

Characteristics of broadleaved woody phytoliths and their preservation in soils in the mid-subtropical zone of China

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Abstract

Background and aims

Phytolith analysis is a promising tool for paleovegetation reconstruction. However, compared with grass phytoliths, there are few studies that have classified the phytoliths of trees. Therefore, reliable definitions of phytolith types in woody plants and studies of representative woody phytoliths in soils are needed to provide an improved basis for reliable phytolith-based vegetation reconstruction.

Methods

To explore the morphology of woody phytoliths and their preservation in soils, we described the phytolith morphology of 60 common broadleaved woody plants in the mid-subtropical zone of China, based initially on anatomical origin, and then on shape and finally on texture. We also for the first time established an index (R value) to explore the preservation of broadleaved phytoliths in soil in this region.

Results

We divided the phytoliths of broadleaved woody plants into 6 major types and 36 sub-types. The 6 major types are: epidermal phytoliths, silicified stomata, tracheid, favose phytoliths, hair, and others. We found some degree of correspondence between different phytolith types and the parent plants. Fagaceae, Lauraceae, Magnoliaceae, Rosaceae, Anacardiaceae and Euphorbiaceae could be clearly distinguished from other families based on phytoliths. Only eight types of broadleaved phytoliths were present in the soils. Elongate attenuate, elongate tenuous psilate, and blocky regulate were better preserved in soils than the other types (elongate attenuate entire, tabular sinuate, hair, sclereid, and favose phytoliths).

Conclusion

Our results provide an important basis for the further application of phytoliths in paleovegetation reconstruction.

Introduction

Phytoliths are an important reference for plant classification (especially Poaceae) and they are also well preserved in soil (Cabanès et al., 2015; Lisztes-Szabó et al., 2019). In recent years, the determination of the characteristic phytoliths of woody plants in the tropical zone and their preservation in soil have shown great potential for studies of the evolution of forest–grassland ecotones and timberlines. Knowledge of phytolith morphologies and assemblages are an important basis for plant classification and a prerequisite for phytolith-based paleovegetation reconstruction. The ability of phytoliths to reliably reconstruct paleovegetation depends on the systematic study and accurate recognition of the morphological characteristics of modern plant phytoliths and their preservation in soil.

Since the discovery by Prat (1936) that different phytolith types were produced in the short cells of different subfamilies of grasses, numerous studies have been conducted on the phytolith morphology of herbaceous plants (e.g., Poaceae, Cyperaceae, Asteraceae) (Barboni et al., 2007; Honaine and Osterrieth, 2012; Wu et al., 2014; Zucol et al., 2019), woody plants (Mercader et al., 2009), and ferns (Mazumdar, 2011). By contrast, previous studies of the phytoliths of modern herbaceous plants, especially Poaceae, were more systematic: numerous morphological descriptions been produced, and several taxonomic systems have been established from different perspectives. Additionally, morphological parameters of phytoliths have been used to describe typical Poaceae phytoliths in more detail, such as bilobate and bulliform; these descriptions have enabled Poaceae to be distinguished to the levels of family, genus, and even species (Li et al., 2005; Lu and Liu, 2003; Mercader et al., 2010; Liu et al., 2013; Tang et al., 2022).

By comparison, descriptions of the phytolith morphology of woody plants are relatively rare. In early studies, the descriptions were not comprehensive, and only 7 types of coniferous phytolith and 8 types of broadleaved phytolith were distinguished (Rovner, 1971; Wilding and Drees, 1971; Klein and Geis, 1978). More recently, there has been an increasing amount of research on woody plant phytoliths in tropical and subtropical regions, and efforts have been made to refine their study based on subtle differences in shape and texture (Wallis, 2003; Xu, Dirk et al., 2005; Mercader et al., 2009; Piperno and McMichael, 2020). Additionally, the discovery of the characteristic phytolith types of palms (i.e., globular granulates) has provided the impetus for phytolith-based paleovegetation and paleoclimate studies in the tropics, including the reconstruction of the evolution of forest–grassland ecotones and plant community evolution during the historical period (Bremond et al., 2005; Barboni et al., 2007; Coe et al., 2013; Crifò and Strömberg, 2020; Rodríguez-Cintas et al., 2020). For example, a forest cover index (D/P) was proposed, which is generally > 1 in the topsoil of tropical rainforest areas, and < 1 in savanna areas (Alexandre et al., 1997). However, in existing studies, globular granulates have been regarded as the sole representative morphotype of woody plant phytoliths in tropical and subtropical regions. The plant composition of existing woody plant communities in tropical and subtropical regions is complex, and different families of woody plants may produce different characteristic phytoliths. Also, changes in the composition of different families will directly affect the true composition of the vegetation in this region. Overall, additional morphological descriptions of woody plant phytoliths are needed in tropical and subtropical regions to determine the differences between the phytoliths of woody plants of different families and genera. Moreover, the morphological classification of woody plant phytoliths is still based mainly on qualitative

descriptions, and the classification of woody plant phytoliths has not yet reached family level. In temperate regions (e.g., in the Changbai Mountains of China), efforts have been made to enumerate and describe the assemblage characteristics of broadleaved and coniferous woody plant phytoliths and their typical phytolith types from the perspective of phytolith anatomical origin (Gao et al., 2018a), and the results have been successfully applied to reconstruct the paleovegetation and tree line changes in the Changbai Mountains (Gao et al., 2018b; Gao et al., 2021). Lisztes-Szabó et al. (2019) also described the phytolith morphology of three deciduous broadleaved trees and three coniferous trees in spruce conifer forest in Norway, from the perspective of anatomical origin, and they identified the characteristic phytolith type (cubical) of *Picea*. Therefore, it is clearly worthwhile to describe the morphology of woody plant phytoliths from the perspective of their anatomical origin and to differentiate different phytolith types, although this approach has long been neglected. In contrast, systematic studies of woody plant phytoliths in the mid-subtropical zone of China are rare, and only a few morphological descriptions and categorization studies of woody plant phytoliths have been conducted in a few regions (Ge et al., 2020). The classification of woody plant phytoliths in this region is not yet systematic enough, and their nomenclature has not been harmonized with the ICPN nomenclature (ICPN 1.0; ICPN 2.0); moreover, the understanding of woody plant phytolith morphology is inconsistent among regions. At the same time, the woody plants in the mid-subtropical zone of China belong to different floras, from north to the south, than those in the Changbai Mountains, and it has been shown that the morphological assemblage and size of phytoliths are very sensitive to environmental factors (Liu et al., 2016); moreover, palms are not abundant in this area. Thus, the clear definition of phytolith types and assemblages in woody plants and the determination of representative woody phytoliths in the soils in the mid-subtropical zone of China has become a significant issue in phytolith research.

In this study we collected a series of samples of broadleaved woody plants in the mid-subtropical zone of China, including 26 families and 60 species, and we also analyzed the phytolith assemblages in 31 representative soil samples and the corresponding plant community samples from this region. Our objectives were to systematically describe the phytolith types of common broadleaved woody plants, to define the relationship between phytolith types and the plants of origin, and to further characterize the broadleaved phytolith assemblages of the surface soils of this region. Our findings potentially provide a more accurate reference for reconstructing the paleoclimate, paleovegetation, and changes in the tree line in the mid-subtropical zone of China.

