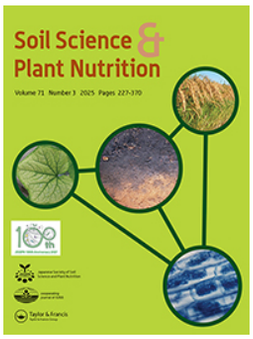

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RESEARCH ARTICLE

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Effects of a chemical dispersant on the dispersibility of Moso bamboo phytoliths in a particle-size analysis

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ABSTRACT

Phytoliths produced by higher plants are biogenic minerals with a wide range of particle sizes that correspond to clay, silt, and sand in mineral soil. Phytoliths supplied to soils through plants' death function as soil particles and affect soil physicochemical properties. Investigations of the functions of phytolith particles as a subset of soil particles are facilitated by fractionation and quantification of the phytoliths based on soil particle-size distribution. Fractionation is typically conducted through particle-size analysis (PSA) methods developed for soil texture analyses. However, the effects of chemicals used to achieve particle dispersion in these methods on phytolith dispersibility remain poorly understood. The objective of this study was to examine the effects of chemical dispersants on phytolith dispersibility. Based on the results, several recommendations are provided with respect to the dispersal conditions. The phytoliths used in this study were extracted from Moso bamboo leaf litter through wet digestion and dispersed by ultrasound followed by two chemical procedures: pH control using hydrochloric acid or sodium hydroxide and the addition of sodium hexametaphosphate as a dispersant. The extent of dispersion was determined by quantifying the clay-sized fraction (<2 µm) through gravity sedimentation and the pipette method. The results showed that the content of clay-sized phytoliths in dispersions remained constant from pH 3 to 9, then increased significantly at pH 10–11, likely due to phytolith dissolution, which also led to overestimation of the clay-sized fraction. This finding demonstrated that dispersion at higher pH (>10), as is often the case in PSA methods for soils, is unsuitable for phytoliths; instead, soil particles should be dispersed at pH < 9 to avoid phytolith dissolution.

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Dispersion; particle-size distribution; pH; sodium hexametaphosphate; soil separates

1. Introduction

Phytoliths, also called opal phytoliths or plant opal, are biogenic minerals consisting of amorphous silicates that are produced by living higher plants, especially gramineous species. In plant organs, as a result of precipitation of silicic acid absorbed from soil water, phytoliths are produced at cell walls, lumens, and intercellular voids, reflecting the shapes of those tissues (Kondo 2010; Ma, Miyake, and Takahashi 2001; Piperno 1988; Watteau and Villemin 2001). The particle size of phytoliths ranges from 0.1 µm (Watteau and Villemin 2001) to 1000 µm (Wilding and Drees 1968). After the death and decay of the plant, these silica pieces are deposited into soils and sediment as discrete, microscopic particles of varying sizes and shapes (Piperno 2006). Because phytoliths are inorganic and thus resistant to the forces of decay that destroy other types of plant materials, they survive in a well-preserved state over long periods of time (Piperno 2006).

Phytolith concentrations in soils are commonly in the range of < 1 to 30 g kg^{-1} on a total soil basis (Drees et al. 1989), but may be as high as 50 g kg^{-1} (Clarke 2003). The highest concentrations have been reported in the soils of coastal and inland swamps, flood plains, grasslands, and forests (Clarke 2003; Vander Linden and Delvaux 2019). Remarkably, silicate minerals composed entirely of phytoliths originating from the accumulation of bamboo phytoliths were recovered from a podzolic bleached layer on the volcanic island of Réunion in the Indian Ocean (Meunier, Colin, and Alarcon 1999). The quantity of phytoliths in soils depends on the type of terrestrial ecosystem and the soil type, which can influence phytolith solubility in soils. Phytoliths are 1–100 times more soluble than primary weatherable lithogenic silicates or pedogenic silicates at common soil solution pH levels (Fraysse et al. 2006, 2009; Fraysse, Pokrovsky, and Meunier 2010; Vander Linden and Delvaux 2019). Thus, only a small portion of the annual input of phytoliths remains undissolved (e.g., ~8% in tropical forests) (Alexandre et al. 1997). The remainder is likely to be a main component of silicon (Si) dissolved through weathering, and thus contribute to the export of Si from terrestrial to aquatic ecosystems (Gérard et al. 2008; Puppe et al. 2017; Sommer et al. 2013) which may act as a driver of the soil – plant Si cycle through re-uptake by plants (Vander Linden and Delvaux 2019). Because the phytolith dissolution rate decreases with increasing acidity (Fraysse et al. 2006, 2009), phytoliths are well preserved in acidic soils (Fraysse et al. 2006). Volcanic substrates are also conducive to the accumulation of soil phytoliths (Clarke 2003). Since phytolith dissolution also depends on the plant species (Alexandre et al. 1997; Blecker et al. 2006; Wilding and Drees 1974) and the types of silicified forms (Piperno 1988),

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the origin of the phytoliths influences their abundance in soils at a given pH level.

