations (2SD) of n repeat MC-ICP-MS analysis are reported relative to NBS28. ................. 3
Table S7: Silicon isotope ratio results from in situ fsLA MC-ICP-MS analysis of international reference materials. Mean δ-values with their 2 standard deviations (2SD) of n repeat fsLA MC-ICP-MS analysis are reported relative to NIST SRM610. .................................................. 3

S-Figure 1: SEM image details of European beech (A) and Norway spruce (B). Both samples consist of finely-ground phytoliths material, indicating a homogenisation process has taken place during the decomposition in the soil or the sample preparation steps. Scale bar are 100 µm. .......................... 4
Table S1: Instrument configuration of Faraday cup and amplifier settings for bulk solution MC-ICP-MS Si isotope measurements. There is a 3 s delay time between line 1 and line 2.

<table>
<thead>
<tr>
<th>Faraday Cup</th>
<th>L3</th>
<th>L2</th>
<th>L1</th>
<th>C</th>
<th>H2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Line 1</td>
<td>$^{28}\text{Si}$</td>
<td>$^{26}\text{Si}$</td>
<td>$^{26}\text{Si}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Line 2</td>
<td>$^{24}\text{Mg}$</td>
<td>$^{24}\text{Mg}$</td>
<td>$^{24}\text{Mg}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplifier</td>
<td>10$^{11}$ Ω</td>
<td>10$^{11}$ Ω</td>
<td>10$^{11}$ Ω</td>
<td>10$^{11}$ Ω</td>
<td>10$^{11}$ Ω</td>
</tr>
</tbody>
</table>

Table S2: Instrument settings for bulk solution MC-ICP-MS Si isotope measurements. *Parameters tuned for best sensitivity, stability and peak shape before each measurement session.

<table>
<thead>
<tr>
<th>Cool Gas Flow</th>
<th>15.00 [L min$^{-1}$]</th>
<th>Extraction Lens</th>
<th>-2000 [V]</th>
<th>Auxilary Gas Flow</th>
<th>0.80 [L min$^{-1}$]</th>
<th>Focus Lens*</th>
<th>-651 [V]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torch:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X*</td>
<td>1.010 [mm]</td>
<td>Rotation Quad 1*</td>
<td>2.03 [V]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y*</td>
<td>-3.760 [mm]</td>
<td>Source Offset</td>
<td>0.0 [V]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z*</td>
<td>-1.250 [mm]</td>
<td>Focus Quad 1*</td>
<td>-19.89 [V]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sampler Cone</td>
<td>Ni Jet</td>
<td>Rotation Quad 2*</td>
<td>0 [V]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skimmer Cone</td>
<td>Ni H</td>
<td>Focus Offset*</td>
<td>50.00 [V]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Focus Quad*</td>
<td>-1.0 [V]</td>
<td>Matsuda Plate</td>
<td>0.0 [V]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dispersion</td>
<td>0.0 [V]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apex: Heat:</td>
<td>Cool: 2 with PFA nebuliser with 160µL/min measured uptake rate.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cycles</td>
<td>30</td>
<td>Integration time</td>
<td>4.194 [s/cycle]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table S3: Instrument configuration of Faraday cup and amplifier settings for fSbLA MC-ICP-MS Si isotope analysis.

<table>
<thead>
<tr>
<th>Faraday Cup</th>
<th>L4</th>
<th>L2</th>
<th>C</th>
<th>H2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Line 1</td>
<td>$^{23}\text{Al}$</td>
<td>$^{28}\text{Si}$</td>
<td>$^{28}\text{Si}$</td>
<td>$^{28}\text{Si}$</td>
</tr>
<tr>
<td>Amplifier</td>
<td>10$^{11}$ Ω</td>
<td>10$^{11}$ Ω</td>
<td>10$^{11}$ Ω</td>
<td>10$^{11}$ Ω</td>
</tr>
</tbody>
</table>

Table S4: Typical laser settings used throughout the study. *Laser repetition rate was adjusted to attain uniform $28\text{Si}$ ion beam intensities during measurements (matched to bracketing standard signal intensity).

| Crater size | 25-30 [µm] | Repetition rate* | 15-125 [Hz] | Scan velocity | 40 [µm/s] | Energy density | 1.2-1.5 J/cm² | Wavelength | 196 [nm] |

Table S5: Instrument settings used for in situ fSbLA fSbLA MC-ICP-MS Si isotope analysis. *Parameters tuned for best sensitivity, stability and peak shape before each measurement session. Auxiliary and nebuliser gas flows were within 0.1 L min$^{-1}$ for the different measurement sessions.