Study Area

The study area (24°38' N–30°08' N, 108°47' E–114°15' E) is located in Hunan Province in the mid-subtropical region of China. This region is surrounded by mountains in the east (the Mufu Mountains and Luoxiao Mountains), west (the Wuling Mountains and Xuefeng Mountains) and south (Nanling Mountains); and it slopes gradually towards the central Xiangzhong hills and basin and the Dongting Lake Plain in the northeast, forming an asymmetrical horseshoe-shaped topography with an opening to the northeast (Wu, 1980). This region has a subtropical monsoon climate with four distinct seasons, hot summers and warm winters, and pronounced monsoons. The average annual temperature ranges from 15°C to 18°C and the average annual precipitation ranges from 1200 mm to 1700 mm, concentrated in the spring and summer. The zonal soils are mainly red and yellow soils, and the non-zonal soils are mainly alluvial soil, paddy soil, limestone soils and purple soils (Li et al., 2021). The favorable climate and diverse habitat conditions lead to significant spatial heterogeneity of the vegetation in this region, resulting in a high plant species diversity and typical central subtropical evergreen broadleaf forest, with significant south–north and east–west differences in vegetation type, species composition, and community structure. The vegetation zone in northern Hunan consists mainly of mixed deciduous/evergreen broadleaved forest, evergreen broadleaved forest, and herbaceous plant communities on lake beaches. The main tree species include evergreens such as *Castanopsis sclerophylla*, *Quercus glauca*, *Lithocarpus glaber*, *Cinnamomum camphora*, and *Ilex chinensis* Sims; and deciduous species such as *Dalbergia hupeana* Hance, *Albizia kalkora* (Roxb.) Prain, *Acer* spp., and *Quercus fabri* Hance. The vegetation zones in central and eastern Hunan are also mainly evergreen broadleaved forest, mixed deciduous/evergreen broadleaved forests and *Pinus massoniana* coniferous forest. The main tree species include *Quercus glauca*, *Castanopsis sclerophylla*, *Quercus glauca* Thunb., *Castanopsis carlesii* (Hemsl.) Hayata, *Castanopsis eyrei* (Champ.) Tutch., and *Carpinus viminea* Lindley. The vegetation in south Hunan is composed mainly of evergreen broadleaved forest, mixed deciduous/evergreen broadleaved forest, and *Pinus massoniana* coniferous forest. The main tree species include *Castanopsis fordii* Hance., *Cryptocarya chingii* W. C. Cheng, *Quercus glauca* Thunb., and *Castanopsis eyrei* (Champ.) Tutch. The vegetation in northwest Hunan is also dominated by evergreen broadleaved forest, mixed deciduous/evergreen broadleaved forest and *Pinus massoniana* coniferous forest. The main tree species include *Castanopsis fargesii* Franch and *Castanopsis eyrei* (Champ.) Tutch. (Qi, 1984).

Materials And Methods

Sample collection

Broadleaved woody plant samples were collected from the vegetation zone of northern and southern Hunan Province. A total of 60 species of broadleaved woody plants, belonging to 26 families and 46 genera, were collected (see Table 1 for detailed information). The plant community and surface soil samples were also collected. The woody plant community was sampled using a 10 m × 10 m quadrat. Based on the plum sampling method, five 1 m × 1 m quadrats were sampled in the four corners and in the center of the quadrat. The litter of all herbaceous and woody plants beneath the forest was collected, and the surface soil (0–2 m) was sampled. A total of 31 plant community samples and surface soil samples were collected, including 9 samples from broadleaf evergreen forest communities, 5 samples from mixed evergreen/deciduous broadleaf forest communities, and 17 samples from deciduous broadleaf forest communities (Fig. 1).

Table 1
List of modern plants used for phytolith analysis and the sampling sites

| Species | Family | Genus | Sampling site | Species | Family | Genus | Sampling site |
|---|--------------|-------------|---------------|--|---------------|--------------|---------------|
| <i>Quercus fabri</i> Hance | Fagaceae | Quercus | Changsha | <i>Vernicia fordii</i> (Hemsl.) Airy Shaw | Euphorbiaceae | Vernicia | Changsha |
| <i>Quercus aliena</i> Bl | Fagaceae | Quercus | Changsha | <i>Tarenna mollissima</i> (Hook. et Arn.) Rob | Rubiaceae | Clerodendrum | Changsha |
| <i>Quercus acutissima</i> Carruth | Fagaceae | Quercus | Changsha | <i>Gardenia jasminoides</i> Ellis | Rubiaceae | Gardenia | Yongzhou |
| <i>Cyclobalanopsis glauca</i> (Thunb.) Oerst | Fagaceae | Quercus | Changsha | <i>Diplospora dubia</i> (Lindl.) Masam | Rubiaceae | Diplospora | Yongzhou |
| <i>Castanopsis hystrix</i> Miq | Fagaceae | Castanopsis | Yongzhou | <i>Mussaenda esquirolli</i> Lévl | Rubiaceae | Mussaenda | Changsha |
| <i>Castanopsis fargesii</i> Franch | Fagaceae | Castanopsis | Changsha | <i>Aidia cochinchinensis</i> Lour | Rubiaceae | Rubia | Changsha |
| <i>Castanopsis sclerophylla</i> (Lindl.) Schott. | Fagaceae | Castanopsis | Changsha | <i>Liquidambar formosana</i> Hance | Hamamelidae | Liquidambar | Yongzhou |
| <i>Castanopsis jucunda</i> Hance | Fagaceae | Castanopsis | Yongzhou | <i>Loropetalum chinense</i> (R. Br.) Oliver | Hamamelidae | Loropetalum | Changsha |
| <i>Castanea seguinii</i> Dode | Fagaceae | Castanea | Shaoyang | <i>Celtis sinensis</i> Pers | Ulmaceae | Celtis | Yongzhou |
| <i>Castanea mollissima</i> Blume | Fagaceae | Castanea | Huaihua | <i>Celtis biondii</i> Pamp | Ulmaceae | Celtis | Yongzhou |
| <i>Cryptocarya chinensis</i> (Hance) Hemsl | Lauraceae | Cryptocarya | Changsha | <i>Broussonetia papyrifera</i> | Moraceae | Broussonetia | Changsha |
| <i>Sassafras tzumu</i> (Hemsl.) Hemsl | Lauraceae | Sassafras | Huaihua | <i>Morus alba</i> L | Moraceae | Morus | Changsha |
| <i>Litsea elongata</i> (Wall. ex Nees) Benth. et Hook. f | Lauraceae | Litsea | Chenzhou | <i>Salix babylonica</i> | Salicaceae | Salix | Huaihua |
| <i>Lindera nacusua</i> (D. Don) Merr | Lauraceae | Lindera | Shaoyang | <i>Urena procumbens</i> Linn | Malvaceae | Urena | Yongzhou |
| <i>Litsea cubeba</i> (Lour.) Pers | Lauraceae | Litsea | Changsha | <i>Pterocarya stenoptera</i> C. DC | Juglandaceae | Pterocarya | Yongzhou |
| <i>Cinnamomum camphora</i> (L.) Presl | Lauraceae | Cinnamomum | Chenzhou | <i>Albizia julibrissin</i> Durazz | Leguminosae | Albizia | Changsha |
| <i>Magnolia officinalis</i> var. <i>biloba</i> Rehder & E.H. Wilson | Magnoliaceae | Magnolia | Huaihua | <i>Melia azedarach</i> L | Meliaceae | Melia | Yongzhou |
| <i>Michelia platypetala</i> | Magnoliaceae | Michelia | Changsha | <i>Vitex negundo</i> L. var. <i>cannabifolia</i> (Sieb. et Zucc.) Hand.-Mazz | Verbenaceae | Vitex | Yongzhou |
| <i>Michelia chapensis</i> Dandy | Magnoliaceae | Michelia | Changsha | <i>Callicarpa rubella</i> Lindl | Verbenaceae | Callicarpa | Changsha |
| <i>Michelia crassipes</i> Law | Magnoliaceae | Michelia | Changsha | <i>Acer albopurpurascens</i> Hayata | Aceraceae | Acer | Changsha |
| <i>Rubus irenaeus</i> Focke | Rosaceae | Rubus | Shaoyang | <i>Styrax confusus</i> Hemsl | Styracaceae | Styrax | Changsha |
| <i>Rubus parvifolius</i> L | Rosaceae | Rubus | Huaihua | <i>Ilex rotunda</i> Thunb | Aquifoliaceae | Ilex | Changsha |
| <i>Rubus corchorifolius</i> L. f | Rosaceae | Rubus | Changsha | <i>Camptotheca acuminata</i> | Nyssaceae | Camptotheca | Huaihua |