Phytoliths are conventionally considered to be particles larger than 5 µm, which enables researchers to identify their origin using microscopy, based on specific shapes that reflect plant cell morphology (Kondo 2010; Sommer et al. 2006; Wilding, Smeck, and Drees 1977). Phytoliths <5 µm in size are regarded as undefined phytogenic Si (Sommer et al. 2006) due to the difficulty of their identification and the inefficiency of their separation from the soil clay fraction (Wilding and Drees 1974; Wilding, Smeck, and Drees 1977). Therefore, in many studies, such as those aimed at restoring paleovegetation, fine fractions <5 µm are removed in the process of purification. However, from a biogeochemical perspective, these fractions drive the Si cycle through dissolution into soil water (Puppe et al. 2017), and are likely to remain in soils briefly as clay-sized particles prior to dissolution. Phytoliths are related to internal soil drainage in the 20–50 µm size range (R. Jones and Beavers 1964). In the 2–50 µm size range, they exhibit high reactivity toward organic anions through Al^{VI} chemisorbed on particle surfaces in an acid medium (Bartoli 1985), or reach surface charge densities higher than those of amorphous silica in the same size range (Fraysse et al. 2006). These findings indicate that phytoliths, even fine fractions <5 µm, have considerable effects on the physicochemical properties of soils, such as soil water permeability and retention or chemical replacement. However, few studies have focused on the contributions of phytoliths to soil genesis.

Investigations of the physicochemical functions of phytoliths (including undefined phytogenic Si <5 µm) as constituents of soil particles are facilitated by their fractionation and quantification based on the soil particle-size distribution to define soil texture rather than on the archeological definition of 5 µm. The particle size of phytoliths ranges from 0.1 to 1000 µm (Watteau and Villemin 2001; Wilding and Drees 1968), corresponding to the size range of soil particles such as clay (<2 µm), fine silt (2–20 µm), coarse silt (20–50 µm), and sand (50 µm to 2 mm) based on the definition of soil separates U.S. Department of Agriculture (USDA) (USDA Soil Science Division Staff 2017). Therefore, phytolith fractionation according to the particle-size distribution of soil separates enables phytoliths to be considered as a kind of soil particle.

To fractionate phytoliths isolated from soils or plants into the desired size classes, researchers typically conduct particle-size analysis (PSA), which was developed to determine the particle-size distribution of soil separates (Bartoli and Wilding 1980; Kanno and Arimura 1958; Piperno 1988; Sase and Kondo 1974; Smithson 1958; Wilding and Drees 1971, 1974; Witty and Knox 1964; Yeck and Gray 1972). The standard PSA method can be summarized as follows: particle aggregates are dispersed in an aqueous solution by physical and chemical means; the sand fraction is separated out by sieving, and the clay and silt fractions are isolated by centrifugation or gravity sedimentation following Stokes' law (Gee and Or 2002; Kilmer and Alexander 1949). In the PSA method, complete separation and dispersion of particle aggregates are essential (Gee and Bauder 1986; Kilmer and Alexander 1949) because inadequate dispersion results in the underestimation of the clay fraction, which also affects the quantitation of other fractions. However, in the case of phytoliths, the standard PSA method for soil separates is often adopted without considering its effect on dispersion. Several studies have shown that electronic physical dispersion using ultrasound is effective for breaking up soil aggregates (Edwards and Bremner 1967; Pritchard 1974; Watson 1971) and to disperse and purify phytoliths extracted from soils or plants (Fujiwara 1976; Kondo and Sase 1986; Kondo, Childs, and Atkinson 1994, 2003; Lombardo, Ruiz-Pérez, and Madella 2016). If physical dispersion according to the standard PSA method is not sufficient, chemical dispersion is often performed through the addition of dispersant agents such as sodium polyphosphate, ammonium hydroxide, sodium hydroxide, or hydrochloric acid, depending on their ability to create and maintain repulsive forces between soil particles (Gee and Or 2002; Kilmer and Alexander 1949; van Olphen 1963, 1977). Piperno (1988) suggested the use of sodium hexametaphosphate under the brand name Calgon, which is now widely used as a chemical dispersant to isolate phytoliths from soil particles. However, the effect of Calgon on phytoliths has not been directly investigated, and Calgon is not always adequate for all soil types (Kilmer and Alexander 1949; Theisen, Evans, and Harward 1968; Nanzyo, Shoji, and Dahlgren 1993). An alternative approach is to control the pH of the dispersion. In this case, although complete dispersion of the soil separates is often achieved under alkaline (pH 9–11) or acidic (pH 3–4) conditions (Tan 1996; Theisen, Evans, and Harward 1968; Wada and Kawano 1978; Warkentin and Maeda 1980; Yazawa 1978), a higher pH can cause rapid dissolution of the phytoliths (Alexander, Heston, and Iler 1954; Barão et al. 2015; Conley and Schelske 1993, 2001; DeMaster 1981; Ma, Miyake, and Takahashi 2001). Moreover, the mechanism by which the pH of the dispersion affects phytolith dispersibility remains unclear. To determine the particle-size distribution of phytoliths in soils and plants using the PSA method developed for soils, the effect of the dispersant agents on phytolith dispersibility must be well understood. Optimization of the PSA method for plant phytoliths will allow evaluations of the input of biogenic mineral particles from plants to soil, and thus their contribution to soil particles.