<table>
<thead>
<tr>
<th>Cool Gas Flow</th>
<th>15.00 [L min$^{-1}$]</th>
<th>Extraction Lens</th>
<th>-2000 [V]</th>
<th>Auxilary Gas Flow*</th>
<th>0.6 [L min$^{-1}$]</th>
<th>Focus Lens*</th>
<th>-582 [V]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nebuliser Gas Flow*</td>
<td>0.6 [L min$^{-1}$]</td>
<td>X-Deflection*</td>
<td>-4.06 [V]</td>
<td>RF Power</td>
<td>1250 [W]</td>
<td>Y-Deflection*</td>
<td>-2.24 [V]</td>
</tr>
<tr>
<td>Torch:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X*</td>
<td>1.090 [mm]</td>
<td>Rotation Quad 1*</td>
<td>2.91 [V]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y*</td>
<td>-3.670 [mm]</td>
<td>Source Offset</td>
<td>-1.0 [V]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z*</td>
<td>-0.900 [mm]</td>
<td>Focus Quad 2*</td>
<td>0.0 [V]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sampler Cone</td>
<td>Ni Jet</td>
<td>Focus Offset</td>
<td>50.00 [V]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skimmer Cone</td>
<td>Ni H</td>
<td>Matsuda Plate</td>
<td>0.0 [V]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Focus Quad*</td>
<td>-2.10 [V]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dispersion</td>
<td>0.0 [V]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFA Nebuliser with a measured uptake rate of 50 µL/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cycles</td>
<td>200</td>
<td>Integration time</td>
<td>1.049 [s/cycle]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 56: Silicon isotope ratio results from solution MC-ICP-MS analysis (after alkali fusion and Si purification) of international reference materials. Mean $\delta$-values with their 2 standard deviations (2SD) of $n$ repeat MC-ICP-MS analysis are reported relative to NBS28.

<table>
<thead>
<tr>
<th></th>
<th>Bulk solution MC-ICP-MS results (this study)</th>
<th>Averages of published values*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\delta^{29}\text{Si}/^{28}\text{Si}_{NBS28}$ (‰)</td>
<td>$\delta^{28}\text{Si}/^{28}\text{Si}_{NBS28}$ (‰)</td>
</tr>
<tr>
<td></td>
<td>2SD</td>
<td>2SD</td>
</tr>
<tr>
<td>BHVO-2</td>
<td>-0.11</td>
<td>-0.14</td>
</tr>
<tr>
<td>ERM-CD281</td>
<td>-0.15</td>
<td>-0.15</td>
</tr>
</tbody>
</table>


Table 57: Silicon isotope ratio results from in situ fsLA MC-ICP-MS analysis of international reference materials. Mean $\delta$-values with their 2 standard deviations (2SD) of $n$ repeat fsLA MC-ICP-MS analysis are reported relative to NIST SRM610.

<table>
<thead>
<tr>
<th></th>
<th>In situ fsLA MC-ICP-MS results</th>
<th>Averages of published values*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\delta^{29}\text{Si}/^{28}\text{Si}_{SRM610}$ (‰)</td>
<td>$\delta^{28}\text{Si}/^{28}\text{Si}_{NBS28}$ (‰)</td>
</tr>
<tr>
<td></td>
<td>2SD</td>
<td>2SD</td>
</tr>
<tr>
<td>ATHO-G</td>
<td>-0.13</td>
<td>-0.19</td>
</tr>
<tr>
<td>BHVO-2</td>
<td>-0.18</td>
<td>-0.14</td>
</tr>
<tr>
<td>GOR132-G</td>
<td>-0.17</td>
<td>-0.15</td>
</tr>
<tr>
<td>NBS28</td>
<td>-0.02</td>
<td>$\equiv 0$</td>
</tr>
<tr>
<td>NIST612</td>
<td>-0.01</td>
<td>-0.02</td>
</tr>
</tbody>
</table>

S-Figure 1: SEM image details of European beech (A) and Norway spruce (B). Both samples consist of finely-ground phytoliths material, indicating a homogenisation process has taken place during the decomposition in the soil or the sample preparation steps. Close inspection of the European beech phytolith fragments reveal a compacted structure of finely-ground fragments. Scale bar are 100 µm.

Literature


Mavromatis, V., Rinder, T., Prokushkin, A. S., Pokrovsky, O. S., Korets, M. A., Chmeleff, J., and Oelkers, E. H.