| Species | Family | Genus | Sampling site | Species | Family | Genus | Sampling site |
|--|---------------|----------------|---------------|--|--------------|--------------|---------------|
| <i>Photinia davidsoniae</i> Rehd. et Wils | Rosaceae | Photinia | Changsha | <i>Ligustrum sinense</i> Lour | Oleaceae | Ligustrum | Changsha |
| <i>Photinia villosa</i> (Thunb.) DC | Rosaceae | Photinia | Changsha | <i>Melia azedarach</i> L | Meliaceae | Melia | Changsha |
| <i>Choerospondias axillaris</i> (Roxb.) Burt et Hill | Anacardiaceae | Choerospondias | Changsha | <i>Maesa japonica</i> (Thunb.) Moritzi | Myrsinaceae | Maesa | Changsha |
| <i>Toxicodendron vernicifluum</i> (Stokes) F. A. Barkl | Anacardiaceae | Toxicodendron | Huaihua | <i>Eurya muricata</i> Dunn | Theaceae | Eurya | Changsha |
| <i>Rhus chinensis</i> Mill | Anacardiaceae | Rhus | Chenzhou | <i>Camellia oleifera</i> Abel | Theaceae | Camellia | Changsha |
| <i>Mallotus philippensis</i> | Euphorbiaceae | Mallotus | Changsha | <i>Rhododendron ellipticum</i> Maxim | Ericaceae | Rhododendron | Shaoyang |
| <i>Sapium sebiferum</i> (L.) Roxb | Euphorbiaceae | Sapium | Yongzhou | <i>Symplocos lucida</i> (Thunb.) Siebold et Zucc | Symplocaceae | Symplocos | Changsha |

Laboratory methods

Plant and soil phytoliths were extracted using the wet ashing method. The procedure for plant phytolith extraction was as follows: First, individual leaves were cleaned using an ultrasonic shaker and then oven-dried overnight at 60°C. The cleaned leaves were then cut into small sections, each weighing ~ 0.2g, and placed in a centrifuge tube, to which 65% HNO₃ was added. The tube was then heated in a water bath at 90°C until the reaction subsided. The samples were then centrifuged for 15 min at 2000 r/min, which was repeated 3 times. Then, a tablet containing a known number of *Lycopodium* spores was added to another centrifuge tube and mixed with 10% HCl; the HCl was then transferred to the centrifuge tube containing the plant sample and the mixture was centrifuged twice, after which the sample was centrifuged in absolute ethyl alcohol. Finally, 1–3 drops of well-shaken the suspension were placed on a glass microscope slide, which was heated over a spirit lamp until all the ethanol was evaporated. Canada balsam oil (1–2 drops) was added and a coverslip placed on top. Observations and identifications were performed with an Olympus microscope at ×900 magnification. At least 300 phytolith grains were counted for each sample. The extraction method for soil phytoliths was slightly different in that it included the flotation of phytoliths using ZnBr₂ liquids, as detailed in Gao et al. (2018b).

Results

A statistical evaluation showed that the majority of the samples of the 60 broadleaved woody plants contained phytoliths of varying amounts and of various types. Phytoliths are classified and named after the International Code for Phytolith Nomenclature (ICPN) 2.0 (Neumann et al. 2019). Comparison of the mean values of the phytolith concentrations showed that the phytolith contents for different families of broadleaved woody plants varied greatly, with the mean total concentration of 8.29×10⁵ grains/g. The concentrations were relatively high (~ 5.96×10⁵ to ~ 53.99×10⁵ grains/g) in Fagaceae, Magnoliaceae, Anacardiaceae, Euphorbiaceae and Ulmaceae, and relatively low in the other families (Fig. 2). We found no or very few phytoliths in plant species such as *Castanea seguinii* Dode, *Castanea mollissima*, *Rubus corchorifolius* L. f., *Diplospora dubia* (Lindl.) Masam, *Mussaenda esquirolli* Lévl. *Aidia cochinchinensis* Lour, *Callicarpa rubella* Lindl, *Camellia oleifera* Abel, *Rhododendron ellipticum* Maxim, and *Symplocos lucida*. The phytoliths of most of the of the broadleaved plants from different families observed in the samples were not only rich in terms of morphotype but each also contained varying combinations of phytoliths from different plants. The main phytolith morphotypes and morphotype frequencies of broadleaved trees are described below.

Phytolith morphotypes of broadleaved woody plants

Silicification In broadleaved woody plants occurs mainly in epidermal cells, stomata, tracheid and hair cells, with occurrences also in mesophyll, vascular tissues and fiber.

Epidermal cells

Three distinct types of phytoliths are produced in the epidermal cells of broadleaved woody plants: first, epidermal phytoliths that still retain the original morphology of the plant epidermal tissue; second, tabular, formed by the silicification of individual cells in the epidermal tissue; and third, elongate, derived from epidermal cells.