In this study, we investigated the effect of chemical dispersants on phytolith dispersibility to determine the relevant considerations and optimal dispersal conditions for applying the PSA method to phytoliths. We tested the effects of the pH of the phytolith suspension, and of Calgon as a dispersant agent, on the dispersibility of phytoliths extracted from leaf litter from Moso bamboo (*Phyllostachys pubescens*), a major Si-accumulating plant distributed throughout eastern Asia.

2. Materials and methods

2.1. Extraction of phytoliths from bamboo-leaf litter

For all analyses in this study, we used phytoliths extracted from Moso bamboo (*P. pubescens*) leaf litters gathered using litter traps in the forest at Kansai Research Center, Forestry and Forest Products Research Institute (34°56'28.4' N 135°46'31.7' E) in April and May 2017. The samples were washed in deionized water for 1 min in an ultrasonic bath (168 W/40 kHz; Ultrasonic Cleaner ASU-10; AS ONE, Osaka, Japan) to remove

external substances adhering to the sample surfaces (Umemura and Tanikawa 2021), followed by rinsing with ultrapure water. After drying at 70°C for at least 48 h, the leaves were cut into approximately 1 cm^2 pieces using pruning shears because phytoliths can be destroyed by fine grinding (Kondo 2005) or even by relatively light comminution using a cooking mill mixer, as observed in our preliminary experiments.

The samples were digested using a wet ashing method modified from conventional methods used to extract phytoliths from plants (L. H. P. Jones and Milne 1963; Kondo 2010; Kondo, Childs, and Atkinson 1994; Piperno 1988). The size distributions of phytolith particles obtained by wet ashing are more representative of those in plants, whereas the high temperatures (450–600°C) used in dry ashing can cause fusing or bonding of the phytoliths, thereby affecting their size distribution (R. Jones and Beavers 1964; L. H. P. Jones and Milne 1963). Briefly, leaf pieces (3 g) were placed in high-purity 300-mL Teflon vessels (Odlab, Geumcheon-gu, South Korea) and preliminarily digested overnight at room temperature in 30 mL of nitric acid (conc. 69–70%, FUJIFILM Wako Pure Chemical Co., Osaka, Japan) under reflux using the EcoPre graphite block acid digestion system (Odlab). Next, they were digested for 3 h at 160°C, followed by 5 h at 220°C. After cooling, 10 mL of nitric acid was added, and then the samples were digested for 27 h at 220°C until the liquid was clear and the waxy residue was completely digested. The decomposed solutions were filtered by suction filtration using 0.45 µm membrane filters (A045A047A; ADVANTEC, Tokyo, Japan). At this point, we confirmed that the filtrate was clear with no suspended particles. The residue on the filter (i.e., phytoliths) was rinsed with ultrapure water. Next, the phytoliths were transferred with 400 mL deionized water into a 500-mL beaker for a series of subsequent PSAs. To confirm the recovery ratio obtained from the PSAs, phytoliths on the filter from another 3-g extraction were transferred to an evaporating dish ($N = 3$). After drying at 105°C for at least 12 h, the phytoliths were weighed; the total amount was 204.3 ± 1.6 mg g^{-1} leaves (mean ± standard deviation [S.D.]).

2.2. Effects of pH on the dispersibility of phytoliths

To investigate the effects of pH on the dispersibility of phytoliths, we prepared nine suspensions of the phytoliths extracted as described above (i.e., approximately 613 mg phytoliths in a suspension, calculated by multiplying 204.3 mg g^{-1} leaves by 3 g leaves). Additionally, we prepared nine corresponding blanks, and then adjusted the pH of all samples over a range of 3–11. The phytolith suspensions were first physically dispersed using ultrasound, as previously described (Fujiwara 1976; Kondo and Sase 1986; Kondo, Childs, and Atkinson 1994, 2003; Lombardo, Ruiz-Pérez, and Madella 2016), and their pH was adjusted using hydrochloric acid (HCl) or sodium hydroxide (NaOH). The extent of dispersion at each pH was verified by quantifying the clay-sized fraction by gravity sedimentation and the pipette method. These operations were based on the standard PSA method as applied to soils (e.g., Gee and Bauder 1986; Gee and Or 2002; Jackson 1956; Kilmer and Alexander 1949; Tan 1996).