The epidermal phytoliths are generally thinly silicified, with silicified edges and unfilled interiors, and they still retain the characteristics of the plant epidermal tissue. A certain quantity of intact epidermal phytoliths were extracted from the broadleaved woody plants (Fig. 3, a–e). In terms of the relative contents of phytoliths in different families and genera, the content of epidermal phytoliths generally reached ~ 10%. The percentages of this

morphotype in *Litsea cubeba* (Lour.) Pers, *Rhus chinensis* Mill, *Sapium sebiferum* (L.) Roxb, *Loropetalum chinense* (R. Br.) Oliver, *Morus alba* L, *Melia azedarach* L, *Acer albopurpurascens* Hayata, *Ilex rotunda* Thunb, was higher, generally up to and exceeding 30%.

Tabular phytoliths formed by the silicification of individual cells can be further classified into square (Fig. 3, h), rectangle (Fig. 3, i), tabular irregular (Fig. 3, f–g), and tabular sinuate (Fig. 3, j–l) morphotypes. Among them, tabular sinuate is characteristic of broadleaved woody plants and it distinguishes them from conifers. The tabular content generally reached ~10%, and within this morphotype, the percentage of tabular in *Sapium sebiferum* (L.) Roxb was as high as 40.93%, while in other plants was generally <30%.

The elongate morphotype produced in epidermal cells mainly consisted of elongate psilate (Fig. 3, m) and elongate tabular (Fig. 3, n), with shallow silicification, a smooth surface and no ornamentation, while elongate echinate (Fig. 3, p–q) and elongate granulate were only found in individual plants, at levels generally <1%. The elongate morphotype derived from epidermal cells in broadleaved woody plants was generally <10% and the content was low. However, several individual plants such as *Quercus fabri* Hance, *Cyclobalanopsis glauca* (Thunb.) Oerst, and *Rubus parvifolius* L. had contents as high as 30% or more.

Stomata

The types of silicified stomata in broadleaved woody plants are diverse (Fig. 3), but one or two dominant types often occur in a particular family, i.e., the dominant types of silicified stomata in plants of the same family are often the same or similar. The silicified stomata content varied widely among different families, but the values were concentrated within the range of 30–80% in Anacardiaceae, Ulmaceae and Magnoliaceae, whereas it was <5% in Lauraceae, Euphorbiaceae, Rosaceae and Hamamelidaceae. The type and content of silicified stomata may, to a certain extent, indicate the class affiliation of their parent plants.

Vascular tissues

Tracheid are widespread in broadleaved woody plants; in broadleaved plants they generally include the compound scalariform tracheid and scattered scalariform tracheid, among which the latter was dominant in most of the broadleaved woody plants, except for *Cryptocarya chinensis* (Hance) Hemsl, *Cinnamomum camphora* (L.) Presl, *Photinia davidsoniae* Rehd. et Wils, *Gardenia jasminoides* Ellis, and *Vitex negundo* L. var. *cannabifolia* (Sieb. et Zucc.) Hand.-Mazz (Fig. 4, a–c). In addition to tracheid, a few unique types in vascular tissues are also produced; for example, wreath structures derived from vascular bundle sheaths were identified in *Mallotus philippensis* and *Albizia julibrissin* Durazz (Fig. 4, t). Among the broadleaved woody plants, the contents of tracheid are large in Rosaceae and Lauraceae, concentrated within the range of 40–60%, and relatively small in Euphorbiaceae, Ulmaceae, and Moraceae. Moreover, the contents of tracheid among different species of plants within families such as Magnoliaceae, Rubiaceae, and Hamamelidae vary widely.

Mesophyll

Generally, the mesophyll tissue in the plant epidermis is clearly differentiated into palisade tissue and spongy tissue, and the phytoliths developed from them are called palisade phytoliths (Fig. 4, e) and favose phytoliths (Fig. 4, f), respectively, and such phytoliths not only have small morphological differences among different plant species, but they also have similar contents, generally <5%.

Hair cells

Hair cells can be subdivided into macro hair, small hair and prickle cells. Macro hair mainly occurs in broadleaved woody plants, which mainly produce two morphotypes: hair and hair with base (Fig. 4). The contents of hair and hair with base in broadleaved woody plants vary greatly among different families, with Ulmaceae, Lauraceae, Rubiaceae, and Moraceae typically having values of 5–40%, while Fagaceae, Magnoliaceae, and Anacardiaceae either do not contain or have only small amounts of these two phytolith types.

Other cells

Phytoliths of other cells generally occur only in certain families and therefore they also tend to be specific to certain broadleaf species. For example, sclereid, derived from thick-walled heteromorphic cells, is distributed in various broadleaved woody plants (Fig. 4, r); and elongate attenuate (Fig. 3, q), elongate tenuous psilate (Fig. 3, s), and elongate attenuate entire (Fig. 3, r), which are produced in fibrous tissue, are mainly distributed within Fagaceae, Lauraceae, and Rosaceae, with contents as high as 10–20%. In a few families and genera there are also a small number of blocky regulate from unknown sources. (Fig. 4, o–p).

These observations demonstrate the abundance of phytolith morphotypes in broadleaved woody plants in the mid-subtropical zone of China, and according to micro-differences in their shapes and texture, they include sclereid, elongate attenuate, elongate tenuous psilate, elongate attenuate entire, tabular sinuate, and blocky regulate which are unique phytolith types in broadleaf plants. The prevalent silicified stomata, hair and tabular have characteristics that differ from those of herbs and conifers.

Relationship between broadleaved woody plant phytoliths and their parent plants

We used principal component analysis (PCA) and discriminant analysis (DA) to investigate the relationship between the phytolith types of broadleaved woody plants and their parent plants. PCA was performed on all the broadleaved woody plant samples and on 12 phytolith types with a relatively high content (Fig. 5). PCA 1 and 2 accounted for 29.4% and 18.9% of the total variance, respectively, with the combined contribution of

48.3%. As can be seen from Fig. 5, Fagaceae, Lauraceae, Magnoliaceae, Rosaceae, Anacardiaceae and Euphorbiaceae are clearly distinguished from the other families, which are not well separated because of their small number (in future research we intend to increase the number of sampled species to further explore the relationship between the phytoliths of different families and their parent plants). From the perspective of the relationship between phytolith type and the parent plants, blocky, elongate and lanceolate are best correlated with Fagaceae, while tabular and tracheid are strongly correlated with Lauraceae and Rosaceae. Epidermal phytoliths and silicified stomata have a relatively strong relationship with Anacardiaceae. Mesophyll and sclereid are closely related to Magnoliaceae. Evidently, the phytolith assemblages can be used to distinguish different families of broadleaved plants to a certain extent, and the corresponding phytolith types of different families are distinctive.

To further clarify the relationship between broadleaved woody plant phytoliths and their maternal plants among different families, the percentages of 12 phytolith types were used to discriminate plants of Fagaceae, Lauraceae, Magnoliaceae, Rosaceae, Anacardiaceae and Euphorbiaceae. The results (Table 2) show that 92.9% of the plants in the initial grouping cases were correctly classified based on the percentage of woody plant phytoliths, which indicates pronounced differences between the phytoliths of broadleaved woody plants of different families. In addition, several plant samples were used for non-repetitive cross-validation, in which the accuracy decreased somewhat, but still correctly classified 46.4% of the plants in the cross-validation group (Fig. 6). The discriminant accuracies of Fagaceae, Lauraceae, Magnoliaceae, Rosaceae and Euphorbiaceae were 62.5%, 50%, 25%, 75% and 33.3%, respectively (Table 2). Thus, to a certain extent, phytolith assemblages can be used to classify broadleaved woody plants into families, and the plants of different families correspond well to different phytolith morphologies.

Table 2
Results of discriminant analysis (DA) of broadleaved woody plants based on phytolith percentages

| Discriminant type | | Actual group | Predicted group | | | | | Total | |
|-------------------|----------------|---------------|-----------------|-----------|--------------|----------|---------------|-------|---------------|
| | | | Fagaceae | Lauraceae | Magnoliaceae | Rosaceae | Anacardiaceae | | Euphorbiaceae |
| Origin | No. of samples | Fagaceae | 8 | 0 | 0 | 0 | 0 | 0 | 8 |
| | | Lauraceae | 1 | 4 | 0 | 1 | 0 | 0 | 6 |
| | | Magnoliaceae | 0 | 0 | 4 | 0 | 0 | 0 | 4 |
| | | Rosaceae | 0 | 0 | 0 | 4 | 0 | 0 | 4 |
| | | Anacardiaceae | 0 | 0 | 0 | 0 | 3 | 0 | 3 |
| | | Euphorbiaceae | 0 | 0 | 0 | 0 | 0 | 3 | 3 |
| | Percentage (%) | Fagaceae | 100 | 0 | 0 | 0 | 0 | 0 | 100 |
| | | Lauraceae | 16.7 | 66.7 | 0 | 16.7 | 0 | 0 | 100 |
| | | Magnoliaceae | 0 | 0 | 100 | 0 | 0 | 0 | 100 |
| | | Rosaceae | 0 | 0 | 0 | 100 | 0 | 0 | 100 |
| | | Anacardiaceae | 0 | 0 | 0 | 0 | 100 | 0 | 100 |
| | | Euphorbiaceae | 0 | 0 | 0 | 0 | 0 | 100 | 0 |
| Cross validation | No. of samples | Fagaceae | 5 | 0 | 1 | 1 | 0 | 1 | 8 |
| | | Lauraceae | 1 | 3 | 0 | 0 | 2 | 0 | 6 |
| | | Magnoliaceae | 1 | 0 | 1 | 0 | 1 | 1 | 4 |
| | | Rosaceae | 0 | 1 | 0 | 3 | 0 | 0 | 4 |
| | | Anacardiaceae | 0 | 2 | 1 | 0 | 0 | 0 | 3 |
| | | Euphorbiaceae | 2 | 0 | 0 | 0 | 0 | 1 | 3 |
| | Percentage (%) | Fagaceae | 62.5 | 0 | 12.5 | 12.5 | 0 | 12.5 | 100 |
| | | Lauraceae | 16.7 | 50 | 0 | 0 | 33.3 | 0 | 100 |
| | | Magnoliaceae | 25 | 0 | 25 | 0 | 25 | 25 | 100 |
| | | Rosaceae | 0 | 25 | 0 | 75 | 0 | 0 | 100 |
| | | Anacardiaceae | 0 | 66.7 | 33.3 | 0 | 0 | 0 | 100 |
| | | Euphorbiaceae | 66.7 | 0 | 0 | 0 | 0 | 33.3 | 100 |

Preservation quality of typical broadleaved phytoliths in surface soil

Our observations and statistical analysis of the phytolith characteristics of the 60 species of broadleaved woody plants enabled the characteristic phytoliths of these plants within the surface soil of this region to be qualitatively summarized, according to subtle differences in phytolith shape and texture. They include elongate (elongate attenuate, elongate attenuate entire, and elongate tenuous psilate), tabular (tabular sinuate), blocky (blocky regulate), hair (hair) and other types (sclereid and favose phytolith), as shown in Fig. 7. To facilitate the use of phytoliths to reconstruct paleovegetation and paleoclimate, we enumerated the phytolith assemblages of the surface soil samples from 31 different plant communities in the mid-subtropical zone of China, and then used the R value of the ratio of the surface phytolith content to the community phytolith content to further explore the preservation characteristics of broadleaved woody plant phytoliths in the surface soil of this region.

The ratio of the concentration of broadleaved phytoliths in the surface soil to that in the corresponding plant communities represents the preservation quality of broadleaved phytoliths in different communities: the higher the ratio, the better the quality. Only ~ 4.43% of the information on existing broadleaved phytoliths is preserved in the surface soil of the central subtropical region; in short, broadleaved phytoliths are poorly preserved. According to the content of different phytolith types, the preservation degree of different phytolith types in different communities can be represented by the ratio of the content of broadleaved phytoliths in the surface soil to that in the corresponding plant community; the higher the ratio, the better the preservation. As can be seen from Fig. 8, blocky regulate, elongate attenuate and elongate tenuous psilate in the surface soils are better preserved, whereas hair, tabular sinuate, favose phytolith, sclereid and elongate attenuate entire are relatively poorly preserved. The use of phytoliths to reconstruct paleovegetation and paleoclimate necessitates more attention being paid to the types of phytoliths that are poorly preserved in the surface soils in this region. The quantitative relationship between phytolith and vegetation can be corrected according to the preservation quality, which may improve the accuracy of phytolith-based paleoenvironmental reconstruction.

Discussion

Morphology and classification of phytoliths in broadleaved woody plants

The phytolith morphology of woody plants is complex and diverse. The impetus for studying their morphological characteristics and representative types is driven by their potential utility for more accurate paleovegetation reconstruction. However, except for Pinaceae and Palmae, research on the phytoliths of woody plants is scarce and not systematic. Following the International Code for Phytolith Nomenclature (ICPN1.0; ICPN 2.0), a small number of woody phytoliths have been described (Fenwick et al., 2011; Das et al., 2013). The characteristics of woody phytoliths have been refined based on shape and texture, which are highly significant for distinguishing several phytolith types with minor differences in shape and texture (Carnellia et al., 2004; Xu et al., 2005; Morris et al., 2009). However, due to the complex and diverse morphology of woody plant phytoliths, descriptions of their morphotypes remain confusing and the different terminologies used are difficult to unify. Moreover, the description of several morphotypes has not adopted the ICPN standard, and the inconsistent description standards among different workers has hindered the communication and sharing of phytolith research results between regions (Kondo, 1977; Wallis, 2003). Up till now, there is no consensus on the morphological characteristics and names of the phytoliths in woody plants, and a relatively comprehensive classification system of phytoliths in woody plants has not been developed. Therefore, based on the International Code for Phytolith Nomenclature (ICPN), a much greater number of studies on the phytolith morphology of woody plants are needed to provide a more systematic understanding. In addition, current studies of the morphology of woody phytoliths have not paid much attention to differences in anatomical origin. However, in the Changbai Mountains in Northeast China and in the alpine coniferous forest region of Norway, phytolith assemblages and the typical phytolith types of individual families and genera of woody plants have been enumerated and described from the perspective of anatomical origin, and the regional paleovegetation has been successfully reconstructed (Gao et al., 2018a; Gao et al., 2018b; Lisztes-Szabó et al., 2019; Gao et al., 2021). Therefore, due to the obvious differences in the external morphology of phytoliths of different anatomical origins, and their important role in identifying the parent plants of phytoliths and in distinguishing herbaceous and woody phytoliths, the classification of woody plant phytoliths based on anatomical origin requires further attention.