In detail, nine phytolith suspensions were sonicated using an ultrasonic homogenizer (VP-60; TAITEC, Saitama, Japan) for 5 min at 220 W/20 kHz to disperse the phytolith particles (modified from Kondo and Sase 1986). The suspensions were transferred into 1-L shaking bottles with a rubber stopper and the volume was adjusted to ~1 L with deionized water. Next, the pH of each suspension was adjusted at 1.0-unit intervals from pH 3.0 to 11.0 using 1 mol L^{-1} HCl or 1 mol L^{-1} NaOH, as monitored using a pH meter (MM-60 R; DKK TOA, Tokyo, Japan) and a glass electrode (GST-5741C; DKK TOA). Equivalent volumes of these reagents were added to the corresponding blanks. The electrode was also immersed in the blanks for the same period of measurement time as in the suspensions to account for elution of the internal standard solution (3.3 mol L^{-1} potassium chloride solution). The suspensions and blanks were adjusted to a 1 L volume with deionized water and shaken for 5–6 h using a TIC TA-25 shaking incubator (50 rpm; Takasaki Scientific Instruments Co., Saitama, Japan) (Kawada and Kojima 1976; Kilmer and Alexander 1949), and then allowed to stand overnight at almost constant room temperature. They were then shaken by hand for 1–2 min and allowed to stand again. After a predetermined settling time for the 2-µm size fraction based on Stokes' law and considering the mean room temperature and the specific gravity of the particles (2.3 g cm^{-3}) (Kondo 2010) (specific gravity of phytoliths: 1.5–2.3 g cm^{-3}, R. Jones and Beavers 1964; Kanno and Arimura 1958; Wilding, Brown, and Holowaychuk 1967), 10 mL of each suspension was gently removed from 10 cm below the water surface using a volumetric pipette that was cut open slightly at one end and transferred to a pre-weighed weighing bottle. After drying at 105°C for at least 12 h, the samples were weighed and the amount of clay-sized particles per total volume of the suspension was calculated by subtracting the value of the blank weighed in the same way. These procedures from hand-shaking to clay sampling were repeated six times over six days, continuously, without readjusting the total volume to 1 L.

After clay-sized particles sampling, the remaining suspensions were filtered using 0.45-µm membrane filters. The residues on the filters were dried at 105°C for at least 12 h, followed by weighing to determine the total phytolith yield. To measure SiO_2 dissolved in the filtrates, the filtrates were adjusted to a 1 L volume with deionized water and neutralized to pH 3–4 with 0–2 mL of 1 mol L^{-1} HCl. The SiO_2 concentration in the filtrates was determined by the colorimetric molybdenum blue method based on Takahashi (2003). Briefly, 5 mL of the solution, 0.25 mL of 2.4 mol L^{-1} HCl, and 2.5 mL of 0.081 mol L^{-1} ammonium molybdate were added to a test tube to bring the pH to 2.4–2.7. After standing for 3 min, 5 mL of 1.35 mol L^{-1} sodium sulfite was added. After approximately 10 min, the absorbance at 690 nm was measured using a spectrophotometer (V-730; Jasco, Tokyo, Japan).

2.3. Effects of a dispersant agent (Calgon) on the dispersibility of phytoliths

To investigate the effects of Calgon on the dispersibility of phytoliths, two sets of phytolith suspensions extracted as described above were prepared and processed as follows based on the method of Kawada and Kojima (1976), modified from the standard PSA method as applied to soils (e.g., Gee and Bauder 1986; Gee and Or 2002; Kilmer and Alexander 1949; Tan

1996). After sonication as described above, the suspensions were transferred into a 1 L shaking bottle and adjusted to a volume of approximately 700 mL with deionized water. Next, as a dispersant agent, 20 mL of Calgon solution freshly prepared by dissolving 40.8 g of Calgon (sodium hexametaphosphate, FUJIFILM Wako Pure Chemical Co.) in deionized water to 1 L was added into one of the shaking bottles, while the other was used as a control (CNT). For each treatment, two blanks with/without Calgon in 700 mL of deionized water were prepared. The suspensions and blanks were shaken for 3 h using a shaking incubator and their volume was adjusted to ~1 L with deionized water. The pH of the suspensions was adjusted at 1.0-unit intervals from pH 3.0 to 11.0 using 1 mol L^{-1} HCl or NaOH. Equal volumes of these reagents were also added to the corresponding blanks, and the electrode of the pH meter was immersed in the blanks for the same period of measurement. After the volumes had been brought to 1 L with deionized water, the suspensions were shaken for another 2–3 h (total duration of 5–6 h after adding Calgon), and allowed to stand at an almost constant temperature overnight. Then, the clay-sized fraction (<2 µm) was sampled through gravity sedimentation and the pipette method and weighed as described above. After adjusting the pH, we shook the suspensions for 1–2 h, and then allowed the samples to stand again at a constant temperature. Next, instead of readjusting the total volume to 1 L, the total volume at every pH after sampling or HCl/NaOH addition was recorded to quantify the clay-sized fractions.