Based on the foregoing, we now focus on three aspects to describe the phytolith types of 60 broadleaved woody plants in the mid-subtropical zone of China: anatomical origin, shape and texture. Previous studies have shown that, in general, herbaceous plants are the main producers of phytoliths, and that the phytolith content of woody plants is lower than in herbaceous plants (Wang and Lu, 1993; Piperno, 2006). Our results are consistent with this conclusion: we found that 10 of the 60 species of broadleaved woody plants selected produced no or very few phytoliths. However, it has been shown that some of the tissues or cells with different morphologies are significant for plant classification (Shao et al., 2005). The morphology of phytoliths in different plants is substantially different, which is mainly related to the cell origin of the phytoliths. For example, phytoliths in Poaceae are mainly deposited in short cells, long cells and hair cells of the epidermis. In this study, phytoliths in broadleaved woody plants were mainly generated in epidermal cells, stomata and tracheid, among which the content of phytoliths in epidermal cells was the largest, followed by silicified stomata, tracheid, hair and hair base, which is consistent with previous studies (Kondo, 2010). In addition, phytoliths have stable genetic characteristics and a consistent significance for plant classification. For example, Poaceae plants typically produce short cell phytoliths, and dicotyledonous plants produce numerous hair and epidermal phytoliths (Wallis, 2003). We found that the phytoliths of different families and genera of broadleaved trees were significantly different, and that species belonging to the same genus usually produced the same morphotype. This suggests that phytolith analysis can identify taxa at the genus level of broadleaved woody plants, although more studies of individual species are needed for confirmation.

Considering the anatomical origin of phytoliths and the differences in their shape and texture, we described six phytolith types from the perspectives of anatomical origin, shape, and texture in this paper—which is consistent with the requirements of the ICPN and is convenient for integration with international phytolith research. Until now, there is no consensus regarding the unique phytolith types of a given family or genus of broadleaved woody plants. Based on our findings and on previous studies of the phytoliths of broadleaved woody plants (Mercader et al., 2010; Ge et al., 2011; Gao et al., 2018a; Lisztes-Szabó et al., 2019; Pearce and Ball, 2019; Ge et al., 2020), the morphotypes of broadleaved woody plants can be summarized as: sclereid, elongate attenuate, elongate attenuate entire, elongate tenuous psilate, tabular sinuate, blocky regulate, hair and favose phytolith. These characteristic morphotypes may be of great significance for studying changes in the alpine timberline and extreme climatic events in the mid-subtropical zone of China. However, due to the limited sample size of this study, further research is needed to evaluate their diagnostic potential.

Preservation of representative phytoliths of broadleaved woody plants

Although the value of herbaceous plant phytolith analysis in the reconstruction of herbaceous plants is well established and recognized (McCune and Pellatt, 2013), research on the phytoliths of woody plants—especially for the reconstruction of the paleovegetation in forested areas—is limited. So far, the application of phytoliths to reconstructing Holocene changes in the forest-grass ecotone in tropical and subtropical regions has only involved the use of globular granulates (Bremond et al., 2005; Barboni et al., 2007), which are relatively rare elsewhere. This is mainly due to the occurrence of unique and abundant globular phytoliths in *Palmae* in tropical and subtropical regions. However, *Palmae* are rare in the mid-subtropical zone of China, where research on the typical phytolith types of broadleaved woody plants is lacking. Therefore, determining the representative phytolith types of broadleaved woody plants in this region is the key to enhancing the application of broadleaved woody phytoliths in this context. Based on previous understanding (Rovner, 1971; Klein and Geis, 1978; Gao et al., 2018a; Ge et al., 2020), eight common broadleaved phytoliths were identified in this study and the elongate attenuate and elongate attenuate entire are new additions. Therefore, in studies of the changes in the forest-grassland ecotone and paleovegetation reconstruction using broadleaved woody phytoliths in the mid-subtropical zone of China, we suggest that these phytolith types should be comprehensively considered, and the phytoliths of different families and genera should be distinguished to provide a true reconstruction of the original vegetation.

Due to the interference of various external factors, after phytoliths enter the soil, only those that are strongly silicified are generally preserved (Cabanés et al., 2011). Previous studies showed that the phytoliths of coniferous woody plants are more easily preserved, and that the types and contents of better-preserved phytoliths are significantly higher than those of broadleaved woody plants (Alberta et al., 2006; Caroline, 2004). Our results are consistent with these findings: only ~ 4.43% of the existing broadleaved woody plant phytolith assemblages is retained in the surface soil in the study area. In terms of the preservation of phytoliths of different types of broadleaved woody plants, previous studies have shown that phytoliths that are relatively thin and have a large surface area and volume are poorly preserved in surface soil. These types include hair and silicified stomata (Cabanés et al., 2011), which is consistent with our findings. It was also found that blocky regulate, elongate attenuate and elongate tenuous psilate are well preserved in surface soil, while elongate is better preserved (Cabanés et al., 2011; Gao et al., 2018a; Liu et al., 2021). Additionally, several characteristic phytolith types, such as tabular sinuate and sclereid, are also produced in broadleaved trees in the mid-subtropical zone of China; however, their content in surface soil is < 5% of total phytoliths, and thus they significantly underestimate the numbers of parent plants. Therefore, in phytolith-based paleovegetation reconstructions in this area, it is important to apply correction factors to the numbers of characteristic phytoliths of broadleaved woody plants to provide a more accurate representation of the broadleaved trees. However, studies of the preservation degree of different broadleaved phytolith types are few and they are not comprehensive, and more detailed studies are needed.

Conclusion

(1) We have comprehensively summarized the rich phytolith morphology of broadleaved woody plants in the mid-subtropical zone of China. Based on anatomical origin, six major morphotypes of the common broadleaved woody plants in this region are: epidermal phytolith, silicified stomata, tracheid, favose phytolith, hair, and others. The phytolith types are further subdivided into 36 sub-types based on micro differences in shape and texture.

(2) The phytolith assemblages of different families of broadleaved woody plants can be clearly distinguished based on phytolith contents. *Fagaceae*, *Lauraceae*, *Magnoliaceae*, *Rosaceae*, *Anacardiaceae* and *Euphorbiaceae* can be clearly distinguished from other families. Among them, *Fagaceae* are characterized by blocky, elongate and lanceolate; *Lauraceae* and *Rosaceae* are characterized by tabular and tracheid; *Anacardiaceae* is characterized by epidermal phytoliths and silicified stomata; and *Magnoliaceae* is characterized by favose phytolith and sclereid. These results indicate some degree of correspondence between different phytolith types and the parent plants, which supports the selection of representative phytolith types of broadleaved woody plants in soil.