2.4. Recovery ratio obtained using soil PSA method

The recovery ratio obtained using soil PSA method was determined based on the ratio of the total contents of fractionated vs. non-fractionated phytoliths. The phytoliths were divided into four size fractions: clay (<2 µm), fine silt (2–20 µm), coarse silt (20–50 µm), and sand (50 µm to 2 mm), referring to the soil particle-size classification scheme of the USDA Soil Science Division Staff (2017). Phytoliths were extracted from separate 3-g leaf samples by nitric acid digestion ($N = 3$) as well as the method described in Section 2.1. After filtration, the phytoliths were transferred with 400 mL deionized water into a 500-mL beaker and dispersed by sonication using an ultrasonic homogenizer, as described above. The suspensions were adjusted to a volume of 1 L, and then adjusted to pH 4.0 by adding a few drops of 1 mol L^{-1} HCl to unify the analytical conditions (at that pH, phytoliths are resistant to dissolution and, in this study, showed stable dispersion). Suspensions were shaken for 5–6 h and allowed to stand at an almost constant temperature overnight. Next, they were shaken by hand for 1–2 min and allowed to stand at a constant temperature again. After the predetermined settling time for fine silt + clay or clay based on Stokes' law, 10 mL of each suspension was gently removed using a pipette and transferred into pre-weighed weighing bottles. After drying at 105°C for at least 12 h, the samples were weighed to calculate the fine silt + clay and clay-sized fractions per total volume of the suspensions. Next, to quantify the coarse silt and sand-sized fractions, the suspensions were again adjusted to a volume of 1 L, and the top 10 cm fraction (clay + fine silt) was removed using a siphon tube after the determined time according to Stokes' law. This process was repeated until the supernatant was clear (more than 10 times). Finally, the particles of the coarse silt (20–45 µm) and sand-sized fractions (>45 µm) were sorted using a testing sieve (45 µm aperture; Tokyo Screen Co. Ltd., Tokyo, Japan), transferred into a pre-weighed evaporating dish, and weighed after drying at 105°C for at least 12 h.

2.5. Statistical analyses

Significant differences in the amounts of the clay-sized fraction from pH 3 to 11 were calculated using Tukey's honest significant difference (HSD) test ($p < 0.05$). Analyses were conducted in R v. 3.4.2 (R Development Core Team 2017).

3. Results

To determine the effect of a chemical dispersant on the dispersibility of phytoliths extracted from bamboo leaves, we first investigated the effects of pH on the clay-sized fraction. The contents of the clay-sized fraction (<2 µm) were almost constant from pH 3 to 9 (mean ± S.D. = 73.0 ± 2.80 mg g^{-1} leaves), and then increased significantly at pH 10 (83.0 ± 4.03 mg g^{-1} leaves) and pH 11 (90.0 ± 1.67 mg g^{-1} leaves) ($p < 0.05$; Figure 1). After six cycles of clay sampling (i.e., 6 days), the total amounts of phytoliths yielded by filtration using a 0.45-µm filter declined remarkably at pH 10 and 11 (Figure 2). By contrast, the dissolved SiO_2 contents of the dispersions increased rapidly at pH > 9, whereas at pH 3–7, they were low and stable. We then examined the effects of Calgon on the dispersibility of the phytoliths; between pH 3 and 11, the clay-sized fractions tended to be similar in dispersions with and without Calgon (Figure 3). In the determination of the recovery ratio obtained through PSA, the phytolith contents of each size fraction in accordance with the soil particle-size classification (dispersed at pH 4) were as follows: clay (<2 µm), 75.2 ± 1.17 mg g^{-1} leaves; fine silt (2–20 µm), 81.4 ± 0.77 mg g^{-1} leaves; coarse

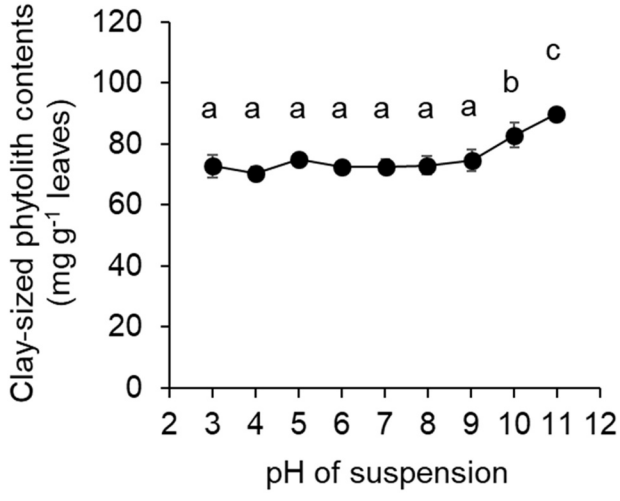

Figure 1. Contents of clay-sized phytoliths extracted from bamboo leaf litter in suspensions at pH 3–11. Error bars indicate standard deviation ($N = 6$). Different lowercase letters indicate significant differences among pH values (Tukey's HSD test, $p < 0.05$).

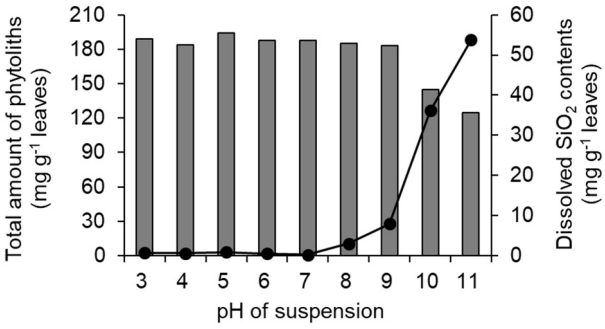

Figure 2. Total amounts of phytoliths yielded by filtration after six cycles of clay sampling (bar, left *Y*-axis) and dissolved SiO_2 contents in the filtrate (solid line, right *Y*-axis) in suspensions at pH 3–11.

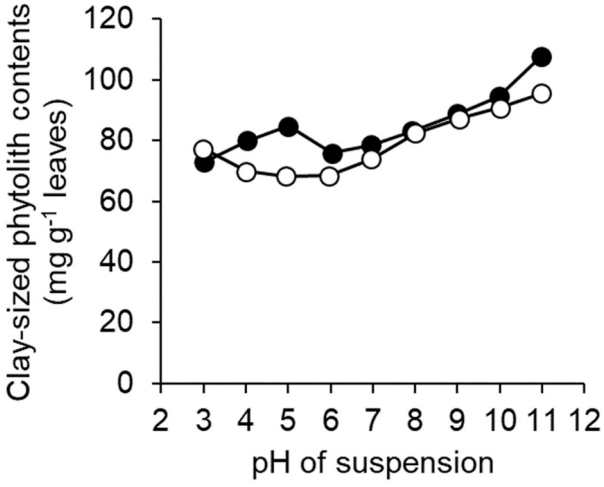

Figure 3. Contents of clay-sized phytoliths extracted from bamboo leaf litter in suspensions at pH 3–11 with Calgon (open symbols) and without Calgon (CNT; closed symbols) ($N = 1$).

silt (20–45 μm), 42.5 ± 1.19 mg g^{-1} leaves; and sand (45 μm to 2 mm), 2.37 ± 0.28 mg g^{-1} leaves (mean ± S.D., $N = 3$). The recovery ratio of the total contents of fractionated vs. non-fractionated phytoliths was 98.6% (201.5 ± 0.54 mg g^{-1} leaves vs. 204.3 ± 1.6 mg g^{-1} leaves).

4. Discussion

4.1. Effects of pH on the dispersibility of phytoliths

Our analysis of the effects of the dispersion pH on the amounts of clay-sized phytoliths showed that the amounts were almost constant from pH 3 to 9, and then increased significantly at pH 10–11 ($p < 0.05$; Figure 1). This tendency was ostensibly the result of increased clay dispersibility at pH 10–11, because complete dispersion of soil separates is often achieved under alkaline conditions (pH 9–11) for soils composed mainly of crystalline clay minerals (Tan 1996; Theisen, Evans, and Harward 1968; Wada and Kawano 1978; Warkentin and Maeda 1980; Yazawa 1978), or sometimes under acidic conditions (pH 3–4) for soils such as those of volcanic ash origin (Wada and Kawano 1978; Warkentin and Maeda 1980). However, as shown in Figure 2, the total amounts of phytoliths obtained by filtration after the series of clay sampling over 6 days declined remarkably at pH 10–11, and the dissolved SiO_2 contents of the dispersions increased rapidly at above pH 9. These results clearly indicate that the increased contents of clay-sized phytoliths at pH 10–11 were because of dissolution of phytoliths, rather than improved dispersibility, resulting in overestimation of the contents of clay-sized phytoliths at high pH levels. The accepted dispersion methods for soils at high pH assume a degree of resistance to dissolution of crystalline minerals. However, compared to primary minerals, the amorphous form of biogenic silica is highly soluble under alkaline conditions (e.g., Barão et al. 2015; Conley and Schelske 1993, 2001; DeMaster 1981), because it is almost 20 times more soluble than crystalline silica (quartz) (Robie and Waldbaum 1968 in Barker, Fontes, and Gasse 1994). Therefore, phytoliths have less resistance to the high pH values used in dispersion of primary minerals. Fraysse et al. (2006) found that the dissolution rate of phytoliths extracted from a bamboo (*Nastus borbonicus*) exhibited pH dependence: a minimal dissolution rate at 2 < pH < 3–4 and increasing with pH values from 4 to 12, with a slope. A roughly similar tendency was described for horsetail, larch, elm, and fern phytoliths (Fraysse et al. 2009); the rates for phytoliths of all types were the lowest at 2 ≤ pH ≤ 3–4, whereas the rates increased linearly for pH values from 4 to 9. In the case of amorphous silica produced by combustion of silane vapors, solubility was constant at pH 5–8 and increased at pH > 9 by dissociation of silicic acid [$Si(OH)_4$] into silicate ion ($H_3SiO_4^-$) (Alexander, Heston, and Iler 1954; Ma, Miyake, and Takahashi 2001). Considering these dissolution properties of amorphous silica, it is possible that in this study the increased contents of clay-sized phytoliths at pH 10–11 was caused both by an increase in the numbers of particles <2 μm due to dissolution of larger particles and an increase in dissociated silicate ions. With regard to the latter, the precipitates weighed as clay-sized phytolith may have included sodium silicates as a compound between sodium hydroxide and silicate ion. These factors may have caused the ostensible increase in the clay-sized fraction at high pH levels.