(3) Based on the observation and description of phytolith morphology of broadleaved woody plants, the representative types were identified, including elongate attenuate, elongate attenuate entire, elongate tenuous psilate, tabular sinuate, blocky regulate, hair, sclereid and favose phytolith. However, there were slight differences in the preservation of the eight phytolith types in surface soil. Blocky regulate, elongate attenuate and elongate tenuous psilate are more readily preserved, while hair, tabular sinuate, favose phytolith, sclereid and elongate attenuate entire were relatively poorly preserved. Studies of the preservation of broadleaved woody phytoliths in soils in the mid-subtropical zone of China can provide a reference for the application of soil phytoliths to the more accurate reconstruction of paleovegetation and paleoclimate in this region.

Declarations

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Figures

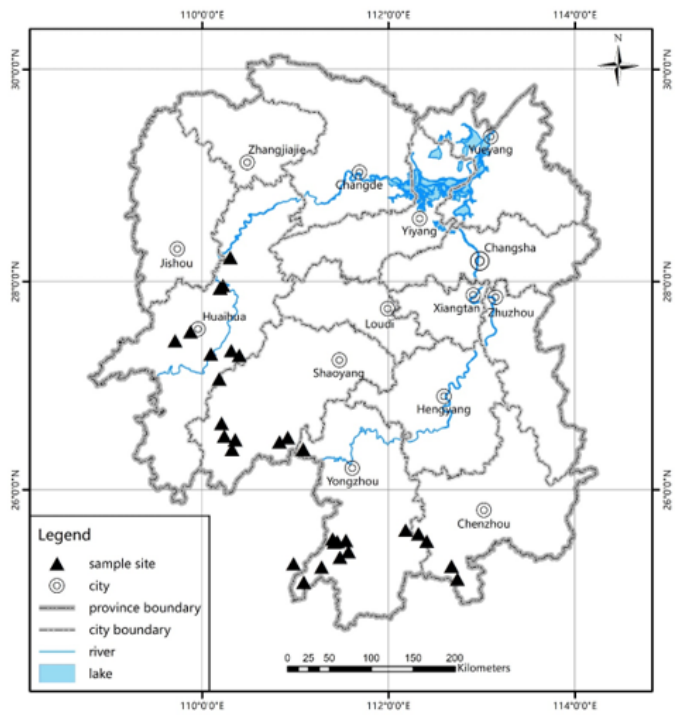


Figure 1
Distribution of sampling sites

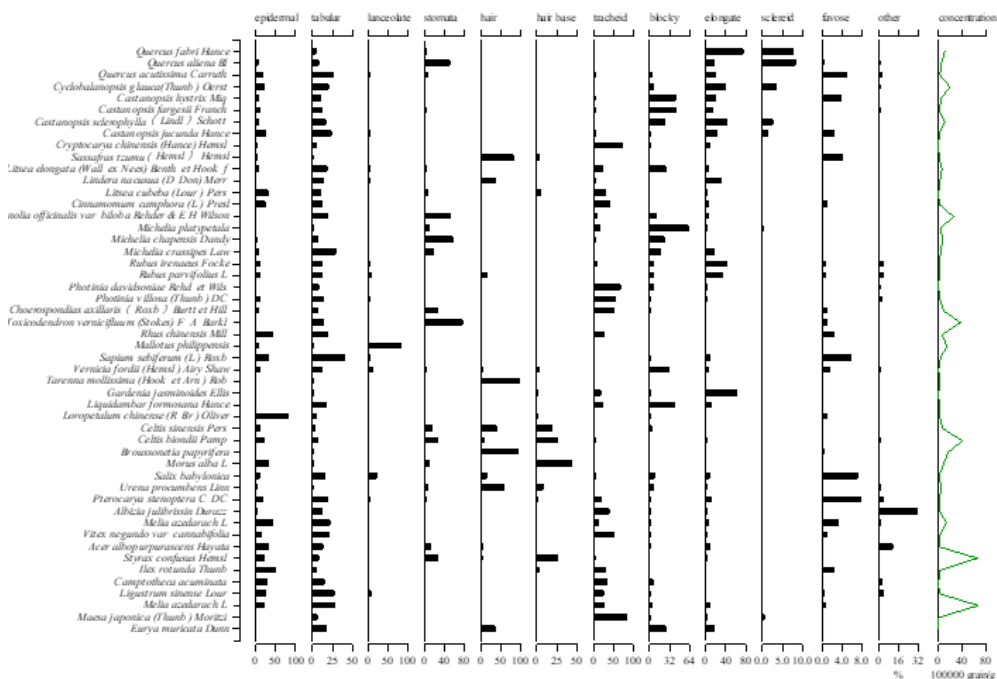


Figure 2
Percentages and total concentrations of phytoliths in the broadleaved woody plant samples

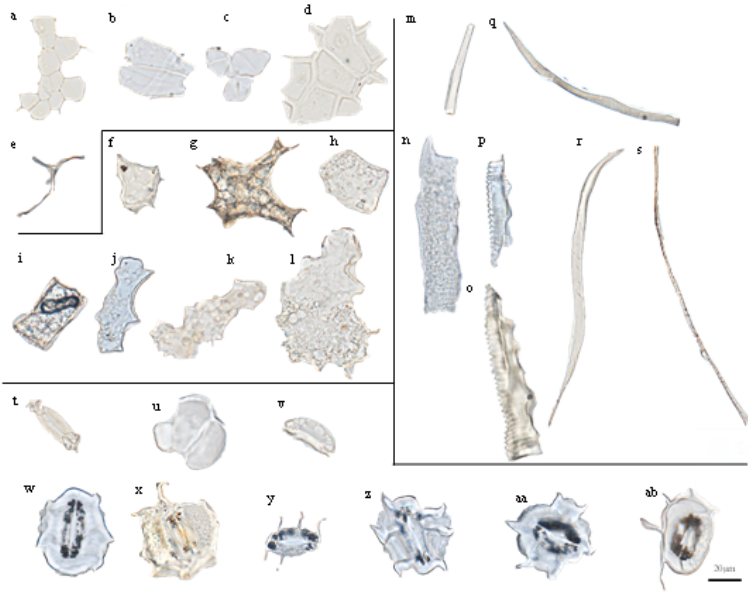


Figure 3
Phytolith morphotypes originating from epidermal cell and stomata in broadleaved woody plants

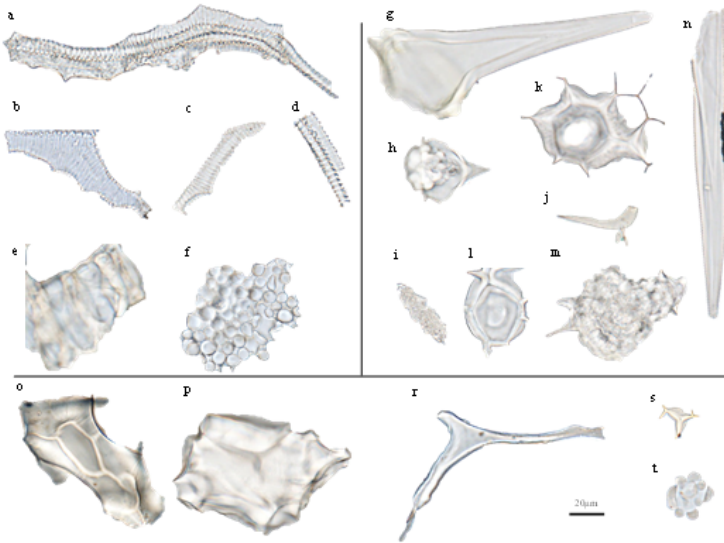


Figure 4
Phytolith morphotypes originating in vascular tissue, mesophyll, hair cells, and other cells in broadleaved woody plants

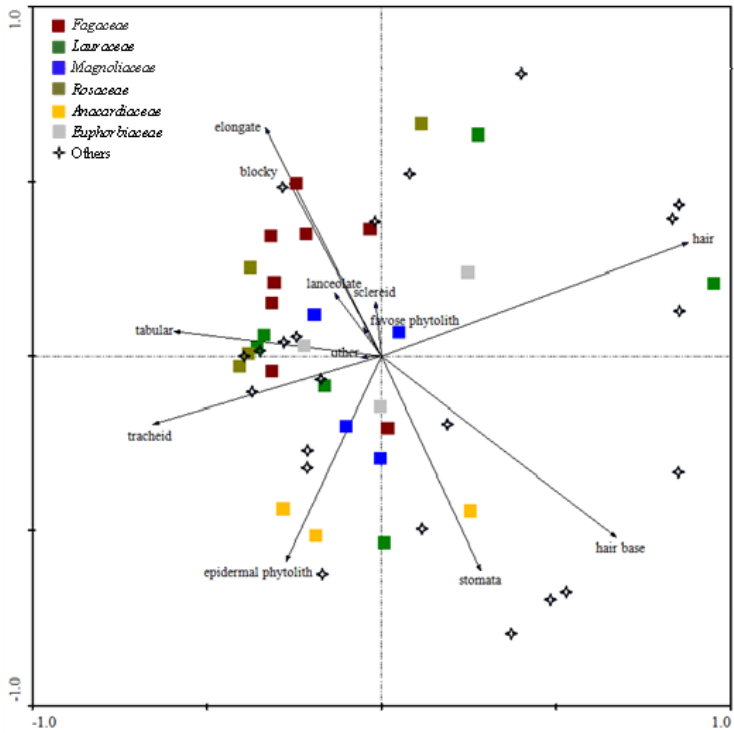


Figure 5
Results of principal component analysis (PCA) of 12 phytolith morphotypes of broadleaved woody plants

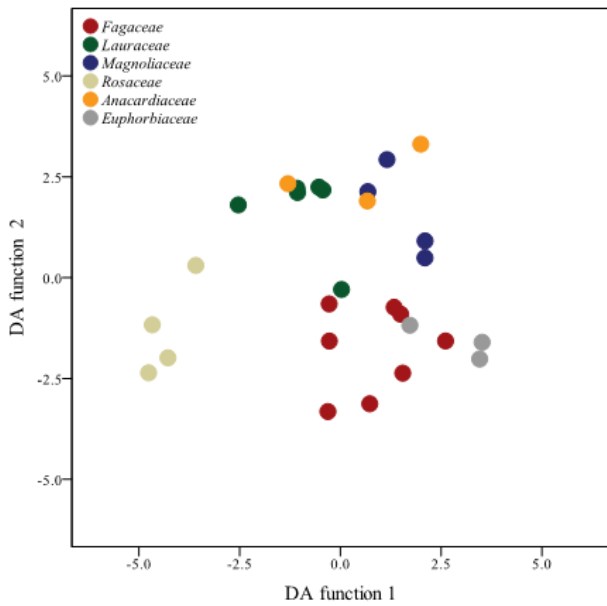


Figure 6
Results of discriminant analysis of broadleaved woody plants of different families based on phytolith percentages

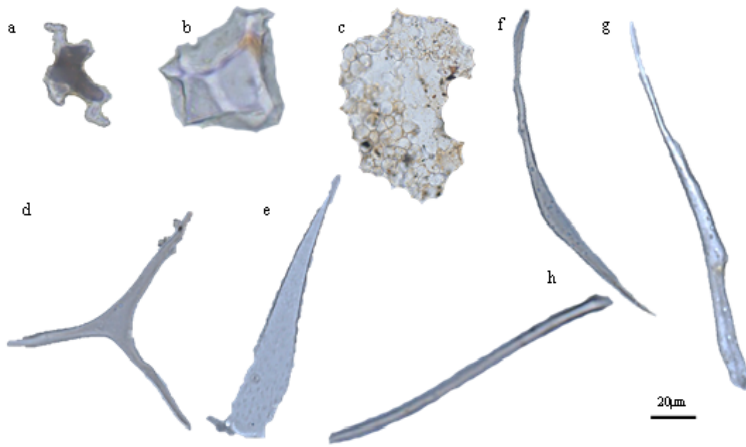


Figure 7

Representative phytolith types of broadleaved woody plants in the surface soil samples

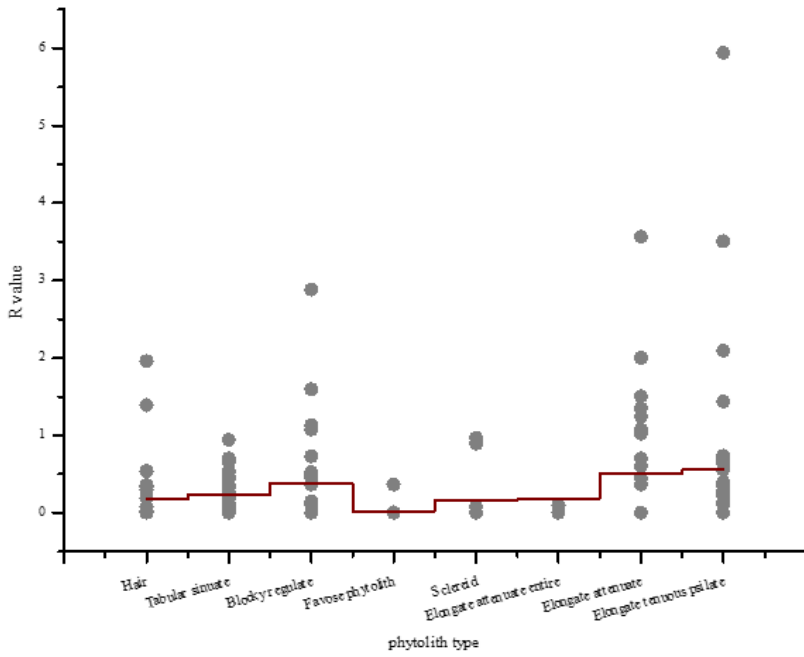


Figure 8

R values of broadleaved phytoliths in the surface soil samples