The overestimation of the clay-sized fraction that occurred under high pH conditions potentially impacted the evaluation of the Si cycle and soil formation. The mean content of the clay-sized fraction from pH 3 to 9 was 73.0 mg g^{-1} leaves (Figure 1), which accounted for 35.7% of the total phytoliths (204.3 mg g^{-1} leaves). In the same way, the contents were 83.0 mg g^{-1} leaves (40.6%) at pH 10 and 90.0 mg g^{-1} leaves (44.1%) at pH 11. Umemura and Takenaka (2014) reported that the amount of phytoliths supplied to soils by litterfall in Moso bamboo forests was 165 to 706 kg ha^{-1} $year^{-1}$, of which 72–88% (approximately. 80%) was comprised of bamboo leaves. Based on this knowledge and our results, the supply of clay-sized phytoliths through bamboo leaf litter was calculated as 47 to 202 kg ha^{-1} $year^{-1}$ at pH 3–9, whereas it was 54 to 229 kg ha^{-1} $year^{-1}$ at pH 10 and 58 to 249 kg ha^{-1} $year^{-1}$ at pH 11 with overestimations of 4.9 and 8.4%, respectively. This estimate clearly showed that the pH of the dispersion caused significant differences in the supply of clay-sized phytoliths and, consequently, that of the other sized fractions. Therefore, we need to consider carefully the analytical conditions during the pretreatment phase of the PSA method in studies of the biological cycle of Si or phytoliths.

Based on these results, we suggest that dispersion should be conducted at pH 3–9, to avoid overestimation of the clay-sized fraction, or under acidic conditions at pH 3–4, whereby the dissolution rate may be much lower. The dispersal conditions established for bamboo phytoliths may also apply to other types of phytoliths, as the dissolution rates of different types of phytoliths extracted from bamboo, horsetail, larch, elm, and fern are almost identical across the full pH range (Fraysse et al. 2006, 2009). Furthermore, our results showed that pH adjustment is not always required when PSA is conducted as described in this study, because the pH of the dispersion obtained after wet ashing, filtration, rinsing with ultrapure water, and sonication was already approximately 4.0 (4.07–4.25).

4.2. Effects of Calgon on the dispersibility of phytoliths

To investigate whether a chemical dispersant agent can improve the dispersibility of phytoliths, we tested the effects of Calgon, the most commonly used agent for soils (Gee and Bauder 1986; Gee and Or 2002; Kilmer and Alexander 1949; Theisen, Evans, and Harward 1968). At pH 3–11, the size of the clay-sized fractions in the dispersions with Calgon tended to be similar to those in the dispersions without Calgon, indicating that Calgon did not enhance phytolith dispersibility. Dispersion of clay particles is basically accomplished by the electrical repulsive force between particles outperforming the attractive force (i.e., intermolecular force) (Goldberg et al. 2011; Hiemenz and Rajagopalan 1997; Overbeek 1952). This electrical repulsive force is enhanced when the particle surface is sufficiently negatively charged (or positively in some cases) and thereby the thickness of the electrical double layer increases (Goldberg et al. 2011; van Olphen 1963, 1977). When Calgon, a polymetaphosphate anion that may have a valence of 30 or more, is adsorbed on the particle surface in excess, some of the negative-charge sites neutralize the positive-charge sites on the particles (produced mainly by exposed aluminum (Al) on the edge of clay plates in soils) and the remaining sites build up a stabilizing negative charge (van Olphen 1963, 1977). Considering such effects of Calgon, the results obtained in this study imply that clay-sized phytoliths are already sufficiently charged for dispersion without the need for Calgon. We can explain the surface-charge property of clay-sized phytoliths from the viewpoint of zeta potential, which is an indicator of dispersion stability; clay particles disperse well when zeta potential increases as the pH moves away from the isoelectric point (IEP) at which the surface charge appears to become zero (Goldberg et al. 2011; Horikawa 1975, 1976; Nanzyo, Shoji, and Dahlgren 1993; Yazawa 1978). In previous studies, Fraysse et al. (2006) used microelectrophoresis measurements to test the isoelectric point of phytoliths extracted from bamboo (*N. borbonicus*) and found that they had an isoelectric point at pH 1.5. That study also found that the phytoliths had a negative zeta potential at pH values higher than the isoelectric point, which reflected their surface bearing a negative charge (> SiO^-). They also observed that the surface-charge densities of bamboo phytoliths were higher than those of two types of silica: amorphous silica (Baker) and vitreous silica (Prolabo). Furthermore, Fraysse et al. (2009) investigated the zeta potential of phytoliths from horsetail, larch, elm, fern, and four grasses, and found that those phytoliths exhibited a negative zeta potential at pH > 1–3 (= pH_{IEP}). The zeta absolute values also increased with increasing pH from pH_{IEP} to 11 (Fraysse et al. 2006, 2009). We did not analyze the isoelectric points or zeta potentials of the phytoliths extracted from bamboo (*P. pubescens*) in the present study, but the findings of these previous studies imply that the bamboo phytoliths also have an isoelectric point at around pH 1–3 and have sufficient negative charge for dispersion at pH 3–11. This dispersibility obtained without Calgon may also result from the physical dispersion achieved by ultrasonication. Electronic dispersion by ultrasonication is an effective method that results in the complete and stable dispersion of soil particles (Edwards and Bremner 1967; Pritchard 1974; Watson 1971) as well as phytoliths extracted from plants or soils (Fujiwara 1976; Kondo and Sase 1986; Kondo et al. 2003; Kondo, Childs, and Atkinson 1994; Lombardo, Ruiz-Pérez, and Madella 2016). Although the effect of Calgon in the absence of physical dispersion by ultrasonication may require further study, our findings showed that Calgon added after ultrasonication did not further improve phytolith dispersibility, implying that the phytoliths were sufficiently charged for dispersion.

The dispersion properties of the phytoliths observed in this study may also apply to phytoliths extracted from other Si-accumulating plants, typified by gramineous species. The Si content of those phytoliths is 82–92% SiO_2 (Arimura and Kanno 1965; Drees et al. 1989; L. H. P. Jones and Milne 1963; Kondo 1988), implying a sufficient negative charge (>SiO^-) on the surfaces of the phytolith particles. By contrast, phytoliths isolated from plants growing on Spodosols or Inceptisols in acid forest ecosystems in eastern France (e.g., beech, fir, red pine, *Festuca*, or *Calluna*) have proportionally lower percentages of SiO_2 (67–80%) and higher percentages of other elements, such as Al, iron, manganese, or titanium (Bartoli and Wilding 1980). Phytoliths extracted from woody species occurring on siliceous bedrock in the Valaisan Swiss Alps contained high proportions of Al in the form of aluminosilicates (Carnelli et al. 2002). The isoelectric point for Al oxides (~8.5–9) is much higher than that of SiO_2, probably because the aluminol surface groups (>$AlOH_2^+$) provide an overall positive charge in the acid pH region (Fraysse et al. 2006). The surface charge characteristics of phytoliths with lower SiO_2 content and higher content of other elements such as Al may differ from those of phytoliths consisting almost entirely of SiO_2, and may lead to differences in dispersibility. For such phytoliths, additional research to test the effectiveness of alternative dispersant agents including Calgon may be required.

5. Conclusions

This study investigated the effect of a chemical dispersant on phytolith dispersibility and determined the optimal conditions for phytolith dispersal when applying the PSA method developed for soils to phytoliths extracted from Moso bamboo leaf litter. Specifically, the effects of pH on phytolith suspensions and of Calgon as a dispersant agent on phytolith dispersibility were examined. Our results showed that the content of clay-sized phytoliths (<2 μm) was almost constant over a pH range

of 3 to 9, but increased significantly at pH 10–11. This increase in dispersibility can be explained by the dissolution of phytoliths at pH > 9, which would also lead to the overestimation of the contents of clay-sized phytoliths. Dispersion at higher pH (>10), as commonly practiced in PSA methods for soils, is therefore unsuitable for phytoliths. Instead, particles should be dispersed at pH < 9 to avoid phytolith dissolution. Compared to untreated samples, those treated with Calgon exhibited no increase in the amount of clay-sized phytoliths over a pH range of 3–11, indicating that the phytolith particles were sufficiently charged for dispersion without Calgon.

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